Abstracts

Plenary Sessions

PL01. Polygenes and the Prevention of Cancer

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Most common cancers show a tendency to familial clustering. The risk to a close relative of a case is increased 2- to 3-fold. Most of this effect is genetic in origin. Our analysis of familial clustering of breast cancer suggests that known strongly predisposing genes such as BRCA1/2 account for only 20% of the total genetic predisposition. A substantial part of the remaining 80% may be attributed to the combination of many common genetic variants, individually of small effect.

If this is so, different individuals in the population will inherit different combinations of variant alleles. By analogy with a hand of cards, some will have a 'strong' hand (kings and queens; high risk), some a 'weak' hand, and most will lie somewhere in between. The result will be a bell-shaped curve that describes the distribution of risk in the population.

From the viewpoint of practical application, the critical question is not 'how much genetic risk is there?' but 'how is the risk distributed?'. If the difference in risk between individuals at high and low risk within the population is small (say 2- to 3-fold), this may not be helpful : but if it is 30- or 40-fold, the implications are very significant.I will discuss these concepts, and the progress and obstacles in finding the genes and other factors that would make up an individual risk profile.

PL02. Prevention and Scar Free Healing of Cleft Lip and Palate: applications from developmental research

***M. Ferguson**;*
School of Biological Sciences, University of Manchester, Manchester, United Kingdom.

No abstract available.

PL03. Life, Sex and WT1 Isoforms

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Mutations in the Wilms' tumour gene, *WT1*, may lead not only to childhood kidney cancer but also to severe glomerular nephropathy and sex reversal. The *Wt1* gene may encode up to 24 slightly different proteins through a combination of alternative splicing, alternative translational start sites and RNA editing. All these isoforms contain 4 C-terminal zinc fingers similar to those found in transcription factors. All non-mammalian vertebrates express only two of these WT1 isoforms differing by just 3 amino acids, KTS, inserted by alternative splicing between zinc fingers 3 and 4. We have shown that these WT1 (+KTS) and WT1 (-KTS) isoforms have remarkably different properties, in terms of subnuclear localisation and interaction with nucleic acids and proteins. Our findings suggest that the –KTS isoforms function as transcription factors, whereas the +KTS isoforms seem to function in RNA splicing. Both human genetics (Frasier's syndrome) and mouse knockouts show that these proteins have different functions during genitourinary development and both are required for survival. The +KTS proteins in particular are required for male determination and function upstream of SRY. Mice lacking WT1 have no kidneys or gonads and the heart is abnormal. I will discuss some of the novel approaches we are taking to identifying the functions of WT1 in the development of these tissues. This includes the use of siRNA to inhibit WT1 expression in kidney organ cultures and the use of a powerful new microscopic technique developed at this Unit, optical projection tomography (OPT).

PL04. Issues in setting up population DNA collections

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No abstract available.

PL05. A survey of practices in biobanking in Europe

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Large population-based collections are only a part of the current biobanking picture. The EU supported EUROGENBANK project investigated human biobanking in Europe, particularly organisational, economical and ethical issues. Data were collected from six EU countries and 147 institutions (mostly public or non-profit private) were investigated by questionnaires and interviews. This activity is increasing in all countries, but most collections are small; only a few are very large. Their purpose is often research, or research and healthcare, mostly in the context of disease studies. Specific budget is rarely allocated and costs are not often evaluated. Samples are usually provided free of charge. Good practice guidelines are generally followed and quality controls performed, but quality procedures are not always clearly explained. Associated data are usually computerised and identifiable. Biobankers generally favour centralisation of data rather than of samples. Legal and ethical harmonisation within Europe is considered likely to facilitate international collaboration. We propose a series of recommendations arising from the project (Hirtzlin I *et al.,* An empirical survey on biobanking of human genetic material and data in six EU countries. *Eur J Hum Genet*, 2003, in press)

In conclusion, biobanking is a lively, diversified and growing activity in Europe. Its rather loose organisation at small scale is not adapted to forthcoming large-scale projects that will need changes in the way ethical issues are dealt with. Empirical studies such as the EUROGENBANK survey, together with wide-ranging exchange of views at European level and multidisciplinary approaches, are useful tools in this challenging field.

PL06. What Use are Population DNA Collections?

***R. Zimmern**;*
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The use of population DNA collections is growing across many different parts of the world. The collections may comprise patients with known diseases and controls, or individuals within a particular country or geographical area. Epidemiologists make use of the data derived from these collections either to correlate specific disease with genetic variants or to study the combined effects of genetic and environmental factors on disease risk. Standard case control methodology is applied for collections based on disease, while for collections of individuals that are followed over a period of time, cohort study methodologies are used. This paper attempts to analyse the utility of these collections from a public health perspective. It investigates the extent to which such collections add to and support the promotion of health and the prevention of disease, and discusses the validity of their data for epidemiological analysis and as predictors of disease risk. The establishment of these collections is not entirely unproblematic. They raise concerns of privacy, consent and confidentiality as well as those of working with the commercial sector. If scientific progress is to be made, these ethical and social implications must be addressed in tandem with the epidemiological issues.

PL07. Genetic Epilepsies

***M. Gardiner**;*
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Epilepsy is a disorder characterised by recurrent episodes of abnormal over activity of the brain known as epileptic seizures. There are many causes but a genetic aetiology is present in about 40% of cases.

Genetic epilepsies are usefully categorised by mechanism of inheritance (mendelian, 'complex' inheritance, chromosomal) and into 'symptomatic' in which there is a recognisable structural or functional

abnormality of the brain, or 'idiopathic' (primary) in which there is no identifiable cause other than a presumed genetic one.
In the last decade, rapid progress has been made in understanding the molecular genetic basis of human mendelian epilepsies. Symptomatic mendelian epilepsies are caused by a wide variety of genes in which mutations cause abnormal brain development, neurodegeneration or altered brain metabolism. Ten genes have now been identified in human mendelian idiopathic epilepsies, all but one of which encode ion channels: *CHRNA4, CHRNB2, GABRA1, GABRG2, SCN1A, SCN2B, SCN1B. KCNQ2, KCNQ3* and *LGI1.* These underlie a wide range of overlapping phenotypes. In parallel, genes underlying spontaneously occurring mouse mutants with spike-wave epilepsy have been shown to encode subunits of voltage-dependant calcium channels.
Progress in the investigation of idiopathic generalised epilepsies with 'complex' inheritance such as juvenile myoclonic epilepsy (JME) and childhood absence epilepsy (CAE) has been much slower. Association studies using SNP haplotypes in candidate ion channel genes may allow advances in the next decade.
References:
1. Elmslie F, Gardiner RM, Lehesjoki A-E (2002) The epilepsies in *Emery and Rimoin's Principles and Practice of Medical Genetics.* Rimoin DL, Connor J, Pyeritz RE, Korf B (eds) Churchill Livingstone, New York pp 3036-3075.
2. Meisler MH, Kearney J, Ottman R, Escayg A (2001) Identification of epilepsy genes in human and mouse. *Annual Review of Genetics* 35, 567-588.

PL08. Congenital muscular dystrophies with stuctural changes of the centralnervous system: disorders of O-glycosylation

T. Voit*;*
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No abstract available.

PL09. Mitochondrial DNA disorders

D. M. Turnbull*;*
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No abstract available.

PL10. Overview on limb development

C. Tickle*;*
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No abstract available.

PL11. Genetics of polydactyly

P. Heutink*;*
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In the past decade rapid advances have been made in the identification of human genes that play a role in upper limb malformations, leading to a better understanding of normal limb development.
One of the most frequently observed limb malformations is polydactly, with a prevalence of between 5 and 17 per 10,000 live births. Depending on the location of the extra digit, polydactyly is divided into pre-axial, post-axial, and central polydactyly.
For post-axial polydactyly, mutations in the *GLI3* gene have been identified and additional genetic loci on chromosomes 13q21,19p and 7q35 have been reported.For pre-axial polydactyly a major locus on chromosome 7q36 exists. The work on this locus is an excellent example of how collaborations between human geneticists and developmental biologists can lead to identification of disease genes. A pre-axial polydactyly locus was identified in 1993 but mutations in genes within the critical region could not be identified. Only by using a combination of linkage data, cytogenetics and animal models it could be demonstrated that the disruption of a regulatory element approximately 800 kb upstream of the *SHH* gene is the most likely explanation for this phenotype.

PL12. Brachydactyly

S. Mundlos*;*
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No abstract available

PL13. Preserving the Heritage of Human Genetics

P. S. Harper*;*
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Although the pedigree and quantitative analysis of human genetic characteristics and disorders began with the recognition of Mendel's laws, the laboratory basis of human cytogenetics and molecular genetics is only 50 years old and the development of medical genetics as a specific field even less. This creates a unique opportunity for documenting the history of our field, since many of the key workers are still alive and their records still in existence.The urgency needed to seize this opportunity has resulted in the creation of the Genetics and Medicine Historical Network, supported by the Wellcome Trust, and closely associated with the European Society for Human Genetics. All interested workers in Human Genetics can contribute in their own areas by helping to ensure that records and correspondence are preserved, older scientists are interviewed and the context of key discoveries documented. This should provide a detailed foundation on which future historians and social scientists can base their studies, as well as making the history of genetics in relation to medicine directly accessible to the wider public.

Scientific Symposia

S01. Mouse bioinformatics from a human geneticist's perspective

G. Borsani*;*
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The laboratory mouse is the most important model organism for studying genetic and multi-factorial diseases in man. As a mammal the mouse has many anatomical, physiological, and metabolic similarities with humans. Furthermore genetic manipulation within the living mouse has been established more than 20 years ago leading to the generation of many mouse models that closely resemble the analogous human pathologies.
The recently disclosed mouse genome sequence revealed about 30,000 genes (with 99% having direct counterparts in humans) and allowed a detailed analysis of conserved synteny between the two species. Comparative genomics between human and mouse is a powerful approach for unlocking some of the hidden features of mammalian genomes such as non-protein coding RNAs, antisense RNAs and conserved regulatory regions.
With knowledge of both genomes, studies of human genes can be complemented by experimental manipulations of corresponding mouse genes to accelerate functional studies. A variety of functional genomics efforts are underway to analyze and to understand the mouse genome. Strategies such as gene traps and chemical mutagenesis are generating a large mutant mouse resource that complements the information already available on spontaneous mutations. Systematic gene expression studies are being carried out on large sets of mouse genes using approaches such as RT-PCR and RNA *in situ* hybridization.
A growing number of mouse bioinformatics resources collect and elaborate data generated by genomics and functional genomics projects, offering a wide range of information on the biology and genetics of the laboratory mouse and providing a huge boost to human genetics researchers.

S02. Bioinformatic analysis of regulatory sequences

E. Wingender*;*
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No abstract available.

S03. Structural bioinformatics: the current view of structure, function and interaction prediction

R. Russell;
Structural Bioinformatics, EMBL, Heidelberg, Germany.

Bioinformatics related to protein three-dimensional structures has come a long way from the days of soothsayers making wrong predictions of protein structures. In this talk I will discuss some of the highlights of the past decade. First, in the context of the recent Critical Assessment of Structure Prediction experiment, I will highlight the current state-of-the art in predicting structure from amino acid sequence, including in effect „solutions" to the protein folding problem. I will then discuss methods to predict function from structure, and the impact of these on Structural Genomics initiatives, and ultimately genome annotation. Lastly, I will comment on how structures, particularly those of proteins in complex with others, can be useful in interpreting interactions found by other methods such as yeast two-hybrids or affinity purifications. The last subject leads to the idea of „Complex" Structural Genomics, that has the ultimate goal of complete structures for whole cells or beyond.

S05. The Eurostem Project

J. Harris;
University of Manchester, Manchester, United Kingdom.

No abstract available

S06. SCIDX gene therapy: Successes and adverse effects

S. Hacein-Bey-Abina;
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No abstract available.

S07. Human hereditary deficits: an unravelling of the sensory systems

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As in the other animal species, human senses are adapted to their environment. Humans perceive the energy of a large variety of stimuli, namely photonic, mechanical, thermic and chemical stimuli, by sensory cells, either dispersed or packed in sensory end organs. Fifteen years ago, our knowledge of the molecular bases of the function of the various modes of sensory reception was very rudimentary with the noticeable exception of the phototransduction cascade.

We considered that the study of hereditary sensory defects in humans would not only have a medical impact, but also should lead to an understanding of some fundamental mechanisms underlying the development and the functioning of the sensory systems. In particular, I proposed explicitly that the study of inherited hearing loss would provide important insights into the biology of the cochlea, the auditory sensory organ, which had thus far escaped molecular characterisation due to the small number of each cell type that it houses.

The present outcome of this research will be discussed in medical and fundamental terms. How the study of these defects enlightens both their pathogenesis and the physiology of the sensory system will be illustrated by the development/functioning of the auditory cells and the development of the olfactory system as illuminated by the functions of the proteins defective in Usher type I syndrome and Kallmann syndrome.

S08. Mouse Mutagenesis for the Genetic Dissection of Auditory Function

S. D. M. Brown;
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Systematic approaches to mouse mutagenesis are vital for future studies of mammalian gene function. We have undertaken a major ENU mutagenesis programme incorporating a large genome-wide screen for dominant mutations (Nolan et al. *Nature Genetics* 25: 440-443). Nearly 30,000 mice have been produced and around 700 mutants have been recovered from the screening programme that included tests for auditory and vestibular function. 54 phenotypes showing balance or circling phenotypes were uncovered, while 28 mice showing hearing impairment, including late-onset sensorineural deafness, were identified. The mouse auditory and vestibular mutants encompassed a variety of phenotypes including patterning and planar cell polarity defects in hair cells in the organ of Corti as well as morphogenetic mutants affecting the development and structure of the vestibular system. Many of these mutants have now been mapped and cloned to elucidate the underlying genetic pathways involved. In addition, these screens for new deafness models have identified a new class of mutants whose hearing loss is due to middle ear inflammatory disease. Otitis Media with Effusion (OME) or "glue ear" disease, inflammation of the middle ear epithelial lining, remains the most common cause of hearing impairment in children. The availability of these novel mouse models represents an exciting opportunity to study the genetics of OME in both mouse and humans.

S09. Development of the inner ear - the zebrafish model

T. Whitfield;
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The zebrafish is a convenient genetic model system for the study of the inner ear. Embryos are accessible at all stages of development, and are optically clear, enabling the otic vesicle to be observed in the live animal. Both forward and reverse genetic approaches are available. We are characterising a number of mutant lines that form models for human disease, including *colourless/sox10* (a model for Waardenburg-Shah syndrome) and *van gogh/tbx1* (a model for DiGeorge syndrome). Both *sox10* and *tbx1* code for transcription factors that are strongly expressed in the otic epithelium, and both mutants have severe sensory and non-sensory otic abnormalities. We are also interested in the signals from surrounding tissues that act to pattern the otic vesicle. We have demonstrated a role for Hedgehog signalling from midline structures (the notochord and floorplate) in the patterning of the anteroposterior axis of the zebrafish ear. A loss of Hedgehog signalling gives rise to a striking partial mirror image duplication of anterior otic structures, concomitant with a loss of posterior otic domains. Ectopic activation of the Hedgehog pathway has the reverse effect: ears lose anterior structures and show a mirror image duplication of posterior regions at the anterior of the ear.

S10. Human Genetic Variation and Human Disease

D. Cox;
Perlegen Sciences Inc., Mountain View, CA, United States.

No abstract available.

S11. Genetic legacy of the European people

R. Villems;
Tartu University, Tartu, Estonia.

No abstract available.

S12. Haplotype analysis of candidate genes for common cardiovascular diseases

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No abstract available.

S13. Phenotypic manifestations of microdeletion syndromes

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During recent years several well known genetic syndromes were found to be caused by submicroscopic deletions. This has lead to a better clinical delineation of these disorders. The identification of genes implicated will lead to insight in the mechanisms underlying malformations, mental retardation and behavioural disorders. Strikingly, several microdeletion syndromes have a highly variable expression, providing an unique opportunity to study mechanisms

causing this variability. Differences in size of the deletion are not a frequent cause of variability, given the frequent origin by unequal crossing-over between flanking duplicons. However, the few unique individuals with smaller deletions will be instumental in delineating the critical deletion region, and thus the implicated genes. Examples are Smith-Magenis syndrome and the del8p23.1 syndromes. Another (rare) cause of variability is the unmasking of recessive alleles, as for instance in a del22q11 deletion patient with Bernard-Soulier disease. The most probable explanation is the existence of genetic and environmental modifiers. Intelligence is multifactorial, and it is therefore not unexpected that in for instance PWS and VCFS, IQ is correlated with parental intelligence. Approximately 50-75% of individuals with a del22q11 have a congenital heart defect. Recently, a functional polymorphism in the VEGF-gene was found to be a modifier for the occurrence of a heart defect in individuals with a del22q11. However, this still does not explain the frequent observation of discordant monozygotic twins with a del22q11 for a heart defect. Possible explanations are second hits, non-shared environment and stochastic factors.

S14. Translocation breakpoints - investigations of what really happens

***C. van Raavenswaay**;*
University Medical Center, Department of Human Genetics, Nijmegen, The Netherlands.

No abstract available.

S15. A Model for the Molecular Basis of FSHD

***R. G. Tupler**[1,2];*
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Facioscapulohumeral muscular dystrophy (FSHD) is a complex disease with a peculiar involvement of muscle groups, highly variable severity, and unpredictable progression. The disease has been causally related to deletion of subtelomeric D4Z4 repeats at 4q35. We observed that a number of genes mapping at 4q35 are over-expressed in the FSHD affected muscle. We showed that an element within D4Z4 specifically binds a multi-protein complex consisting of YY1, HMGB2, and nucleolin. We demonstrated that this multi-protein complex binds D4Z4 *in vitro* and *in vivo* mediates transcriptional repression of 4q35 genes. Based on these results, we propose a model for the molecular basis of FSHD. In normal individuals, the presence of a threshold number of D4Z4 repeats leads to repression of 4q35 genes by virtue of a DNA-bound multi-protein complex that actively suppresses gene expression. In FSHD patients, deletion of an integral number of D4Z4 repeats reduces the number of bound repressor complexes and consequently decreases (or abolishes) transcriptional repression of 4q35 genes. As a result, these genes are inappropriately over-expressed, ultimately leading to disease onset and progression. Our results also provide insights into the biological function of DNA repetitive elements in gene transcription and their potential role in human diseases.

S16. Fat and thin genes

***S. O'Rahilly**;*
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No abstract available.

S17. Gonadotrophin function and sexual development

***I. Huhtaniemi**;*
Institute of Reproductive and Developmental Biology, Imperial College London, London, United Kingdom.

The unraveling of structures of gonadotrophin and gonadotrophin receptor (R) genes, and the human mutations detected in these genes, have provided us with novel tools to clarify the role of gonadotrophin action in sexual differentiation and in molecular pathogenesis of certain disturbances of sexual differentiation, development and fertility. Moreover, the mutations detected are very elucidating by providing further information about known and less well elucidated actions of gonadotrophins. Only one inactivating mutation of the LHβ subunit has so far been described; a man with normal intrauterine masculinisation but complete blockage of postnatal sexual development. The phenotype can be explained by the stimulatory effect of choriongonadotrophin on fetal testicular androgen production, inducing the intrauterine masculinisation. However, pituitary LH is crucial for stimulation of the postnatal testicular activity. Both loss-of-function (inactivating) and gain-of-function (activating) mutations of gonadotrophin receptors are known. Completely inactivating LHR mutations cause in males pseudohermaphroditism, with external female genitalia, missing uterus and lack of female secondary sex characteristics. Incomplete LHR inactivation causes micropenis and hypospadias with varying severity. In females, the phenotype is relatively mild with anovulation and infertility. Activating LHR mutations cause in males early-onset gonadotrophin-independent precocious puberty (testotoxicosis) but no phenotype has been found in females, for unknown reasons. Inactivating complete FSHR mutation causes in women hypergonadotrophic hypogonadism with lack of follicular development. If inactivation is incomplete, the phenotype is secondary amenorrhoea with arrested follicular maturation. In men, FSHR inactivation causes disturbances in spermatogenesis, but no azoospermia or absolute infertility. This is at variance with men reported with FSH ligand mutation, who all are azoospermic. The reason for the discrepancy remains unclear. Only one activating mutation of FSHR has been described in a hypophysectomised male, who had persistent spermatogenesis despite absent gonadotrophin secretion. However, the point mutation detected may represent a polymorphism. No activating FSH-R mutations have been detected in women.

S18. Bone dysplasia

***V. Cormier-Daire**;*
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No abstract available.

S19. Exon-Skipping Mutations in Human Disease

***A. R. Krainer**;*
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Accurate splicing depends on the canonical consensus sequences that define exon-intron boundaries, as well as on information present in the coding regions. Exonic splicing enhancer (ESE) elements are very prevalent and might be present in most, if not all, constitutive and alternative exons. ESEs are recognized by a class of splicing factors, the SR proteins, which promote splicing by recruiting spliceosomal components to the correct splice sites.
Point mutations in coding exons can disrupt critical ESEs, thereby causing the entire exon to be skipped. Such an alteration in the splicing process has drastic consequences for the expression of the gene product, as it can result in deletion, frameshifting, mRNA decay, or a combination of these changes. Predicting or determining which mutations cause exon skipping is important for accurately assessing phenotypic risk.
I will review our work on prediction, identification, and mutational analysis of ESE motifs. I will also describe an approach we recently developed, which is based on small synthetic compounds that can be specifically targeted to an inefficiently spliced exon, promoting its inclusion. These compounds were designed to emulate the function of natural SR proteins, and should be a valuable tool to understand the mechanisms of ESE-dependent splicing, and to modulate alternative splicing of specific pre-mRNAs. They might also provide a new therapeutic approach to treat diseases associated with exon skipping.

S20. Spinal muscular atrophy: Perspectives of a therapy by restoring the splicing pattern of SMN2

***B. Wirth**, L. Brichta, Y. Hofmann, K. Haug;*
Institute of Human Genetics, University of Bonn, Bonn, Germany.

Proximal spinal muscular atrophy (SMA) is the second most frequent autosomal recessive disorder in human caused by homozygous absence of the survival motor neuron gene (SMN1). An almost identical copy of the gene, SMN2, fails to compensate for the loss

of SMN1 due to a silent mutation in exon 7 that disrupts an exonic splicing enhancer, resulting in major production of exon 7 skipped SMN2 transcripts. Thus, SMN2 mainly produces a defective protein and only to a minor amount a full-length SMN2 protein which is identical to the SMN1 protein. We identified three trans-acting splicing factors (Htra2-beta1, hnRNP-G and RBM) being able to restore the splicing pattern of SMN2 with high efficiency when they are over-expressed. hnRNP-G and RBM bind to Htra2-beta1 which directly binds to SMN pre-mRNA forming a functional complex that modulates/enhances SMN2 exon 7-inclusion. Furthermore, we were able to show that increasing amounts of valproic acid (VPA), a histone deacetylase inhibitor and a drug used in long-time epilepsy treatment for three decades, restores the splicing pattern and increases the SMN2 protein level 2-4 fold in cell cultures of SMA patients. A clinical trial firstly in SMA parents and in case of success in SMA patients afterwards is in preparation. For the very first time, there is a real chance for a therapy of a monogenic inherited disease based on restoring the splicing pattern of a gene.

S21. Exon skipping as a therapy for DMD: From nonsense to sense by antisense

***G. J. van Ommen**;*
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No abstract available.

S22. Candidate pathways in cancer genetics

***P. G. Pelicci**;*
European Institute of Oncology, Department of Experimental Oncology, Milan, Italy.
No abstract available.

S23. Overview of the Hedgehog pathway

***A. Bale**;*
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No abstract available.

S24. Array-based expression profiling in breast cancer

***L. van't Veer**;*
Netherlands Cancer Institute, Amsterdam, The Netherlands.
No abstract available.

S25. Overview of Alzheimer's disease

***C. M. van Duijn**;*
Genetic Epidemiology Unit, Department of Epidemiology & Biostatistics, Erasmus MC, Rotterdam, The Netherlands.
Considerable progress has been made in unravelling the genetic etiology of Alzheimer's disease (AD), the major cause of dementia. Three genes have been identified involved in the autosomal dominant forms of AD, the ß-amyloid precursor protein gene (*APP*) and two homologous genes presenilin 1 (*PSEN1*) and presenilin 2 (*PSEN2*). Although the risk of AD for carriers of mutations in these genes approaches 100%, the contribution of these genes to the occurrence of disease is lower than 0.5%. On the population level, the apolipoprotein E (*APOE*) gene is a more important genetic determinant for the early-onset AD as well as the predominant late-onset form, explaining up to 15% of the incidence of disease. In addition to the evidence for other Alzheimer loci on chromosome 9, 10 and 12, there is increasing evidence for a role of cholesterol metabolism and vascular factors in Alzheimer's disease, i.e. atherosclerosis and blood pressure. These findings open opportunities for new approaches for etiologic research as well as preventive strategies.

S26. Cholesterol-Lowering approaches to Alzheimer's Disease – From Basic Science to Diagnosis and Treatment

***K. Beyreuther**[1], Tobias Hartmann[1], Colin L Masters[2];*
[1]ZMBH, University of Heidelberg, Heidelberg, Germany, [2]Department of Pathology, University of Melbourne, Parkville, Australia.
Several lines of evidence suggest a role for cholesterol on beta-amyloid production. Suppression of cholesterol neosynthesis by statins strongly reduces the formation of Aß40 and Aß42 species in vivo and in vitro. A recent clinical study revealed that this reduction of Abeta production could also be achieved in the brains of patients with AD that were treated with simvastatin. Lowering of CSF Abeta and 24S-hydroxycholesterol, the brain-specific cholesterol metabolite, was most pronounced in patients with mild to moderate AD. CSF Abeta was determined with a novel commercial ELISA assay for the determination of the Abeta42/Abeta40 ratio. The latter has been shown to be a valid biological marker for the diagnosis of MCI and AD.
Experiments in cell culture suggest that the processing of APP by beta-secretase (BACE) is affected when cholesterol levels are lowered. Because cholesterol is required to transport APP, BACE and presenilin to compartments in which beta- and gamma-cleavage does occur, intracellular cholesterol transport regulates the amyloidogenic processing of APP. Alterations in cholesterol transport have important consequences for both, APP-processing and the localization of the presenilins, which are essential components of the gamma-secretase complex. Exposure of neuronal cells to cholesterol-transport inhibiting agents resulted in a marked decrease in beta-secretase cleavage products of full-length APP. Alpha-secretase activity was not increased because the corresponding APP fragments released by alpha-secretase were not elevated.
In conclusion, our results suggest that amount and subcellular distribution of cholesterol may be an important factor in how cholesterol alters Abeta production and the risk of AD.

Concurrent Sessions

C01. Selective loss of HOXD13 function produced by a missense mutation in the homeodomain causes a novel human limb malformation

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The 5' members of the *HoxA* and *HoxD* gene clusters play major roles in vertebrate limb development. One such gene, *HOXD13*, is mutated in the human limb malformation syndrome synpolydactyly. Both polyalanine tract expansions and frameshifting deletions in HOXD13 cause similar forms of this condition, but it remains unclear whether other kinds of *HOXD13* mutations could produce different phenotypes. Here we describe a six-generation English family in which a novel combination of brachydactyly and central polydactyly co-segregates with a missense mutation that substitutes leucine for isoleucine at position 47 of the HOXD13 homeodomain. We have compared the HOXD13(I47L) mutant protein both in vitro and in vivo to the wild-type protein and to an artificial HOXD13 mutant, HOXD13(IQN), which is completely unable to bind DNA. We have found that the mutation causes neither a dominant negative effect nor a gain of function, but instead impairs DNA binding at some sites bound by wild-type HOXD13. Using retrovirus-mediated misexpression in developing chick limbs, we have shown that wild-type HOXD13 can upregulate *cEphA7* in the autopod, but that HOXD13(I47L) cannot. In the zeugopod, however, HOXD13(I47L) produces striking changes in tibial morphology and ectopic cartilages which are never produced by HOXD13(IQN), consistent with a selective rather than a generalised loss of function. Thus a mutant HOX protein which recognises only a subset of sites recognised by the wild-type protein causes a novel human malformation, pointing to a hitherto undescribed mechanism by which missense mutations in transcription factors can generate unexpected phenotypes.

C02. Localization and identification of the gene responsible for Dyggve-Melchior-Clausen syndrome

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Dyggve-Melchior-Clausen syndrome (DMC) is a rare autosomal-recessive disorder characterized by spondylo-epi-metaphyseal dysplasia and mental retardation. Linkage analyses in nine inbred and non-inbred families (from Morocco, Tunisia, Portugal and Lebanon) recently allowed us to localize the disease causing gene to chromosome 18q21.1 (Zmax=9.65 at t=0 at locus D18S1126, interval of 1.8 cM). Several known genes within the critical region were first regarded as possible candidate genes but were finally excluded by direct sequencing. One predicted transcript proved to be located in the interval, FLJ20071 indicated a gene initially composed of nine exons but detailed in-silico analysis finally deduced a total of 17 exons the first of which is non-translated. Genomic sequencing of this gene, that we propose to name Dymeclin, identified non-sense (580c>t, 610c>t, 656t>g, 1447c>t), frameshift (1877del a) and splice mutations (IVS10 1125+1g>t, IVS11 1252-1g>a) in all affected patients of our series. One patient with non-consanguineous parents was found to be a compound heterozygote (656t>g and 1877del a). RT-PCR and northern blot analyses indicated that Dymeclin/FLJ20071 is widely distributed, especially in fetal chondrocytes and brain. The predicted protein product (669 aminoacids) yields little insight into its likely function as no homology to any known protein family was found. However, electron microscopic study of cutaneous cells of an affected child revealed dilated rough endoplasmic reticulum, enlarged and aberrant vacuoles and numerous vesicles in several cell types of the skin. We conclude that DMC is consequent upon loss of function of the Dymeclin/FLJ20071 gene, presumably involved in intracellular digestive process.

C03. Skeletal muscle is the primary tissue involved in the pathogenesis of murine X-linked myotubular myopathy.

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X-linked myotubular myopathy (XLMTM) is a severe muscle disorder characterized by hypotonia and generalized muscle weakness in newborn males. Patients muscles present hypotrophic fibers with centrally located nuclei. The MTM1 gene mutated in XLMTM encodes a ubiquitously expressed phosphatase, called myotubularin, that dephosphorylates phosphatidylinositol 3-monophosphate PI(3)P. Myotubularin belongs to a large family of phosphatases conserved from yeast to human. We have recently identified novel members of this family and extended to 14 the number of myotubularin-related proteins in human. Eight are predicted to be enzymatically active, whereas the remaining proteins present substitutions at catalytically essential residues. Mutations in the MTMR2 gene, the closest homologue to MTM1, result in the demyelinating neuropathy Charcot-Marie-Tooth type 4B1. We developped an animal model for XLMTM by homologous recombination. The constitutive Mtm1 knockout mice present a severe generalized myopathy with reduced life expectancy, and show the presence of hypotrophic muscle fibers with central nuclei. To determine the primary tissue involved in XLMTM pathogenesis, we have generated skeletal muscle and neuronal myotubularin deficient mouse lines by a conditional gene targeting approach. Only the Mtm1 skeletal muscle deficient line developped a phenotype identical to that observed in the classical knockout model. In contrast, the neuronal mutant line did not show obvious clinical or histopathological symptoms. These results demonstrate that muscle is the primary tissue involved in XLMTM pathogenesis. Although MTM1 and MTMR2 share very high protein similarity, act on PI(3)P and are both ubiquitously expressed, these two genes implicated in different pathologies do not functionally compensate.

C04. An isoform of hPANK2, deficient in pantothenate kinase–associated neurodegeneration, localises to mitochondria

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Mutations in the human *PANK2* gene have been shown to occur in autosomal recessive pantothenate kinase–associated neurodegeneration (PKAN), a syndrome originally described by Hallervorden and Spatz. The phenotype includes progressive dystonia, rigidity, choreoathetosis, spasticity, retinitis pigmentosa, optic atrophy, parkinsonism or seizures, reminiscent of the spectrum of symptoms seen in mitochondriopathies.

The pantothenate kinase catalyses the first and rate limiting step in the biosynthesis of coenzyme A, a key molecule in energy metabolism.We determined the genomic structure of *hPANK2* and identified two alternatively used first exons. The resulting transcripts encode distinct isoforms of hPANK2, one of which carries an N-terminus with a predicted mitochondrial targeting signal. Using an *in vitro* import assay and *in vivo* immunolocalisation experiments mitochondrial localisation of this isoform could be demonstrated, while the other localised to the cytoplasm.

To test for additional functional pantothenate kinase activities in human cells which could complement hPANK2 mutations, a complementation assay was used. In this experiment hPANK4 could be demonstrated to possess pantothenate kinase activity.

Loss of function mutations in the hPANK2 core domain are known and are predicted to abolish both hPANK2 isoforms. Mutations in the N-terminal domain leave the cytosolic isoform intact. Taking into account that the phenotype is indistinguishable in patients with either of these mutations, the following conclusions can be drawn: First, it is the deficiency of the mitochondrial hPANK2 activity which is responsible for the symptoms of the syndrome and second, the deficiency of the cytosolic activity can be compensated by other kinases.

C05. A mixed epigenetic-genetic-environmental model for autism with a principal imprinted gene in chromosome 15q11-q13

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We define epigenetic disease as an abnormal phenotype caused by altered gene expression in the absence of nucleotide sequence variation. There is evidence that maternal but not paternal duplications of chromosome 15q11-q13 can cause autism. The Angelman gene (*UBE3A*) maps in 15q and is imprinted with paternal silencing in brain. Based in part on the duplication data, we hypothesize that over-expression of *UBE3A* is the major cause of autism, perhaps most commonly through brain-specific failure of paternal silencing. Using the AGRE and NIMH/Stanford sib pairs, we found evidence for increased sharing of paternal alleles in 15q11-q13, with the greatest sharing centromeric to the imprinting center (IC) at *D15S817*; sharing was greater for the 77 AGRE than for the 56 NIMH sib pairs with a Chi square of 5.48 (P = 0.02) for the combined data. We propose that autism may be caused in the majority of cases by epigenetic imprinting defects arising on normal or particularly susceptible paternal 15q chromosomes during spermatogenesis or after fertilization, but before the time of MZ twining, leading to brain-specific over-expression of *UBE3A*. Rather than the widely accepted multilocus model for autism, we propose a mixed epigenetic-genetic-environmental model with a principal imprinted gene in chromosome 15q. A combination of de novo and inherited contributions could explain why concordance for autism is high in MZ but low in DZ twins. Evidence will be presented for an epigenetic modifier locus for autism. A mixed epigenetic-genetic model may be relevant to other complex disease traits.

C06. Homozygosity mapping of Marinesco-Sjögren syndrome to 5q32.

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The Marinesco –Sjögren syndrome (MSS), first described in 1931, is an autosomal recessive condition characterised by somatic and mental retardation, congenital cataracts and cerebellar ataxia. Progressive myopathy was reported to be among the cardinal signs of MSS with myopathic changes on muscle biopsies and in several reports a specific dense membranous structure associated with nuclei in muscle fibres. Hypergonadotrophic hypogonadism and skeletal deformities related to a major hypotonia can also be observed. The major differential diagnosis of MSS is Congenital Cataracts, Facial Dysmorphism and peripheral Neuropathy (CCFDN) syndrome, which is localised to 18qter while no genetic localisation has yet been reported for typical MSS.
Using homozygosity mapping strategy in two large consanguineous families of Turkish and Norwegian origin respectively, we have identified the MSS locus on chromosome 5q32. Indeed a whole-genome scan performed in the Turkish family identified two candidate regions (5q32 and 7p14.2) with homozygosity by descent in affected siblings. High-density mapping of these two regions in the Norwegian family excluded linkage to the 7p14.2 region and revealed homozygosity and haplotype sharing between the six affected individuals over 6 consecutive 5q32 markers. LOD score calculation, including the consanguinity loops, gave a maximum value of 2.9 and 5.2 at θ = 0 for the Turkish and the Norwegian families respectively, indicating linkage between the disease and the D5S1995-D5S638 haplotype spanning an 8.7 cM interval. This localisation represents the first step towards the identification of the MSS gene and will allow to test the genetic homogeneity of this syndrome.

C07. Genetics and human behaviour: the ethical context

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Research into the genetic influences on behavioural traits in the normal range raises important ethical issues. Public perceptions of the research and its potential applications are influenced by its historical context, in particular, the eugenic programmes that were widely instituted in the early 20th century. This presentation examines these historical issues and considers the ethical and social implications of modern behavioural genetics. Issues such as the medicalisation of normal behaviour, and the potential application of research findings in areas such as prenatal selection, criminal justice are discussed. The presentation draws on the conclusions and recommendations made by the Nuffield Council on Bioethics in its recent report on Genetics and human behaviour: the ethical context.

C08. Long-term follow-up in FOS – the Fabry Outcome Survey – confirms beneficial effects of agalsidase alfa in Fabry disease

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Enzyme replacement therapy (ERT) has recently been introduced to treat Fabry disease, an X-linked disorder caused by deficient activity of the lysosomal enzyme α-galactosidase A. FOS -the Fabry Outcome Survey - is a European outcomes database for patients who are receiving, or candidates for, ERT with Replagal™ (agalsidase alfa; TKT-5S, Danderyd, Sweden). As of September 2002, 285 patients (122 female, 163 male) were enrolled in FOS, constituting the world's largest group of Fabry patients. About 70% have received agalsidase alfa, most for >12 months. Initial data analysis supports the results from previous clinical trials, that ERT has positive effects on renal, neurological and cardiac manifestations of the disease. On entry into FOS, nearly 50% of patients had impaired renal function (proteinuria, 34%; renal failure, 5%; dialysis, 3%; renal transplantation, 6%). Agalsidase alfa stabilized renal function over 12 months, as assessed by serum creatinine and glomerular filtration rate. FOS data also confirm that females can be severely affected; the mean number of organ systems affected ranged from 4.5 in girls <10 years to 10 in women >60 years. At baseline, 85% and 63% of men and women, respectively, suffered chronic or acute pain, assessed using the Brief Pain Inventory (BPI). For the whole group, mean BPI scores for 'pain at its worst' fell from 4.1 at baseline to 2.3 after 12 months of ERT (n=23, p=0.032). These first results are encouraging, although continued follow-up of patients in FOS will be important in determining the longer-term efficacy and safety of ERT.

C09. Evaluation of Breast Cancer Risk Assessment Packages in the Family History Evaluation and Screening Programme

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Background: Accurate individualized breast cancer risk assessment is essential to provide risk-benefit analysis prior to initiating interventions designed to lower breast cancer risk. Several mathematical models for the estimation of individual breast cancer risk have been proposed, however, no single model integrates family history, hormonal factors and benign breast disease in a comprehensive fashion. A new model by Tyrer and colleagues has addressed these deficiencies. Therefore, this study has assessed the predictive and discriminatory value of the Tyrer-Cuzick model against established models namely: Gail, Claus and Ford. *Methods:* The goodness of fit and discriminatory accuracy of the models was assessed using data from 3151 women attending the Family History Evaluation and Screening Programme. All models were applied to these women over a mean follow up of 5.26 years to estimate risk of breast cancer. *Results:* The ratios of expected to observed numbers of breast cancers (95% confidence intervals [CI]) were 0.68 (0.53-0.88) for Gail, 0.75 (0.59-0.97) for Claus, 0.65 (0.51-0.84) for Ford and 1.07 (0.84-1.39) for Tyrer-Cuzick. Of the 65 cases of breast cancer, 59.7% of women were assigned risks using the Gail compatible with chemoprevention under the IBIS protocol. The proportion for Claus, Ford and Tyrer-Cuzick were 48.4%, 38.7% and 74.2% respectively. *Conclusion:* The Tyrer-Cuzick model is the most consistently accurate model for prediction of breast cancer. Gail, Claus and Ford all significantly underestimate risk although the accuracy of Claus may be improved by adjustments for other risk factors. Tyrer-Cuzick model also shows good sensitivity.

C10. Mutations of *EDA, EDARADD* and *TRAF6* genes in anhidrotic ectodermal dysplasia genotype/phenotype correlation.

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Anhidrotic ectodermal dysplasia results from the abnormal ectodermal-mesodermal interaction and the defect in the differentiation of skin appendages during embryonic development. Initiation of the differentiation process requires protein products of *EDA, XEDAR* and *NEMO* genes, localised on the X chromosome, *EDAR* gene, localised on chromosome 2, *EDARADD* gene localised on 1 chromosome and *TRAF6* gene on chromosome 11. These

genes encode proteins involved in pathway transducting signal from ectoderm to mesenchyme, which initiate skin appendages formation. Affected individuals harbouring mutations in mentioned genes exhibit symptoms of anodontia or oligodontia with conical shape of teeth, hypertermia caused by characteristic severe deficiency of sweat glands and hypotrichosis - sparse hair.
The structure of *EDA*, *EDARADD* and *TRAF6* genes was investigated in 40 patients with anhidrotic ectodermal dysplasia and 100 relatives. Appropriate fragments of *EDA, EDARADD* and *TRAF6* gene were amplified by PCR using specific primers and were subjected to multitemperature single-stranded conformation polymorphism analysis (MSSCP). The fragments that exhibited aberrant patterns in MSSCP analysis were sequenced using an automated DNA sequencer. Sequence analysis revealed several novel mutations including novel deletion of 37bp in exon 5 of the *EDA* gene resulting in premature termination of translation and truncated form of ectodysplasin – protein product of *EDA* gene; novel 383ΔA in exon 7 of *EDARADD* gene resulting in premature stop codon. Our investigations allow to establish strategy for future molecular diagnostics of anhidrotic ectodermal dysplasia and genotype/ phenotype correlation.

C11. Autosomal dominant and recessive ectodermal dysplasia anhidrotic are allelic diseases at the EDARRAD locus

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Anhidrotic ectodermal dysplasia (EDA) is a disorder of ectodermal differenciation characterized by sparse hair, abnormal or missing teeth and unability to sweet. The X-linked EDA is the most common form caused by mutations in the EDA gene which encodes ectodysplasin, a member of the TNF family. Autosomal dominant and recessive forms of EDA, indistinguishable from the X-linked form, have been also described. Two different disease genes have been hitherto identified. Mutations in EDA-3 encoding EDAR, a TNF receptor, result in both dominant and recessive forms. In addition, mutations in a recently identified gene, EDARADD (for the Edar-Associated Death Domain) have also been shown to cause recessive EDA.
We report on a large moroccan family with an autosomal dominant EDA. The eight affected individuals presented with hypotrichosis, hypodontia, and anhidrosis. Genetic analysis showed linkage to chromosome 1q42-q43 where the Edaradd gene is located. We screened the 7 exons of this gene and found a novel missense mutation (G335-->T) which changed a leucine residue into an arginine (Leu 112-->Arg). Thus the EDARADD gene accounts for both recessive and dominant EDA. EDAR is activated by its ligand, ectodysplasin and uses EDARADD to build an intracellular complex to activate NF-kB. This missense mutation probably affects the interaction of EDARADD with EDAR and the dowstream signaling pathway which plays a crucial role in the differentiation of skin appendages. This might explain the phenotypic similarity of the X-linked and autosomal forms of anhidrotic ectodermal dysplasias.

C12. Huntington's disease like phenotype due to trinucleotide repeat expansions in the *TBP* and *JPH3* genes

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We report a group of 252 Huntington's disease-like (HD-L) patients, including 60 with typical HD, who had tested negative for pathological expansions in the IT15 gene, the major mutation in Huntington's disease. They were screened for repeat expansions in two other genes involved in HDL phenotypes; those encoding the junctophilin-3 (JPH3/HDL2) and prion (PRNP/HDL1) proteins. In addition, because of the clinical overlap between patients with HDL disease and autosomal dominant cerebellar ataxia or dentato-rubro-pallido-luysian atrophy (DRPLA), we investigated trinucleotide repeat expansions in genes encoding the TATA-binding protein (TBP/SCA17) and atrophin-1 (DRPLA).
Two patients carried 43 and 50 uninterrupted CTG repeats in the JPH3 gene. Two other patients had 44 and 46 CAA/CAG repeats in the TBP gene. Patients with expansions in the TBP or JPH3 genes had HDL phenotypes indistinguishable from HD. Taking into account patients with "typical HD", their frequencies were evaluated to be 3% each in our series of typical HDL patients. Interestingly, incomplete penetrance of the 46 CAA/CAG repeat in the TBP gene was observed in a 59 year old transmitting but healthy parent and his severely affected children with repeats of the same size. Furthermore, we report a new configuration of the expanded-TBP allele, carrying 11 repeats on the first stretch of CAGs. Expansions in the DRPLA gene and insertions in the PRNP gene were not found in our group of patients. Further genetic heterogeneity of HDL phenotype therefore exists.

C13. Definition of a critical region on chromosome 18 for congenital aural atresia by arrayCGH

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Deletions of the long arm of chromosome 18 occur in approximately 1 in 40,000 live born infants. Congenital aural atresia (CAA) or narrow external auditory canals occur in approximately 66% of all patients with a terminal deletion 18q. The present paper describes a series of 20 CAA patients of which 18 contained microscopically visible 18q deletions. The extent and nature of the chromosome 18 deletions was studied in detail by array-based comparative genomic hybridization (CGH). High resolution chromosome 18 profiles were obtained from all patients, and a critical region of 5 Mb that was deleted in all CAA cases, could be defined on 18q22.3-18q23. Therefore, this region can be considered as a candidate region for aural atresia. Moreover, the array-based high resolution copy number screening enabled a refined cytogenetic diagnosis in 10 patients. This screening appeared to be applicable to the detection of genetic mocaicisms and, in particular, to a detailed delineation of ring chromosomes. This study clearly demonstrates the power of the arrayCGH technology in high resolution molecular karyotyping. Deletion and amplification mapping can now be performed at the submicroscopic level and will allow high throughput identification of genomic regions harboring disease genes.

C14. Detection of trisomy 21 and other aneuploidies by Paralogous gene quantification.

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The link between trisomy 21 and DS was established in 1959, and since then diagnosis has been performed mostly by Karyotyping and later FISH, both of which are based on counting the number of chromosomes present in human cells. These methods are highly accurate but labour intensive.
The development of alternative methods for quicker, higher throughput and semi-automated diagnosis, as accurate as presently used methods would be highly desirable.
We have developed a universal method based on the use of paralogous genes to detect the presence abnormal chromosome numbers.
Paralogous genes, are genes that have a common evolutionary origin but have been duplicated over time in the human genome. These genes which in many cases are located on different chromosomes, can retain a high degree of sequence similarity. We exploit sequence similarities in order to co-amplify paralogues sequences located on different chromosomes with identical primer pairs. Single nucleotide differences between the paralogue sequences are then quantified using the pyrosequencing technology, and the ratio of the ‚alelles' reflects the relative frequency of the chromosomes tested.We have

now developed and validated tests for trisomies of chromosomes 21, 13 and 18, as well as all X Y chromosome abnormalities in over 100 individuals. In addition we have used the same principle to detect common deletions such as those in DiGeorge and Williams-Beuren syndromes.

C15. Mapping the Wolf-Hirschhorn syndrome phenotype outside the currently considered WHS critical region, and defining a new critical region, WHSCR-2

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Wolf-Hirschhorn syndrome (WHS) is a MR/MCA syndrome caused by a partial 4p deficiency. The currently considered WHS-critical region (WHSCR) is restricted to the 165 kb in 4p16.3 defined by the loci D4S166 and D4S3327. Three genes have been so far described as candidate for WHS: WHSC1 and WHSC2, falling partially or completely within the WHSCR, and LETM1, mapping distally the WHSCR. A unique patient was reported with a small interstitial deletion restricted to the WHSCR, who presented with a questionable WHS phenotype.

We analyzed 8 microdeletion patients, all presenting with a mild phenotype, with deletion between 3.5 and 1.9 Mb. The currently considered WHSCR was fully preserved in the 1.9 Mb deletion patient, in spite of a typical WHS phenotype. The deletion, in this patient, spanned the region from 190b4 cosmid to the telomere.

We also observed one patient without WHS, who was deleted from FGFR3 to the telomere, due to an unbalanced t(4p;12p) translocation.

We conclude that: 1) the distinctive WHS phenotype is defined by the association of mental retardation, typical face, growth retardation, congenital hypotonia and seizures; 2) this basic phenotype maps distally the currently considered WHSCR; 3) the new critical region we propose, WHSCR-2, falls within an about 400 kb interval in 4p16.3, between 190b4 cosmid (D4S3327) and D4S98; 4) WHSC2 is no longer a candidate gene for WHS; 5) D4S98 (FGFR3) is the most specific haploinsufficiency locus for the molecular test; 6) splitting the WHS phenotype in a „classical“ and in a „mild“ form is recommended.

C16. Abnormal chromosome condensation on chromosome 4p16.1 linked with familial inherited microtia

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A family presented with an autosomal dominant form in three generations of microtia, absent nasolacrimal duct and variable eye coloboma, a syndromic disorder not yet reported as such. High resolution cytogenetic analysis revealed an aberrant chromosome 4pter in 2 affected family members. Linkage analysis confirmed linkage to chromosome 4p16.

Multicolor FISH, chromosome 4 paint and 4pter region specific paint indicated that the der(4) contains only chromosome 4 DNA. However, CGH and microarray-CGH at 1 Mb resolution did not detect a dupliction or deletion. FISH analysis was performed with a series of BAC probes covering the region at 2 Mb resolution. Two consecutive BACs were labeled with different fluorophores. If an amplification of a smaller DNA region would have occurred, the two consecutive probes would become spaced apart. Since each set of two probes co-localize on metaphases, no large amplification has occurred. In addition, the hypothesis that no duplication is present was reinforced by the observation that none of the BACs used in this analysis showed two signals in interphase nor metaphases and that none of the markers used for the linkage analysis are duplicated.

Since no chromosomal amplification, duplication or insertion could be detected at this locus, we hypothesize that the chromosomal anomaly is due to a cytogenetically visible epigenetic change. Since methylation can influence chromosome condensation and gene expression, we investigated the methylation status of this locus by immunostaining with anti-MeC antibodies. This analysis showed that the locus is hypermethylated, thus supporting our hypothesis.

C17. An investigation of factors associated with the breakpoints of recurrent inversions

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We have ascertained 169 apparently independent inversions detectable on the light microscope excluding those generally considered to be common polymorphisms. Of these, 54 were represented more than once; two occurred 6 times, one 4 times, two 3 times and the remaining 32 twice. Thus the great majority appeared unique. To see whether our 21 different recurring inversions resulted from repeated independent events or were identical by decent, we examined 3 previously reported large series of inversions and a compilation of records of all abnormalities seen in a large number of UK cytogenetic laboratories (the Oxford Database). We found that only 8 our 21 recurring inversions were also seen more than once in at least two other series.

The human genome contains many repeated sequences including many hundreds of olfactory receptor (OR) genes and duplicons. Among our 42 recurrent inversion breakpoints we found only 6 which occurred in OR regions and, of these, only 2 involved both breakpoints of a single inversion. We identified 15 breakpoints that could have involved known duplicons but, again, only two of these involved both breakpoints of a single inversion. Thus while illegitimate recombination between repeat sequences is strongly associated with genomic disorders, OR genes and duplicons do not appear to play a major role in the genesis of inversions detectable at the light microscope level.

Our results will be discussed in the light of breakage hot spots versus founder effects and experiments to differentiate between these two possibilities.

C18. Expression of cohesin proteins in human germ cells

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Cohesion between sister chromatids is an essential prerequisite of correct chromosome segregation at anaphase, and failure of cohesion is associated with non-disjuntion. Sister chromatids are held together in both mitotic and meiotic divisions by a protein complex termed cohesin. The proteins constituting the cohesin complex differ between the two types of cell division and components of the meiotic cohesion complex have recently been identified. We have characterised two of these proteins, STAG3 and REC8, in human germ cells. Using polyclonal antibodies we have been able to determine expression and location of these proteins in the initial stages of meiosis. In cells at pre-leptotene and leptotene of prophase I, prior to formation of axial elements and the synaptonemal complex (visualised using an antibody against the protein SCP3), STAG3 and REC8 are present as diffuse nuclear signals. In these early meiotic stages, SCP3 accumulates within nucleoli. Both STAG3 and REC8 colocalise from zygotene to diplotene, forming linear structures along chromosome arms. In these cells, SCP3 changes from being nucleolar to colocalising with the cohesin proteins. In human fetal oocytes that show abnormal disruption of the axial elements or synaptonemal complexes, SCP3 staining shows a correlated disruption whereas the cohesin proteins may show normal linear elements. Our observations suggest a model of meiotic chromosome organisation in which the cohesin proteins first form a core structure (the cohesin axis) which acts as a scaffold for the subsequent formation of the synaptonemal complex.

C19. Ataxia with oculomotor apraxia type 1 (AOA1): a phenotypical and genetic study

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Ataxia with ocular motor apraxia type 1 (AOA1) is a rare autosomal recessive cerebellar ataxia (ARCA) associated with oculomotor apraxia, hypoalbuminemia and hypercholesterolemia. The gene *APTX*, which encodes for Aprataxin, has been recently identified (Moreira et al., 2001; Date et al., 2001).
Objectives. To determine the relative frequency and the phenotypical characteristics of AOA1.
Methods. A group of 152 families with non Friedreich's ataxia (FA) progressive ARCA was selected and a subgroup of 58 families with ocular apraxia and/or hypoalbuminemia and/or hypercholesterolemia was analysed for mutations in the *APTX* gene by sequencing. A detailed neurological, neuropsychological, electrophysiological, oculographic and brain imaging evaluations were performed in mutation carriers.
Results. Three missense mutations (A198V, D267G, W279R) and one truncating mutation (W279X) were identified in 8 families (13 patients). The mean age at onset was 6.5±5.1 years (range: 2-18). Choreic movements were frequent at onset (78%) then disappearing with the course of the disease. Cerebellar ataxia and severe sensorimotor neuropathy were present in all patients. In contrast, oculomotor apraxia (61%), hypercholesterolemia (69%), hypoalbuminemia (77%) were inconstant findings. The mutation A198V was associated with a remarkably severe and persistent chorea.
Conclusion. The relative frequency of AOA1 is approximately 10% of ARCA after exclusion of FA. The presence of chorea, sensorimotor neuropathy, absence of Babinski sign, oculomotor and biological abnormalities can help to distinguish AOA1 from FA on a clinical basis. The frequency of chorea at onset suggests this diagnosis should be also considered in children with chorea lacking the *IT15* mutation, responsible for Huntington's disease.

C20. Genetic heterogeneity in Walker-Warburg syndrome: two genes found, at least two more to go.

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Three rare autosomal recessive syndromes form a spectrum of molecularly related diseases. Muscle-eye-brain disease (MEB), Walker-Warburg syndrome (WWS) and Fukuyama congenital muscular dystrophy (FCMD) share the combination of congenital muscular dystrophy and brain malformations including a neuronal migration defect. The genes underlying FCMD (*Fukutin*) and MEB (*POMGnT1*) are implicated in O-glycosylation. To unravel the genetic basis of WWS, we adopted a candidate-gene approach in combination with homozygosity mapping in 15 consanguineous WWS families. Analysis of the locus for O-mannosyltransferase 1 (*POMT1*) revealed homozygosity in 5 of 15 families. Sequencing of the *POMT1* gene revealed causative mutations in 6 of 30 unrelated WWS patients. These results indicate that WWS is genetically heterogenous. A genome-wide linkage analysis indeed suggested the existence of at least two additional WWS loci. In order to elucidate the remaining WWS patients we have extended our candidate gene-based mapping strategy. Homozygosity was observed for four WWS families at the *Fukutin* locus. So far, *Fukutin* mutations had been restricted to Japanese FCMD patients, whose phenotype is clinically much less severe than WWS. These FCMD patients are predicted to maintain residual Fukutin activity. In contrast, mutation analysis in one of our WWS families revealed a homozygous nonsense mutation. This result shows that homozygosity for a null mutation in the *Fukutin* gene causes a WWS phenotype.
In conclusion, evidence is presented that WWS can be due to mutations in two genes, *POMT1* and *Fukutin*, and that at least two more genes are required to explain the majority of remaining WWS patients.

C21. Autosomal recessive primary microcephaly: genes and phenotype

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During the third trimester of pregnancy the human brain continues to grow, relative to body size, at a greater rate than that seen in our higher primate relatives e.g. gorilla and chimpanzee. This leads to a human brain that is three times greater in size. Perturbations of this process result in congenital microcephaly. We have studied a cohort of 57 consanguineous Northern Pakistani families with the clinical diagnosis of "autosomal recessive primary microcephaly" (MCPH) and now report our clinical and molecular findings.
Affected individuals have a head circumference of <-4SD and mild/moderate mental retardation, but no other abnormal findings. MRI scans show a general reduction of brain size but particularly of the cerebral cortex.
Using autozygosity mapping we have found 7 MCPH loci. Genotyping of all families against all loci showed that MCPH5 is the common locus accounting for 27/57 families.
We identified the ASPM gene (the orthologue to the drosophila abnormal spindle protein which is involved in neuroblast asymmetric cell division) at the MCPH5 locus using a common haplotype and positional cloning approach. All mutations identified to date cause premature truncation of the 3442 amino acid protein. We are currently investigating the function of ASPM in humans.
ASP(M) has increased in size progressively comparing C.Elegans, Drosophila, mouse and human. The majority of this is accounted for by a change in the number of the IQ domains present from 2 to 21 to 60 to 72, which parallels the increase in central nervous system size and complexity.

C22. Homozygous mutations in the Indian Hedgehog gene cause a new autosomal recessive skeletal dysplasia with cone-shaped epiphyses in hands and hips

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We recently delineated a new autosomal recessive skeletal dysplasia in two consanguineous families. The clinical phenotype is characterized by short stature with short limbs and brachydactyly, relatively large head and narrow thorax with pectus deformities. Radiographically cone-shaped epiphyses are observed in the hands, the hips and to a variable degree in the shoulders, knees and ankles. We named the disorder acrocapitofemoral dysplasia (ACFD) because of the characteristic radiographic abnormalities in hands and hips in all affected individuals. Homozygosity mapping linked the disorder to a locus on 2q35-q36 with a maximum two point lod score of 8.02 at θ =0 for marker D2S2248. Using a candidate gene approach, we identified two missense mutations in the amino-terminal signaling domain of the gene encoding Indian hedgehog (*IHH*). Both affected individuals of family 1 were homozygous for a 137C>T transition (P46L) and the three patients in family 2 were homozygous for a 569T>C transition (V190A). The two mutated amino acids are strongly conserved residues. Previously, heterozygous missense

mutations in another part of the amino-terminal signaling domain of *IHH* have been identified in patients with brachydactyly type A-1 (BDA1). Interestingly, the heterozygous parents in our families do not show signs of BDA1. A loss of function of IHH in ACFD and a dominant negative effect of the BDA1 mutations could be a good explanation of this observation. Additional in vitro and in vivo experiments are necessary to investigate the consequences of the different (BDA1 and ACFD) mutations on Ihh signaling in the growth plate.

C23. Localised mutations in the gene encoding the cytoskeletal protein filamin A cause diverse malformations in humans

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Remodelling of the cytoskeleton is central to the modulation of cell shape and migration. Filamin A, encoded by the gene FLNA, is a widely expressed protein that regulates re-organization of the actin cytoskeleton by interacting with integrins, transmembrane receptor complexes and second messengers. We have identified localised mutations of FLNA in 30 individuals with diagnoses spanning four X-linked disorders - otopalatodigital (OPD) syndromes types 1 and 2, frontometaphyseal dysplasia and Melnick-Needles syndrome. These clinically related syndromes are characterised by a broad range of congenital malformations affecting craniofacial structures, the skeleton, brain, viscera and the urogenital tract. All of the identified mutations in FLNA conserve the reading frame and cluster within four discrete regions of the gene; several occur recurrently. These findings contrast with previous observations that loss-of–function of FLNA leads to a localized neuronal migration disorder, X-linked periventricular nodular heterotopia, that manifests in females but is embryonic lethal in males. The patterns of mutation, X-inactivation, and phenotypic manifestations associated with the OPD-spectrum disorders suggest mechanisms by which mutations in filamin A may disrupt signalling pathways that mediate organogenesis in multiple systems during embryonic development.

C24. Spermine Synthase Deficiency is Associated With the Snyder-Robinson X-Linked Mental Retardation Syndrome

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Polyamines are essential for normal cell growth and differentiation. However, little is known about the specific cellular functions of these compounds and despite extensive laboratory investigations, there are no known heritable conditions in humans in which polyamine synthesis is perturbed. Studies of mouse fibroblasts from the gyro (Gy) mutant mouse, in which spermine was completely absent due to the disruption of spermine synthase (Sms), an X-linked gene, found no alteration in growth rate or overt morphological changes. In contrast, the mutant mouse exhibits neurological abnormalities. Based on this observation, mutation analysis of SMS was undertaken in 9 XLMR families linked to Xp22.1. We found a splice mutation (329+8G→A) in the Snyder-Robinson syndrome (SRS; OMIM 309583). The mutation leads to aberrant splicing such that exon 4 is removed in a significant population of the SMS mRNA. This altered transcript gives rise to a truncated protein with no enzyme activity. Affected males have decreased activity of spermine synthase, and correspondingly very low levels of intracellular spermine in lymphocytes and fibroblasts. These findings clearly indicate a role for spermine in cognitive function and may assist in explaining the importance of spermine's function as an "intrinsic gateway" molecule for the rectification properties of some K^+ channels. Furthermore, the ability to measure decreased activity of spermine synthase and an altered ratio of spermine to spermidine in white cells makes it possible to diagnose SMS deficiency in male patients with mental retardation of unknown etiology.

C25. Comprehensive characterisation of 11q amplicons including the MLL gene in AML/MDS patients

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Structural rearrangements involving mixed lineage leukemia (MLL) gene (11q23) are among the most common recurring abnormalities in de novo and therapy-related hematologic disorders, including myelodysplastic syndromes (MDS), acute lymphoid (ALL), acute myeloid (AML) and biphenotypic leukemias. Most often, MLL is rearranged due to involvement in a diversity of chromosomal translocations, less commonly by partial tandem duplications and subsequent self-fusion. Recent reports have implicated MLL amplification as another potential mechanism of leukemogenesis. We identified 18 AML/MDS cases with MLL gene amplification in form of ring chromosomes, dmins or hsr by clasical and molecular cytogenetic studies. In an attempt to define the entire amplified region, we performed a restriction landmark genomic scanning (RLGS) analysis. Eight amplified fragments were uncovered in two analysed AML samples. Virtual genome scan (VGS), a novel informatic tool for sequence prediction of RLGS fragments, rendered sequence information for six of these fragments. The amplification status of the chromosomal regions covered by these fragments was tested by semiquantitative PCR in seven AML/MDS samples. Subsequent array CGH analysis confirmed and extended the RLGS results. Taken together, our data indicate that in all analysed AML/MDS samples the minimally amplified region extends from the MLL gene further in telomeric direction and contains additional genes with potential involvement in leukemogenesis. Further, this analysis revealed the presence of an additional independent 11q amplicon in three of the patients.

C26. Screening for genomic rearrangements of the mismatch repair genes must be included into the routine diagnosis of HNPCC

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In HNPCC, *MSH2* and *MLH1* mutations are detected in approximately half of the families fulfilling the Amsterdam (AMS) criteria. Screening by Quantitative Multiplex PCR of Short

Fluorescent Fragments (QMPSF) of *MMR* genes in AMS+ HNPCC families without detectable point mutations led us to identify genomic rearrangements of *MSH2* in 20% of the cases. We have now integrated the QMPSF into the routine diagnosis of HNPCC and have analyzed, using QMPSF, a total of 304 families. In 29 families, we have identified 19 distinct exonic rearrangements of *MSH2*, removing exon(s) 1, 1-2, 1-4, 1-6, 1-7, 1-8, 1-15, 2, 3, 4-6, 5, 5-6, 7, 8, 9-10, 12-13, 13-15 and a single case of duplication involving exons 9-10. Deletions removing exon 1 also remove the promoter and we detected four distinct 5' breakpoints. We also identified a *MSH2* 1.7 kb genomic rearrangement affecting the promoter, selectively. We detected 8 exonic rerrangements of *MLH1*, removing exon(s) 1-19, 2, 4-6, 6, 7-9, 11, 13-16 and 14, in families in which immunostaining of the tumours had revealed a selective extinction of the MLH1 protein. We conclude that *MSH2* rearrangements are remarkably heterogeneous and are involved in at least 10% of the AMS+ families, which justifies to include their search in the routine diagnosis of HNPCC. Even if genomic rearrangements of *MLH1* appear less frequent, except in certain populations where they are associated to a founder effect, their presence should be considered in HNPCC patients, when the immunostaining of the tumours reveals an absence of MLH1 expression.

C27. Mono- and biallelic mutations in the human MutY homologue, MYH, predispose for hereditary colorectal polyps and carcinoma in a large Dutch cohort

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Background:
Biallelic germline mutations in the human MYH gene lead to somatic mutations of the APC gene and predispose carriers to multiple colorectal polyps and carcinoma.
Methods:
We screened the MYH gene in 250 families affected by either classical familial adenomatous polyposis (FAP) or an attenuated form of polyposis, previously shown not to carry APC germline mutations. In addition, we selected probands without MLH1, MSH2 or MSH6 germline mutations from a cohort of 40 HNPCC-like families not exhibiting a clear-cut autosomal dominant inheritance pattern. Mutation screening by DGGE was performed in only four of the 16 MYH exons. The remainder of the MYH gene is currently being investigated by direct nucleotide sequencing.
Results:
We identified 34 MYH germline mutation carriers, 15 of whom were homozygous for the previously described Y165C or G382D missense mutation. Six probands were found to be compound heterozygotes for the Y165C mutation and the G382D missense mutation.
The probands exhibited a relatively young age-at-diagnosis of colorectal cancer (mean 45 years, range 22-65 years), and a wide variety in number of polyps (ranging from a few to hundreds). Pedigree studies of these families suggests the presence of late onset colorectal cancer in the parental generation of probands.
Conclusions:
Preliminary results in our large and clinically well-defined series confirm that biallelic mutations in the MYH gene play a major role in the predisposition of colorectal polyps and carcinoma. However, the alleged risk of colorectal cancer in heterozygous carriers still remains to be ascertained.

C28. *PMS2* mutation as a cause of primitive neuroectodermal tumours of childhood

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Supratentorial primitive neuroectodermal tumour (PNET) is a highly lethal paediatric malignancy that is usually sporadic and of unknown etiology. We report here on a consanguineous family in which supratentorial primitive neuroectodermal tumours and café-au-lait patches (CALS) segregated in a pattern suggesting autosomal recessive inheritance.
In this family, individuals in two sibships developed CALS. In one sibship, two siblings developed PNETs, and a further sibling developed a primary cerebral non-Hodgkin`s lymphoma. The parents of both sibships were first cousins and clinically unaffected. There was no family history of colorectal cancer, and the patients did not meet diagnostic criteria for neurofibromatosis type I, or atypical HNPCC/Turcot syndrome.
We performed whole genome autozygosity mapping, and unexpectedly found linkage to a region of 7p22 including the *PMS2* gene. Using previously published mutation analysis methods, however, no mutation could be found in the entire *PMS2* gene. We then performed bioinformatic analysis that indicated the existence of undescribed *PMS2* pseudogenes that interfere with mutation analysis. Using these data to design new specific amplicons, a novel homozygous nonsense mutation in *PMS2* exon 14 was found. Published *in vitro* data suggest this mutation will ablate the ability of PMS2 to interact with MLH1.
This unexpected finding suggests that central PNET of childhood may result from *PMS2* mutation, significantly widening the spectrum of disorders associated with mismatch repair gene defects. Our initial failure to detect this mutation further suggests that the paucity of published *PMS2* mutations may reflect underreporting due to technical difficulties.

C29. CHEK2*1100delC identifies a hereditary breast and colorectal cancer phenotype

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Background: We recently identified the 1100delC variant of the cell cycle checkpoint kinase CHEK2 gene as a low-penetrance breast cancer susceptibility allele (Meijers-Heijboer et al, Nature Genet. 31:55, 2002). Here we have investigated the involvement of CHEK2*1100delC in colorectal cancer susceptibility.
Methods: DNA from blood samples was analyzed for the presence of the CHEK2*1100delC variant by a PCR-based allele-specific oligonucleotide hybridization assay.
Results: The CHEK2*1100delC variant was identified in none of 95 FAP families and in 2.6% of 234 HNPCC or HNPCC-like families. Although the prevalence of CHEK2*1100delC among HNPCC or HNPCC-like families was increased as compared to its 1.1% population frequency, this difference was not statistically significant (P=0.07). When non-BRCA1/BRCA2 breast cancer families were classified by stringent clinical criteria that defined a putative hereditary breast and colorectal cancer (HBCC) phenotype, we identified CHEK2*1100delC in 18% of 55 HBCC families compared to 4% of 380 non-HBCC breast cancer families (P<0.001). Importantly, co-segregation of the CHEK2*1100delC genotype with the cancer phenotype was incomplete in five of nine informative CHEK2*1100delC breast cancer families.
Conclusions: The CHEK2*1100delC variant provides conclusive genetic evidence for the existence of an HBCC subtype of familial breast cancer. Although CHEK2*1100delC strongly associates with the HBCC phenotype, the mutant allele is not the only cancer predisposing factor in the variant-positive families but appears to act in synergy with another, as yet unknown susceptibility gene(s). The unequivocal definition of the HBCC phenotype opens new avenues to search for this putative HBCC susceptibility gene or genes.

C30. Results of the CAPP1 study:aspirin and resistant starch are beneficial in familial adenomatous polyposis

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In 1993 the consortium we represent launched CAPP1, a European Concerted Action on Polyp Prevention. Carriers of Familial Adenomatous Poylposis (FAP) were recruited to a randomised placebo controlled trial of 600mg aspirin and/or 30 grams of resistant starch. Substantial epidemiological evidence supports the view that these interventions might prevent the development of adenomatous polyps, the precursor of colorectal cancer. 206 FAP carriers received treatment. Completed data on 133 subjects followed for at least one year have been analysed. Neither intervention resulted in a significant reduction in polyp number as assessed either by the endoscopist or blinded review of rectal videos. The mean size of largest polyps was, however, significantly reduced in the aspirin only group (p=0.01). A secondary analysis used data from those who had stayed more than one year, suggesting higher compliance. Here, both aspirin alone and the combined aspirin/resistant starch group achieved significance (p=0.04 and p=0.03).

Those treated with starch had significantly shorter crypts (p<0.0001,95% CI 0.87,0.96); those treated with aspirin had longer crypts and a 37% increase in crypt cell proliferation. These data suggest that aspirin and resistant starch are protective against cancer but with different modes of action. Aspirin may act later, preventing progression of small adenomata.

FAP carriers are an ideal study group since their molecular defect is shared with a majority of sporadic colorectal cancers and any FAP chemoprevention strategy is likely to have general relevance. Such studies need sustained infrastructure investment as planned by the new International Society for Gastrointestinal Hereditary Tumours InSiGHT.

C31. Genome scan for multiple sclerosis : a Franco-American collaboration

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Multiple sclerosis (MS) is a chronic inflammatory disorder of the central nervous system. Although it is well established that genetic factors influence susceptibility to MS, the only one identified to date resides in the HLA region. Several genome scan studies have already highlighted regions of interest, but with small overlap of the regions they identified.

The present sample consists in 243 multiplex families, with either affected sibs only (205 families) or more distantly related affecteds (38 families). The families were recruited in France (94 families) and the US (149 families), using the same clinical inclusion criteria.

A genome scan with 356 microsatellite markers was carried out to search for linkage using the Z statistics of Kong and Cox (1997). The strongest signal is observed on chromosome 1q in the total sample (Z = 3.38). Among the other regions with Z >2, are the HLA region (Z = 2.26), and the 5q31-33 region (Z = 2.17), with a possible interaction of the risk factors in these two regions (uncorrected p = 0.03). The presence of a risk factor in region 5q31-33 is particularly interesting since it has already been shown in several auto-immune diseases. Furthermore, this region is homologous to the rat chromosome 10 region where a susceptibility locus for Experimental Autoimmune Encephalomyelitis has been mapped.

C32. A major non-HLA locus in coeliac disease maps to chromosome 19

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Coeliac disease (CD) is a multifactorial disorder, characterized by villous atrophy of the small intestine. It is strongly associated with HLA-DQ2 and DQ8, but other genes are expected to contribute to CD as well. We therefore performed a genomewide screen in 67 Dutch families with affected sibpairs that met strict diagnostic criteria. Fifteen additional families were available for follow-up of interesting regions. As expected, highly significant linkage to the HLA-region was detected. More importantly, significant linkage was also present on chromosome 19, with a multipoint maximum lod score (MMLS) of 4.43 (nominal p = 6.2 x 10^{-6}). The importance of this region was further supported by single point analysis, showing ten consecutive markers in this region with a lod score >1. This locus is distinct from other regions reported on chromosome 19 showing weak evidence for linkage. It accounts for a 2.6-fold increased risk to siblings, compared to a risk of 4.6 of the HLA-region. Furthermore, we identified suggestive linkage to chromosome 6q21 (MMLS = 2.79, nominal p = 3 x 10^{-4}). This locus lies ~70 cM downstream from the HLA-region, and has also been implicated in other autoimmune disorders like type 1 diabetes mellitus, suggesting the presence of an autoimmunity susceptibility gene in this region.

Our results clearly demonstrate the presence of a major non-HLA locus involved in CD in the Dutch population. This novel, high-risk CD locus is located on chromosome 19p13.1 and is the first non-HLA locus in CD showing genomewide significant linkage in an outbred population.

C33. Copy number polymorphism of a beta-defensin antimicrobial gene cluster

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Beta defensins are a family of 29 genes which encode small cationic antimicrobial peptides that counter and contain infection at the epithelial surface, and are an important part of the innate immune system. These genes occur in clusters, and have evolved by gene duplication followed by adaptive selection, and some appear to have a signalling role in addition to antimicrobial properties. By using a combination of multiplex amplifiable probe hybridisation (MAPH) and semi-quantitative fluorescence in situ hybridisation (SQ-FISH), we have found that a cluster of beta defensin genes at chromosome 8p23.1 which is polymorphic in copy number. Chromosomes have between 1 and 8 copies, and that high copy number alleles (≥7 copies) are visible by G-banding cytogenetics as a euchromatic variant at 8p23.1. The repeat unit is a minimum of 240kb in size and contains at least three beta defensins (DEFB4, DEFB103, DEFB104), a defensin-like antigen SPAG11, and part of an olfactory repeat region. Iincreased copy number of the repeat unit was correlated with increased expression of DEFB4 in a panel of cell lines derived from individuals with different copy number of this beta defensin cluster. Variation in defensin levels due to copy number polymorphism may be functionally important in the susceptibility to infection. Duplication alleles may provide the raw material on which divergent adaptive selection can act on each duplicate defensin gene.

C34. Digenic alteration (HFE/HAMP) associated with adult Hemochromatosis phenotype

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Background. Hereditary Hemochromatosis (HH) is a genetically heterogeneous disease. Feder *et al* identified the most common and the most prevalent form of hereditary iron disorder (HH1, OMIM 235200) in 1996. Since then other types of HH have been described: Juvenile or type 2 HH (OMIM 602390), type 3 HH (OMIM 604250) associated with mutation of *TFR2* and type 4 HH (OMIM 606069) associated with ferroportin. The hepcidin antimicrobial peptide (HAMP, OMIM 606464) was described to play an important role in iron metabolism and recently Roetto *et al* reported that *HAMP* mutations in two Greek families were associated with a phenotype of severe HH classified as juvenile. **Methods**. We studied the *HAMP* gene in 31 patients with HH phenotype and one or no mutation in *the HFE* gene, and 100 blood marrow donors as controls. We used D-HPLC analysis to scan the *HAMP* coding sequence in our subjects and sequencing in the patients presenting an altered profile. **Results**. We identified two novel mutations (175C>G and 212G>A) in *HAMP* in four patients who already carried one mutation at the *HFE* locus. **Discussion.** We report for the first time a digenic inheritance in Hereditary Hemochromatosis between the *HFE* and *HAMP* genes. These data shed new light on the complexity of molecular basis of Hemochromatosis.

C35. Transcriptional profiling of Parkinson's disease genes in vitro.

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Three genes have been described as being definitively causative for inherited forms of Parkinson's disease (PD), with mutations reported in α-synuclein, parkin, and more recently DJ-1 (Bonifati *et al*., 2003). Two recessive mutations in DJ-1 have been reported that appear to encode loss of function alleles. Although the precise function of DJ-1 is unclear, it has some possible effects on transcriptional regulation. The aim of the current work was to examine the effects of DJ-1 on the expression of genes in the dopamine synthesis pathway.
We have shown that that the expression of genes involved in the dopamine synthesis pathway is altered in response to over-expression of wild type, but not mutant, α-synuclein. We are currently exploring three strategies to identify common effects of different PD genes on transcriptional regulation. These experiments have been performed in a human neuroblastoma cell line, M17 that express the full complement of dopamine synthesis genes. First, we have used quantitative RT-PCR to look for effects of α-synuclein expression on DJ-1 steady state mRNA levels. Second, we are examining if DJ-1 has any effects on dopamine synthesis genes. Third, we are analyzing the transcriptosome of cell lines whose expression of DJ-1 or α-synuclein is altered. Using these three approaches we hope to identify common pathways that are regulated by different PD genes.
Bonifati V et al., (2003) Science 299(5604): 256-259.

C36. Haplotype-based survival analysis of MMP-9 gene polymorphisms in relation to long-term prognosis of patients with coronary artery disease

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By its role on remodelling events, matrix metalloproteinase 9 (MMP9) plays a key role in coronary artery disease (CAD). We investigated the association between two polymorphisms of the MMP9 gene (C-1562T in the promoter and R279Q in the coding sequence) with plasma levels of MMP9 activity, and long-term prognosis in 1054 patients with proven CAD followed up for a mean period of 3.8 years. MMP9 activity was increased in patients suffering from future cardiovascular event (66.3 vs 59.7 ng/ml; p = 0.02). The two polymorphisms were in strong linkage disequilibrium, generating three haplotypes CR, CQ and TQ with frequencies of 0.64, 0.22 and 0.14, respectively. Haplotype analysis revealed that the TQ haplotype was associated with increased MMP9 activity (+5.4 [0.04 – 10.4], p = 0.003) by comparison to the CQ and CR haplotypes. In order to investigate the potential association between MMP9 gene haplotypes and long-term prognosis in CAD patients, a new statistical model for haplotype-based survival analysis was developed. In the subgroup of patients with stable angina (n = 716), the CQ and TQ haplotypes were associated with increased risk of future cardiovascular event (HRR = 1.55 [1.06 – 2.28] and HRR = 1.45 [0.90 – 2.35], respectively; p = 0.04) independently of MMP9 activity. These two HRRs were not significantly different (p = 0.44), suggesting an effect of the Q279 allele alone on prognosis.
In conclusion, our study suggests that genetic variation of MMP9 influences both MMP9 activity and prognosis in CAD patients.

C37. Preimplantation Genetic Diagnosis (PGD) for b-thalassaemic haemoglobinopathies based on mutation analysis using real-time PCR (LightcyclerTm)

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PGD represents an alternative to prenatal diagnosis, selecting unaffected IVF embryos for transfer in couples at risk for transmitting a severe genetic disorder. PGD for monogenic disorders commonly involves blastomere biopsy from 3-day cleavage-stage embryos, and PCR-based diagnosis. However, single-cell DNA amplification has inherent difficulties, including contamination risk, PCR failure, and allelic drop-out (ADO). Furthermore methods must reliably and accurately characterize the embryo genotype for the relevant disorder. We have >5years experience with single-cell genotyping and PGD, using a method based on denaturing gradient gel electrophoresis (DGGE). Although accurate, the method is technically demanding, time consuming, and not highly sensitive, providing results in about 80% embryos. To simplify protocols, reduce diagnosis time, improve sensitivity, whilst maintaining accuracy and monitoring ADO, we established a protocol based on real-time PCR, using fluorescent hybridisation probes for mutation detection. We designed, standardized and validated mutation detection probes for the common b-thalassaemia mutations world-wide (and HbS), through mutation analysis in >200 carriers, and additionally 25 prenatal diagnoses (Clinical Chemistry, accepted for publication). We adapted the method to PGD using nested PCR, with the second reaction in LightcyclerTm capillaries, including fluorescent detection probes for melting curve analysis for allele assignment. The LightcyclerTm protocol was applied in 10 PGD cycles. Results (available within 5hours) were obtained in 81/89 blastomeres (91%), of which genotypes in 69 blastomeres also analysed with the DGGE-PGD protocol, were completely concordant. Thus PGD using mutation detection with real-time PCR is more sensitive, accurate and moreover very rapid.

C38. Indications and outcome analysis of 50 consecutive cycles of PGD for reciprocal translocations.

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Chromosomal reciprocal translocations are found in 1 in 500 of the population, and are the most common reason for referral to our preimplantation genetic diagnosis (PGD) centre. Ovarian stimulation, oocyte retrieval, in vitro fertilisation, embryo culture, biopsy of 3-day embryos and in situ hybridization testing of blastomere nuclei using locus-specific and centromeric probes were as previously described. Out of 175 cycles started, 50 were for reciprocal translocations, for 28 different couples. 57% of these couples were referred for recurrent miscarriage, 14% for male infertility, 7% for pregnancy termination, 4% because of a previous affected child, and the rest for a combination of these reasons. Seven cycles were cancelled, 43 reached oocyte retrieval and 31 reached embryo transfer. 327 embryos were tested, of which 71 (21.7%) were normal or carrier; 56 of these were transferred, resulting in 10 pregnancies and 14 babies born. Pregnancy rate per oocyte retrieval was 23.3%, per embryo transfer was 32.3% and per couple was 35.7%. There were no pregnancy misdiagnoses. 202 (61.8%) embryos were abnormal, 38 (11.6%) had an inconclusive result, and the test failed on 16

(4.9%) embryos. 9.8% of embryos showed the presence of more than one cell line, and 5.1% had chaotic chromosome constitutions. Calculation of the analytical performance of the test gave a negative predictive value of 1.00 (95% CI 0.9494 -1.000) and a positive predictive value of 0.9261 (95% CI 0.8864 -0.9659), demonstrating that PGD for reciprocal translocations is a robust, successful procedure, providing a realistic alternative to other reproductive choices.

C39. Very early prenatal diagnosis on coelomic cells using quantitative fluorescent polymerase chain reaction

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Quantitative fluorescent PCR (QF-PCR) has been shown to be an accurate assay for the rapid prenatal diagnosis of chromosome disorders. The extraembryonic coelom develops during the 4th week of gestation and it can be aspirated from the following week making coelocentesis the earliest possible method of prenatal diagnosis after implantation.
We have evaluated the possibility of using the QF-PCR assay performed on DNA extracted from cells presents in the extraembryonic coelom for the detection of common chromosome aneuploidies. QF-PCR amplification using several markers for chromosomes X,Y, 21, 18 and 13 was successfully achieved on all 15 samples of coelomic fluid (CF), placental tissue and maternal blood. Multiplex analyses of maternal blood samples and chorionic tissues allowed to confirm the fetal origin of the cells and, eventually, to identify maternal contamination of the CF samples. Prenatal detection of fetal gender was successful in all cases. When tested with autosomal primers, seven samples were found to contain exclusively fetal DNA. Eight samples contained small amounts of maternal DNA that did not interfere with the QF-PCR analysis. One fetus was correctly diagnosed as affected by trisomy 13. If compared to cell culture, QF-PCR requires very small volumes of sample suggesting that, using this approach, coelocentesis may prove to be useful for very early prenatal diagnosis.

C40. Chromosome instability in female early embryonic development

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The predominance of females segregating chromosome aberrations to their offspring has been explained mostly by selection disadvantage of unbalanced products of spermatogenesis. However, analysis of data from the literature supports the idea that somatic cells of early female embryos are similar to female germ cells in that they are prone to malsegregation. The goal of this study was to compare the sex ratio (male to female ratio) of carriers of presumably mitotic-occurring chromosome abnormalities to identify any sex biases. In examining the literature, we found a female prevalence in cases of mosaicism associated with uniparental disomy. This predominance was highest at gestational age < 16 wk (sex ratio is 0.17), which diminished at later stages of fetal development indicating potential correction of trisomies predominantly in females. There is a threefold prevalence of 46,XX/45,X mosaics over 46,XY/45,X mosaics in prenatally diagnosed cases, which also suggests a gender-specific postzygotic chromosome loss. Finally, there is a female predominance in carriers of de novo homologous acrocentric translocations/isochromosomes, both balanced and unbalanced, with or without mosaicism. Homologous acrocentric rearrangements mostly result from post-fertilization events, not meiotic. Mosaicism results from later gestation postyzygotic formation, while formation in the first zygotic clevage results in nonmosaics. Thus, these data suggest that females may have gonadal mosaicism for aneuploidies and structural rearrangements more often than males. This may lead to the maternal origin bias in offspring with trisomies or structural rearrangements (especially Robertsonian translocations). We conclude that there is likely a sex-specific difference in chromosome instability in early embryogenesis.

C41. Is there an increased risk of imprinting disorders after assisted reproductive technologies ?

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Beckwith-Wiedemann syndrome (BWS) is a model imprinting disorder with an estimated incidence of 1 in 15,000. Most sporadic BWS cases result from epigenetic errors resulting in methylation changes at differentially methylated sites on the maternal chromosome within an 11p15.5 imprinted gene cluster. Angelman syndrome may also result from epigenetic alterations at a maternally imprinted gene cluster. Recently two children diagnosed with Angelman syndrome born after intracytoplasmic sperm injection (ICSI) with sporadic imprinting defects were described (Am J Hum Genet 2002;71). This has led to suggestions that artificial reproductive techniques (ART) may increase the risk of imprinting errors. We have reviewed our referrals of BWS to assess if there is an association between BWS and ART. We identified 6 BWS cases conceived using ART out of 149 BWS cases, or 4%. Since the precise nature of conception of the BWS cases studied was not always documented, the actual figure may be higher than this. In the UK, 1.0% of pregnancies occur after assisted conception. Based on these data, if the proportion of births after ART in BWS and the general population were similar, we would have expected 1.4855 of the 149 BWS patients studied to have been born after ART. To test the significance, a Poisson approximation to the binomial gave a two-tailed p value of 0.009.
As 3 of our cases were after IVF and 3 after ICSI, it may be that in vitro cell culture, rather than a specific ART predisposes to an increased risk of imprinting disorders.

C42. Screening for fragile X syndrome in women of reproductive age

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Fragile X syndrome is the most common cause of inherited mental retardation and the discovery in 1991 of the molecular basis for the disorder made routine population screening feasible. Since early 1994 the Danek Gertner Institute of Human Genetics has offered both pre-conceptional and antenatal screening to women with no known history of fragile X syndrome. Prenatal diagnosis is offered to those with a full or pre-mutation (FM or PM). The results for the 9,459 tests done until March 1999 have already been published (1) and the carrier frequency was 1:70 when the cut-off for PM was defined as 52 repeats. An FM was diagnosed in 1 in 12 fetuses of carriers and all those pregnancies were terminated.
By October 2002 a total of 30,838 women had been tested. In Israel, the cut-off for a PM was recently raised from 52 to 55 CGG repeats and the overall carrier frequency is now 1 in 149 (5 FM and 202 PM). Prenatal diagnosis was carried out in 194 pregnancies of carriers and 19 fetuses had an FM (1 in 10). All these pregnancies were terminated.
Owing to the high PM rate in our population, we conclude that screening for fragile X syndrome among women of reproductive age

should be more widely available.
1. Pesso et al. Prenatal Diagnosis 20: 611-614 (2000).

C43. Towards an electronic altas of gene expression in early human development.

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Now that the human genome sequence is essentially known, we need to develop comprehensive human gene expression maps [1]. Extrapolation from mouse studies has been extremely useful, but there is an important need to develop high resolution human gene expression maps. Four justifications are: (a) human and mouse gene lists are different; (b) some human and mouse orthologues have diverged very considerably in sequence; (c) there are important examples of expression differences between highly conserved human and mouse orthologues [2]; (d) some human attributes (notably cognitive functions) can not be modelled in mouse. Because of the importance of the brain, and the considerable human-mouse differences in brain development, special priority should be accorded to studies of brain gene expression, and to identifying novel molecular markers of human embryonic brain development. We are systematically studying gene expression in early human development (CS 10-23; weeks 4-8), and have developed electronic 3-D reconstructions of embryonic anatomy at various stages (using both conventional approaches – which provide cellular resolution – and high throughput optical projection tomography-based models). We have electronically digitised ISH/ ICC expression patterns and entered the data into a customised 2D- expression database (EurExpress database). We have also been producing 3D expression data which we have mapped back onto 3D reconstructions of embryonic anatomy. We will show examples of 2-D reconstruction data, and where possible, we will present movies to show the 3-D expression reconstructions.
(1) Strachan et al. (1997) Nature Genet. 16, 126-132
(2) Fougerousse et al. (2000) Hum Molec Genet. 9, 165-173

C44. Functional genome analysis of rats suffering from cardiac hypertrophy with a new cDNA indication array

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Cardiac hypertrophy was induced in nephrectomized Sprague-Dawley rats, followed by a whole genome RNA profiling study on cDNA arrays (Rat Unigene-1, 27,000 clones). After data mining, about 1,000 genes potentially involved in cardiac remodeling were identified: growth factors, cytoplasmic signaling molecules, cytoskeletal proteins, as well as members of cell-cell and cell-extracellular matrix contacts. With this information, 1,100 clones were extracted, re-arrayed, PCR-amplified, quality controlled and spotted onto nylon membranes and glass chips. This sub-array was again hybridized with samples from animals confronted with the disease for 2, 8, or 12 weeks, respectively. Already in the 2 weeks samples it was obvious that the remodeling of cell contacts and extracellular matrix (ECM) had started: Components of adherens junctions (cadherin 2 type 1, beta-catenin, alpha-actinin), tight junctions (claudin-3), and the extracellular matrix (collagens) were up-regulated. Strong expression of tissue inhibitors of matrix metalloproteinases (TIMP-1, -3) is another indicator for ECM stabilization. Moreover, we could identify members of the renin-angiotensin hormonal system (endothelin-converting enzym, renin-binding protein), growth factors (TGFbeta, TGFbeta receptor binding protein), and second messengers (protein kinase C receptor) as potential signal transducers. Interestingly, the pattern changes at later time-points (8, 12 weeks). In particular cytoplasmic cytoskeletal proteins (keratin, kinesin), focal adhesion proteins (integrin alpha-1, actin, tropomyosin 6, dynactin, plectin), and ECM components (laminin) seemed to contribute to fibrotic growth of the heart and increase of blood-pressure. We could show that this new indication array is a good basis for the identification of potential drug targets of cardiac myopathies.

C45. High resolution whole genome microdeletion screening by arrrayCGH

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There is an increasing awareness that microdeletions, not visible by routine chromosome analysis, are a major cause of human malformation and mental retardation. In order to detect these microdeletions, novel high resolution whole genome technologies are needed such as array-based comparative genomic hybridisation (arrayCGH)[1]. For whole genome profiling we have established a colony-purified, FISH-verified, phage-tested, 3.5K framework cloneset which is fully integrated in the human genome browser.
The aim of this study was to test the potential of the arrayCGH technology for genome-wide microdeletion screening. We developed an approach that leads to a significant reduction of false positive results, thus minimizing the need for laborious validation experiments and facilitating implementation in routine diagnostics. The sensitivity and specificity of the technology were tested in normal versus normal control experiments and through the screening of patients with known microdeletion syndromes. Finally, a series of 20 clinically selected mentally retarded patients (with/without malformations) with normal karyotypes were analysed. Validation of putative novel microdeletions was performed by FISH analysis of patients and their parents. The results show that deletions and duplications from 1 to 5 Mb can reliably be detected by this approach. These results demonstrate the power of the arrayCGH technology for genome-wide microdeletion screening. High throughput identification of microdeletions constitutes a major step towards the identification of causative genes. We also show that it can be implemented in diagnostics.
[1] Veltman et al. High-throughput analysis of subtelomeric chromosome rearrangements by use of array-based comparative genomic hybridization. AJHG 70:1269-76 (2002).

C46. Multiple-species analysis of conserved sequence blocks on human chromosome 21 allows functional sub-classification

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Chromosome 21 (Hsa21) is the smallest human chromosome. Several diseases are associated with genes or genomic regions of Hsa21 including Down syndrome. Comparative analysis of sequences between species could identify functional conserved features of the genome. We have previously presented a detailed analysis of conserved blocks (>100 bps, >70 % identity) between human and mouse and have concluded that the majority (2262) of them on Hsa21 are not genes (Nature, 420: 578). Here, we further investigate the pattern of nucleotide changes in 220 of these non-genic conserved blocks by PCR amplification and sequencing in 12 additional species representing all mammalian clades. We find that a large fraction of these regions (total of 55519 bps) are highly conserved in multiple mammals ranging from 25% to 90% of the 220 blocks, depending on the species, which supports functional conservation. We also detect patterns of conservation consistent with the presence of distinct sub-classes of functional non-genic sequences. In addition, we observe a highly significant heterogeneous pattern of species-specific substitutions that suggest species-specific selective constraints. Finally, we have compared the pattern of substitution within these non-genic sequences with alignments of equal-species coverage from protein-coding and non-coding RNA genes. Discriminant analysis shows significant differences between these three classes of conserved sequences. We have built models that make use of the conservation characteristics to classify a given sequence in one of the above 3 categories. These models will be very useful for the functional characterization of conserved sequences in the human genome.

C47. Neocentromeres in 15q24-26 map to duplicons which flanked an ancestral centromere in 15q25

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The existence of latent centromeres has been proposed as an explanation for ectopic emergence of neocentromeres in man. This hypothesis predicts an association between the position of neocentromeres and the position of ancient centromeres inactivated during karyotypic evolution. Multiple neocentromeres have been reported in 15q24-26, which contains a high density of chromosome specific duplicons, rearrangements of which have been implicated as a susceptibility factor for panic and phobic disorders with joint laxity. We have investigated the evolutionary history of this region in primates and found that it contains the site of an ancestral centromere which became inactivated about 25 million year ago. This inactivation has followed a non-centromeric chromosomal fission of an ancestral chromosome which gave rise to phylogenetic chromosomes XIV and XV in human and great apes. Detailed mapping of the ancient centromere and two neocentromeres in 15q24-26 has established that the neocentromere domains map approximately 8 Mb proximal and 1.5Mb distal of the ancestral centromeric region, but that all three map within 500kb of duplicons, copies of which flank the centromere in Old World Monkey species. This suggests that the association between neocentromere and ancestral centromere position on this chromosome may be due to the persistence of recombinogenic duplications accrued within the ancient pericentromere, rather than the retention of “centromere competent” sequences *per se*. The high frequency of neocentromere emergence in the 15q24-26 region and the high density of clinically important duplicons are, therefore, understandable in the light of the evolutionary history of this region.

C48. Systematic screen for human disease genes in yeast

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Mitochondria are essential organelles of eukaryotic cells. High similarity between yeast and human mitochondria allows functional genomic study of *Saccharomyces cerevisiae* to be used to identify human genes involved in disease. To date, more than hundred heritable disorders have been attributed to defects in nuclear-encoded mitochondrial proteins in man. Many mitochondrial diseases remain unexplained, however, in part due to the fact that only a fraction of the mitochondrial proteins have been identified. Systematic functional screens using whole-genome pool of yeast deletion mutants have identified 160 proteins, which presumably play important roles for the maintenance of respiratory-competent mitochondria. High-throughput protein-protein interaction analyses, in silico predictions and expression profiling have delivered additional sets of potential new mitochondrial proteins. We developed a database (http://ihg.gsf.de/mitop), which integrates these data and allows their analysis regarding mitochondrial functions by including the knowledge of published studies listed in databases such as SGD and MIPS. These data provide a comprehensive picture of the cellular processes and molecular components required for mitochondrial function.
With this mitochondrial proteome set in hands we determined the human orthologs, which provide a list of 150 human proteins with a potential mitochondrial localisation. Among these are known disease proteins with unknown subcellular localisation. For identifying new candidates of mitochondria-related disorders, we screened the OMIM entries and established a catalogue of at least 250 potential mitochondrial diseases. The correlation of the genomic map positions of the two datasets allows a focused screen for disease genes.

C49. Inbreeding coefficient estimation by genomic control: application to Charcot-Marie-Tooth disease

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Many linkage studies are done in inbred populations, either small isolated populations or large populations where marriages between relatives are encouraged. In such populations, there exist very complex genealogies with unknown loops. So the true inbreeding coefficient of an individual is often unknown. Good estimations of the inbreeding coefficient (*f*) are important as it has been shown that underestimation of *f* may lead to false linkage conclusions.
When an individual is genotyped for markers spanning the whole genome, it should be possible to use this genomic information to estimate his *f*. To do so we propose a maximum likelihood method that takes into account marker dependencies through a hidden Markov model. This methodology also allows us to infer the full probability distribution of the identity-by-descent (IBD) status at each marker along the genome and provides a variance for the estimates. We simulate a full genome scan mimicking the true autosomal genome for (1) first cousin pedigree (2) quadruple-second cousin pedigree. In both cases, we find that our method accurately estimates *f* for different marker maps. The interest of the approach is illustrated with data on a demyelinating autosomal recessive Charcot-Marie-Tooth study.

C50. Patterns and origins of genetic diversity in minority populations (minzu) of the Peoples Republic of China

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There are 55 goverment-recognized minority populations in PR China, which collectively account for 10% of the total population. These minorities have existed for 800 to over 4,000 years, and currently range in size from 2,300 to 15.5 million. DNA samples from the majority Han and nine minorities in north, south, west and central China, the Hui, Bai, Kuchong, Maio, Yao, Tibetans, Bo‘an, Dongxiang and Salar, were analyzed by biparental markers on chr 13 and 15 and uniparental markers on the Y-chr and mtDNA. The number, size range and frequency of alleles differed by population, with unique alleles detected at autosomal loci in each minority. Deviations from Hardy-Weinberg equilibrium ($p<0.05$) were present in all populations except the Yao. Heterozygote deficiency also was found in specific populations, especially the Muslim Hui, Bo‘an and Salar, with Fis estimates of 0.390, 0.140 and 0.150 reflecting their traditions of community endogamy and consanguineous marriage. On the Y-chr ancient unique event polymorphisms (UEPs) were shared across populations, but the Y-STR results indicated male lineage diversification within a historical time-frame. While virtually all populations shared the common Asian C-T mutation at position 16223 in mtDNA, a T-C mutation at 16234 was present at high frequency only in Tibetans. Phylogenetic analysis clustered the populations into four groups: the Han and Hui, the Bai and Tibetan, the Kuchong, Miao, and Yao, and the Bo‘an, Dongxiang, Salar, matching their present-day geographical locations. However, Y-chr STR and SNP analysis suggested language replacement had occurred in the Bo‘an, Dongxiang and Kuchong.

C51. Apparent mismatches between genetic and linguistic phylogenies reflect different approaches to contact

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The assessment of correlations between linguistic and genetic phylogenies is central to the ‘new synthesis‘ (Cavalli-Sforza 2000, Sykes 1999), which seeks to use genetic, linguistic and archaeological evidence in tracing population histories. In a range

of studies, linguistic – genetic correlations (Sokal 1988, Poloni et al 1997, Gray and Jordan 2000, Rosser et al 2000) are significant for populations separated by significant physical barriers. However, although geographically close populations: are typically less divergent in genetics than distant ones (as one would expect), this increased similarity is not paralleled in linguistic distance. We argue that this apparent mismatch in geographically close populations results from an interdisciplinary discrepancy over admixture. While geneticists accept gene flow between populations, and factor this into their calculations, comparative linguists typically remove loan-words when establishing language family relations, hence underplaying the contribution of admixture. Addressing this discrepancy may reveal more accurate matching of linguistic and genetic trees for close as well as distant populations. However, linguists must reconsider their current practice in excluding evidence of contact, investigating new methods of diagnosing and using borrowings. We report one approach, using lists of more and less conservative meanings, and applying the tree-selection programs of Felsenstein (2000) to language data. Our results are also shown to correlate significantly with independent measures of phonetic distance. We argue finally that this foregrounding of contact-induced change necessitates the investigation of network diagrams as well as family trees for the representation of genealogical linguistic relationships.

C52. The first metric linkage disequilibrium map of a human chromosome.

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Recent descriptions of linkage disequilibrium (LD) patterns have focussed on delimiting blocks corresponding to regions of low haplotype diversity. The HapMap project aims to determine common haplotype patterns within these blocks so that a few haplotype tag single-nucleotide polymorphisms (htSNPs) can be used to define each haplotype. In comparison, a metric LD map, with map distances analogous to the centiMorgan scale of linkage maps, provides additional information by characterising inter-block regions which define the relationship between blocks. Such maps avoid arbitrary block definitions and give an additive scale that is useful to determine optimal marker spacing for positional cloning. LD maps also provide insights into the relationship between sequence motifs and recombination since the pattern of LD is closely related to recombination hot-spots and their resolution is higher than existing linkage maps. Using LDMAP to analyse SNP data spanning chromosome 22 (Dawson et al., 2002), we have obtained the first whole-chromosome metric LD map. This map identifies regions of high LD as plateaus or blocks and regions of low LD as steps reflecting variable recombination intensity. The intensity of recombination is related to the height of the step and thus intense recombination hot-spots can be distinguished from more randomly distributed historical events. The map defines optimal SNP spacing for positional cloning and suggests that the minimum number of SNPs required to cover the genome is around 50,000. The map is also closely correlated with the most recent high-resolution linkage map and a range of sequence motifs including GT/CA repeats.

C53. Automatic Detection of Informative Combined Effects in Genetic Association Studies of Complex Traits

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There is a growing body of evidence suggesting that the relationships between gene variability and common disease are more complex than initially thought and require the exploration of the whole polymorphism of candidate genes as well as several genes belonging to common (or interacting) biological pathways. When the number of polymorphisms investigated within a study is large and the hidden structure of the relationships among them complex, the use of data mining tools to extract the relevant information is a necessity. Here, we propose a fully automated method for detecting informative combined effects among several polymorphisms (and other non-genetic covariates) within the framework of association studies. The sequential heuristic algorithm, called DICE, combines the advantages of the regressive approaches with those of large-scale data exploration. Importantly, DICE considers the problem of interaction between polymorphisms as an effect of interest and not as a nuisance effect. The algorithm compares at each step a wide variety of candidate models and chooses the one(s) that provide(s) the best approximation to the data while having the smallest number of parameters. To avoid difficulties related to the null-hypothesis testing theory, the selection for the best model(s) is based on an information criterion to be minimized. DICE identifies a subset of polymorphisms and/or non-genetic variables, which individually or in combination, are associated with the phenotype. Several applications on real data sets demonstrated that the method was able to recover results already found using other approaches, but in addition detected biologically-sensible effects not previously described.

C54. The rhesus macaque as an animal model for endometriosis in women: prevalence and familial aggregation

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Endometriosis is a complex trait in women characterised by endometrial-like tissue found outside the uterus, causing pelvic pain and infertility. Diagnosis can only be established through surgical visualisation, complicating the study of its aetiology. Since endometriosis occurs spontaneously in nonhuman primate species with menstrual cycles, we investigated the prevalence and familial aggregation of endometriosis in a large colony of rhesus macaques. 142 Rhesus macaques with endometriosis were identified mainly from necropsy and surgical records between 1981 and 2002 of a colony at the University of Madison-Wisconsin. Females that had died aged ≥10 years without endometriosis, had both ovaries until at least one year prior to death, and had a full necropsy, were considered unaffected. All affecteds were used to build one large multigenerational pedigree and 9 nuclear families comprising 1,602 females in total.

The prevalence of endometriosis among necropsied rhesus macaques in the colony was 31.4% (95% CI: 26.9-35.9%); prevalence increased with rising age at death and calendar period. The average kinship coefficient was significantly higher among affecteds compared to unaffecteds ($p<0.001$), and a higher recurrence risk for full sibs (0.75, 95% CI: 0.45-1.0) was found compared to paternal half-sibs (0.47, 95% CI: 0.42-0.52) and maternal halfsibs (0.26, 95% CI: 0.10-0.41). Preliminary variance component analyses suggest heritability estimates between 0.2 and 0.6.

The results support familial aggregation of endometriosis in the rhesus macaque, and indicate this is a promising animal model for the investigation of its genetic epidemiology.

Posters

P001. Polymorphisms of *CYP450* and *GST* genes and Breast Cancer in Russian Population

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Activating and detoxifying enzymes from cytochrome P-450 and glutathione S-transferase families play an important role in the metabolism of carcinogens, but association studies of polymorphism in their genes and breast cancer (BC) susceptibility are controversial. Using PCR and RFLP, we evaluated common polymorphisms in *GSTT1*, *GSTM1* (null and non-null alleles), *CYP2C19* (a G to A substitution in exone 5), and *CYP2E1* (a T to A substitution in introne 6) genes in 155 BC patients and 67 unaffected controls from Tomsk Region, Russia. These groups consisted of Russian women similar in age (42.5 and 38.5, respectively).

We detected no significant differences between BC cases and controls on either allele frequencies or genotypes distributions for the variants of *GSTT1*, *GSTM1* and *CYP2E1*.

Allele frequency of *CYP2C19**2 variant was higher in BC patients: 27.7 % vs. 18.2 % in controls (OR=1.72; p=0.041). There was no difference in *CYP2C19**1/*2 heterozygous genotype distribution between BC cases and controls (see table; p=0.543), whereas the frequency of *CYP2C19**2/*2 genotype was insignificant higher in BC patients (p=0.070), suggesting a recessive effect of *CYP2C19**2 variant on BC risk.

*CYP2C19**2 polymorphism produces an aberrant splice site, resulting in a non-functional truncated protein, and it is responsible for poor metabilizer phenotype. We found that this variant in *CYP2C19* gene represented 18 % of Russian population (vs. 8 % in Caucasians, and 35 % in Asians) and probably associated with BC susceptibility. The last suggestion demands a confirmation in other ethnic groups.

Samples	*CYP2C19* genotypes, %		
	*1/*1	*1/*2	*2/*2
BC cases, n=155	52.9	38.7	8.4
Controls, n=67	65.2	33.3	1.5

P002. Cyp1A1 Gene Polymorphism And Risk Of Gynecological Neoplasm In Turkish Population

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The role of CYP1A1*3 gene polymorphism has been noticed for several cancers. We assessed the association of this gene polymorphism in patients with gynecological cancer in Turkish population.

CYP1A1*3 polymorphism was analyzed in 53 patients with cervical intraepithelial neoplasia (CIN), 59 patients with cervical cancer, 27 patients with endometrial hyperplasia (EH), 80 patients with endometrial carcinoma (ECa), and 202 control subjects.

In the CIN, patients with CYP1A1 Ile/Val had a 5.6-fold higher risk of CIN (95%CI:2.9-10.9) There was no statistically significant difference in the distribution of CYP1A1 Val/Val genotype. The frequency of any Val genotype was found to be statistically different with an OR of 4.6 (95%CI:2.4-8.8). In patients with invasive cervical cancer, Ile/Val genotype increased the risk for developing cancer (OR:4.2; 95%CI: 2.2-7.9). No statistically significant difference in the distribution of Val/Val genotype was observed among the cervical cancer patients. However, patients with any Val genotype revealed a 3.7-fold higher risk of having cervical cancer (95% CI:2.-6.8).

Among EH, there were no statistically differences in the distribution of Ile/Val, Val/Val and any Val genotypes, respectively. In the ECa populations, patients with Ile/Val revealed a 2.8-fold higher risk of having endometrial cancer (OR:2.8; 95%CI:1.5-5). The frequency of any Val genotype was found to be statistically different with an OR of 2.3 (95%CI:1.3-4.1).

Our data suggest that the variant alleles of CYP1A1 gene in cervical cells may contribute to CIN or invasive cervical cancer. We also suggested that CYP1A1 gene polymorphism is correlated with the genetic susceptibility of patients with endometrial carcinogenesis.

P003. Polymorphisms of xenobiotic detoxification genes and susceptibility to blood cancer in children

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Biotransformation enzymes play an important role in the metabolism of different chemicals. We determined whether any association exists between genetic polymorphisms in cytochromes P450 (CYP1A1 and CYP2E1), glutathione S-transferases (GSTM1/GSTP1) and individual susceptibility to acute leukemia (AL) in children.

Genotyping assays based on PCR – RFLP method were used to determine the frequency of polymorphisms in CYP1A1, CYP2E1, GSTP1 , GSTM1 in a case-control study composed of 83 patients with AL - 35 with high risk (HRAL) and 48 with standard risk of disease and 102 age- and gender-matched healthy individuals.

AL seemed to be associated with Val/Ile-genotype of CYP1A1 gene, especially among HRAL group (x2=5,729, P=0,0173). Although, distribution of polymorphic variants CYP2E1 and GSTP1 genes did not have significant difference, we have found higher frequency of c1c2 genotype of CYP2E1 gene among patients with HRAL – 12% versus 6% in controls and high frequency of VV-genotype of GSTP1 gene (6,2% versus 3%, respectively). We have not observed significant difference in the distribution of GSTM1-null genotype between studied groups.

The results suggest that genetic polymorphisms of cytochromes P450 and GSTP1 genes may play significant role in the susceptibility to high risk form of acute leukemia in children. There is no association between GSTM1-null genotype and AL.

P004. Do parents and grands-parents of patients with achondroplasia have a higher cancer risk ?

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Several studies, performed according to hypotheses based on teratogenesis and carcinogenesis have tried to answer the question: do parents of children with congenital anomalies have a higher cancer risk ? Thegeneral answer is no, however a higher risk for cancer was reported in the parents of children with cleft lip/palate. In achondroplasia *de novo* mutations are of paternal origin, raising the hypothesis of the existence of a "mutator" gene which may favour also the occurrence of cancer.

A questionnaire was sent to members with non familial achondroplasia of two associations of little people, one French, and one from Quebec. We asked for cancer, lymphoma and leukemia in their parents and grandparents. In the hypothesis tested the maternal lineage was the control.

148 answers were obtained from 76 males and 72 females with achondroplasia. Out of them 68 have parents and/or grandparents with cancer. Eight fathers and 2 mothers of patients with achondroplasia had cancer. Among the grandparents of achondroplastic dwarfs there were 36 cancers including 2 lymphomas in the paternal grandfathers, 20 cancers including 2 chronic myeloid leukemia (CML) in the paternal grandmothers, 22 cancers including 2 CML in the maternal grandfathers, and 4 cancers in the maternal grandmothers.

In conclusion, paternal grandfathers and grandmothers had significantly more cancers than maternal grandfathers and grandmothers (X^2=4.43, p<0.05). This result raises hypotheses in relationship with the paternal origin of *de novo* mutations in achondroplasia.

P005. Promoter polymorphisms of interleukin 6 and tumor necrosis factor α genes in patients with multiple myeloma

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Interleukin 6 (IL-6) and tumor necrosis factor α (TNFα) are pleiotropic cytokines which play important role in the pathogenesis of multiple myeloma (MM). Promoter polymorphisms G/C at the position -174 in the IL-6 gene and -308 G/A at the position –308 in the TNFα gene appear to influence the regulation of these genes. We determined the effect of polymorphic variations in the IL-6 and TNFα promoters on the predisposition to and on the outcome of MM. Genotype distribution was determined in 69 patients with MM from Bashkortostan (19 - with severe outcome (SO) and weak response to chemotherapy, 26 – with benign outcome (BO) and good response to chemotherapy, 24 with unspecified form of disease) and in 102 age- and sex-matched healthy individuals. We did not find associations between distribution of genotypes and allele frequencies in IL-6 and TNFα genes and development of MM. However, we observed that CC genotype of IL-6 promoter which is associated with low IL-6 production, was present exclusively in the BO subgroup with a frequency of 0.35 versus 0.00 in SO subgroup (x2=7.3; p=0.03). We suggest that CC polymorphism in the IL-6 gene may be predictive of the benign MM outcome in Bashkortostan.

P006. DNA-polymorphisms of CYP19 and CYP17 and predisposition to breast cancer risk.

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Breast cancer is one of the most frequently discovered malignancies in women, and responsible for approximately 20% of deaths from cancer. There is evidence for genetic predisposition to breast cancer; about 5% of patients have a family history of the disease. Candidate low penetrance genes are involved in a variety of pathways, for example, steroid hormone metabolism. As increased exposure to estrogens is considered to be a risk factor for breast cancer, the human aromatase gene (***Cyp19***) and gene cytochrome P450c17α (***Cyp17***) are plausible candidates for low penetrance breast cancer susceptibility. These enzymes catalyze the conversion of androgens to estrogens and estradiols synthesis in the estrogen biosynthesis pathway. We have assessed the frequency of allelic variants of the ***Cyp19*** intron 4 [TTTA]*n* repeat in 123 breast cancer cases and 119 controls. It had been previously reported that the $[TTTA]_8$, $[TTTA]_{10}$ and $[TTTA]_{12}$ repeat variants represent low penetrance breast cancer susceptibility alleles. Our breast cancer cases had a statistically significant positive association with the $[TTTA]_8$ allele (11,8% versus 6,3%; P=0,04; OR=1,99 (95% CI 1,04-3,81)). The frequency of the $[TTTA]_{10}$ and $[TTTA]_{12}$ alleles was not significantly elevated in our breast cancer sample compared with controls. ***Cyp17*** gene was studied for SNP T27C (MspA1). ***Cyp17*** genotype (*A2/A2* allele; MspA1+/+) was associated with an increased risk of breast cancer (for *A2/A2*): 32,5% versus 20,2%; P=0,03; OR=1,91 (95% CI 1,06-3,43). The risk of breast cancer increased significantly at a combination of $[TTTA]_8$ allele and *A2/A2* genotypes (7,3% versus 0%; P<0,01; OR=19,83 (95% CI 1,14-344,89).

P007. Correlation of the blood group O and the risk of the development of gastric cancer under the age of 50 in Northern Iran

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Background: Developement of gastric cancer (GC) before 50 is likely to have a genetic basis. Blood group A has been shown as a risk factor for GC. Northern Iran is an endemic region for GC.
Methods: In this prospective case-control study, we enrolled Iranian gastric cancer patients under the age 50 and sex-matched controls over 50. All the patients and (if alive)/or their family members were interviewed and their pedigrees were drawn. The blood group of the patients were also tested or obtained from the in-patients records.
Results: 44 cases (mean age: 36.2, 18-49; m/f=1) under 50 years old and 44 sex-matched controls (mean age: 67.1, 50-88) were enrolled in the study. 41% of the study group were dead and 59% were alive at time of study. Distribution of blood groups is as follow: 68.1% O, 13.6% A, 13.6% B and 4.5% AB in cases and 27.7%, 63.6%, 6.8% and 4.5 in controls, respectively. 50% of the cases and 9% of controls had some first or second-degree relatives with gastric or other types of cancers (p<0.01). Breast, lung, gynecological and hematological malignancies constituted other type of cancer in their families.
Conclusions: It seems that cancer before 50 is accompanied with a familial aggregation. Interestingly, our study shows the significant correlation between blood group O and the development of gastric cancer under 50. This arises the need for more linkage analysis study on the role of blood group genetic area in familial aggregation of gastric cancer.

P008. The role of GGC repeats in gene expression and its association with cancer.

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The eucaryotic releasing factor 3 (GSPT1/eRF3) has multi-funtional properties in eucaryotic cells. It controls the regulation of the cell cycle mediating the G1 to S phase transition and it regulates protein synthesis as a GTP-dependent stimulator of eRF1 in termination of translation and also as an initiator of the mRNA degradation machinery. It was also reported to have a key role in the recycle of ribosomes in successive cycles of translation, and probably in transcription regulation.
In this study we intended to understand the influence of the alterations in this gene (GSPT1/eRF3) that can be involved in susceptibility for the development of gastric carcinoma.
Gene expression was investigated by Real Time PCR and a significant overexpression of eRF3 was observed in about 40% (7/17) of gastric tumour samples (mainly adenocarcinoma intestinal type, with different states of differentiation), when compared with the noncancerous adjacent tissue. A significant correlation between the presence of the 149 bp allele of the STR region and cancer (freq= 5%, χ^2 =15,506; p=0,008) was also detected using fluorescent STR allelotyping. LOH was found in circa 30% (19/55) of the informative cases. Breast (mainly ductal carcinoma), lung (mainly adenocarcinoma)and endometrium tumours were also investigated and similar expression patterns were detected.
Our results shows that GSPT1/eRF3 may have a role in (de)controlling cell cycle or (de)regulation protein synthesis.

P009. Identification of polymorphisms in multidrug resistance gene in leukemia patients

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Multidrug resistance(MDR) is a serious limitation to effective chemotherapeutic treatment of many cancer types including leukemia. One of the most important proteins the overexpression of which is responsible for the MDR in many cancer types is P-glycoprotein. This protein is the product of the *MDR1* gene. In many studies, single nucleotide polymorphisms C3435T, G2677T, T-129C in *MDR1*were shown to correlate with P-glycoprotein expression in normal tissues.I n this study, these polymorphisms were compared in 16 resistant and 12 non-resistant acute leukemia patients and 17 healthy individuals. In the acute leukemia patient group the C3435T polymorphism seems to have a preventive effect since 58.3% patients having T/T genotype are non-resistant while 59.3% patients having C/C genotype are resistant to chemotherapy. The G2677T

polymorphism conferred no advantage for the drug resistance phenotype. In this case resistant and non-resistant patients have approximately same allele ratio, having G/G genotype about 80% (p=1). Any effect of the T-129C polymorphism on resistance could not be detected since all the patients were homozygous for the wild type T/T genotype. In the Turkish population the incidence of the T allele (73.5%) for C3435T polymorphism was significantly higher (p=0.025) than the C allele (26.4%), indicating that the Turkish population shows greater P-gp mediated resistance than populations that have higher C allele. The incidence of G allele for G2677T polymorphism is high (84%). The incidence of T-129C C allele is too low to be detected in 45 individuals.

P010. Association of NQO1 with spontaneous breast cancer

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To search for an association of possible candidate genes for spontaneous breast cancer we collected 220 DNA samples from patients with spontaneous breast cancer and 600 DNA samples from unselected blood donors from the Tyrol. Eight SNPs from six candidate genes were selected, and the allele frequency of each SNP was compared between tumor patients and controls.

Gene	SNP	Rare Allele frequency in patients	Rare Allele frequency in controls	p
BRCA1	Q356R	0,07	0,09	n.s.
BRCA1	P871L	0,33	0,32	n.s.
BRCA2	N372H	0,266	0,28	n.s.
TP53	R72P	0,28	0,25	n.s.
GNB3	C825T	0,33	0,33	n.s.
ApoE	R112C	0,15	0,14	n.s.
ApoE	C158R	0,07	0,08	n.s.
NQO1	P187S	0,21	0,17	0,034

The SNPs were determined employing the 5'-exonuclease assay and MGB-probes. The allele frequency was determined by allele counting and the statistical analysis was done by χ^2-test. Only the P187S polymorphism of *NQO1*-(NADH-dependent-quinone-oxireductase) showed a significant association of the S187-allele with breast cancer, RR = 1.34 and p=0.034. Homozygotes for the S allele are highly significantly more frequent within the patients compared with the controls (RR = 3.75 and p= 0.006).
The NQO1-enzyme is involved in the detoxification of carcinogenic substances from tobacco smoking. The enzyme with a Serine in position 187 is inactive and might therefore not be able to protect the mammalian tissue of carcinogenic substances, conferring a higher risk to developing breast cancer.

P011. The seroactivity frequencies of SOX Group B and ZIC2 antigens in Turkish Small Cell Lung Cancer Patient Sera and its Correlation with Clinical Parameters

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Lung cancer is divided into two main histological groups: small cell lung cancer (SCLC) and non-small cell lung cancer. SCLC accounts for approximately 20% of all lung cancer. SCLC, a disease of unknown etiology, although initially responsive to treatment is ultimately fatal. SOX group B (SOX 1, SOX2, SOX3 and SOX21) and ZIC2 antigens, which are embryonic neural proteins, were identified as highly immunogenic tumor antigens by SEREX (serological analysis of expression cDNA libraries) in small cell lung cancer (A.O. Gure et al). In order to identify the frequency of antibodies reactive with SOX and ZIC2 antigens among Turkish small cell lung cancer (SCLC) patient population, sera from 70 Turkish SCLC patients and 30 normal adults (controls) were analysed against phage clones coding for SOX1, 2, 3 or ZIC2. Sera from SCLC patients were collected before and after chemotherapy. 27/70 of patients were seroreactive for at least one of the 4 antigens (SOX1, 2, 3 or ZIC2). The seroactivity results are shown in Table I. No antibody was detected against SOX or ZIC2 proteins in 30 control samples. Seroreactivity frequencies in these SCLC patients against SOX1, SOX2, SOX3 and ZIC2 antigens were 17/70 (24.2%), 15/70 (21.4 %), 7/70(10%) and 11/70 (15.71%), respectively. We will furher evaluate the possible correlation of seroreactivity to these antigens with clinical parameters of progress and outcome of SCLC.

Table I. Number of patient sera reactive against SOX and ZIC2 antigens during pre- and post-chemotherapy

Antigens	Pre-chemotherapy Patients' sera	Post-chemotherapy Patients' sera
SOX1	1	3
SOX2	2	_
SOX3	_	_
ZIC2	2	5
SOX 1, 2,3	4	2
SOX 1, 2	3	1
SOX1,2 and ZIC2	_	2
SOX1,3 and ZIC2	_	1
SOX2 and ZIC2	_	1
Total	**12 /35 (34.2%)**	**15/35(42.8%)**

P012. Genetic alterations in gastric cancer among the Iranian population.

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Genetic alterations during the tumorgenesis of gastric cancer are not well characterized. APC, β-catenin, MCC, p53 and E-cadherin are involved in gastric cancer tumorgenesis. APC controls β-catenin as a proto-oncogene in WNT signaling pathway playing a major role in progression of early dysplasia of gastric epithelium into adenoma and adenocarcinoma particularly in well differentiated gastric cancer. P53 mutations exist in adenoma and adenocarcinoma of same type of tumors. Expression of E-Cadherin is diminished in gastric adenocarcinoma resulting in metastasis of these tumors. Mutational analysis and expression of these genes were studied in early and advanced gastric adenocarcinoma to elucidate their role in tumorigenesis of gastric cancer. Paraffin-embedded gastric adenocarcinoma including the normal margins from 40 patients, with endoscopy and/or gastrectomy (age group32-70yrs) were screened for mutations in APC, β-catenin genes and LOH in 5q21-22 the APC and MCC regions. The APC coding region, including 4 Mutation Cluster Regions (MCRs) in Exon 15 (1267-1588bp) that are the hot-spots for nonsense mutations were examined by PCR-SSCP followed by sequencing. 4/40 of adenocarcinoma had a mutation. PCR-SSCP and PCR-RFLP detected 12/40 mutations in β-catenin gene. The LOH analysis revealed LOH in 5/40 specimens in APC region and 4/40 in MCC region. Overall 62% of genetic changes were detected in gastric cancer particularly in intestinal type. 39/52(75%) of patients showed mutated p53 and 28/52(54%)were negative for expression of E-cadherin as determined by Immunohistochemistry. These data correlates with the reported results helping the formulation of a pathway in tumorgenesis and progression of gastric cancer.

P013. Polymorphisms in Biotransformation Enzymes and Breast Cancer

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Our study aimed at determining whether any association exists between genetic polymorphisms in epoxide hydrolase (*EPHX1*), NADPH-quinone oxidoreductase (*NQO1*), glutathione S-transferases (*GSTM1/P1/T1*) and individual susceptibility to breast cancer in

Czech women. A case-control study comprised of 191 patients with breast cancer and 232 age-matched healthy individuals.
The distribution of genotypes in exon 6 of *NQO1* was significantly different between the control group and breast cancer cases. Odds ratio (OR) for variant genotype *NQO*2/*2* was 6.76 (CI=1.92-23.81, P=0.003). Association of *GSTM1-null* with breast cancer was on the borderline of significance (OR=1.50, CI=1.02-2.21, P=0.049). Individuals lacking *GSTM1* and simultaneously carrying at least one *NQO1* variant allele were at significantly higher risk of breast cancer (OR=2.18, CI=1.35-4.68, P=0.003). Patients with higher EPHX1 activity had larger and well-differentiated tumors, were more probably in the premenopausal group and were bilaterally affected i.e. generally had worse prognosis. The variant *GSTP1*2/*2* genotype was prevalent in breast cancer cases (OR=1.78, CI=0.94–3.34, non-significant) and associated with positive estrogen receptor status (P=0.036) and family anamnesis of breast cancer (P=0.032). *GSTT1-null* genotype seemed to be a protective factor as it was overrepresented in the group of cases at stages 0 and I.
In conclusion, the results suggest that genetic polymorphisms in biotransformation enzymes may play a significant role in the development and progression of breast cancer.
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P014. Evaluation of genetic background of acquired aplastic anemia

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Acquired aplastic anemia (AA) is a rare disease in which pancytopenia most probably derives from an autoimmune attack towards the hematopoietic stem cell by autoreactive T-cell. The initial activation may be due various triggers such as genetic events, drugs or infections. Genetic factors related to the immune response may influence T-cell activation and the release of myelotoxic cytokines. In order to evaluate if genetic variations of the mechanisms of T cell control and of the capability of producing myelosuppressive cytokines may have a role in the origin of AA, we analyzed the distribution of the polymorphisms of CTLA4, TNF-α, IFN-γ, and IL 10 (see table) in 52 patients with AA and in 100 normal controls.
The lack of difference between patients and controls regarding IL10, TNF-α stands against a role for these polymorphisms. It is still to be evaluated whether these polymorphisms may impact on the outcome of the disease. The increased expression of CTLA4 in AA patients vs controls seems to indicate a pathogenic role for these polymorphisms. It is a further support to the autoimmune pathogenesis of AA. Furthermore analysis of CTLA4 haplotype suggest a role as a risk factor in this condition. In addition our results suggest a role of IFNγ in the pathogenesis of this condition.

	Genotype	Controls %	AAA %
IL-10 **–1082G->A**	AA	33	34
	AG	51	42
	GG	16	24
TNFα –308**G->A**	GG	84	82
	AG	15	18
	AA	1	0
IFNγ : **VNDR1349** (allelic frequency)	(CA)11	48	63
	(CA)12	46	30
	(CA)13	6	7
CTLA4 Haplotype frequency	C-318 G+49	56	30
	C A	35	62
	T A	9	7
	T G	Extremely rare	0

P015. The sequence alteration at codon 289 of BRCA2 gene in Turkish breast cancer families

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Breast cancer is one of the most common malignancies affecting women. Inherited gene mutation may be responsible for 5-10% of breast cancer cases. Germ-line mutations in breast cancer susceptibility genes BRCA1 and BRCA2 account for the majority of families with hereditary breast and ovarian cancers. Carriers of these mutations have an increased life-time risk of developing breast and ovarian cancers. Population genetics studies aimed at determining the relative contributions of these genes in hereditary breast and/or ovarian cancers have shown a wide variation among different populations .The frequency and the types of germline mutations involved in Turkish breast-ovarian cacers are not well known. Exons 10 and 11 of BRCA2 gene are hot spot regions for determining of susceptibility to breast cancer. In this study, we screened exon 10 of BRCA2 gene in 35 families with breast/ovarian cancer by heterodublex analysis with non-radioactive silver staining technique. Altered fragments were determined at exon 10-1 of BRCA2 gene. BRCA2 (codon 289; 1093 A to C) different sequence alteration was identified in 17 individuals of 6 (17.1%) families by sequencing analysis. This point may reflect candidate mutation for BRCA2 gene. Consequently, it may be a new mutation to determine genetic susceptibility for Turkish population.

P016. Cloning and characterization of the promoter region of human rad51 (HsRad51) gene

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Rad51 is a homologue of bacterial RecA, which is required for meiotic and mitotic recombination and promotes ATP-dependent homologous pairing and DNA strand exchange. The interaction of HsRad51 with several tumor suppressor genes namely p53, BRCA1 and BRCA2 implies possible role(s) of this protein in tumorigenesis. Overexpression of Rad51 protein have been reported in different human cancers like pancreatic adenocarcinoma and invasive ductal breast cancer.
In the present study we describe the isolation, sequencing and first functional characterization of the promoter of human Rda51 gene. We cloned and sequenced an 8.1 kb 5´-flanking region of HsRad51 gene and identified the promoter region of this gene. Analysis of 1.5 kb of DNA upstream of the initiation of translation revealed that this sequence has a high GC content, lacks a TATA element and contains a number of putative transcription factor binding sites.
The regulatory sequence crucial for rad51 promoter activity were characterized between -543 and +106 bp. In this region, a minimal promoter sequence including E2F /GABP or/and Ets1/PEA3 transcription factor binding sites, as well as a proximal promoter sequence with an P53 binding site were identified. The sequence between -43 and +204 bp shows down-regulate function, which can be effected by a single base substitution of C to G.
We demonstrate here the primary data about rad51 promoter organization and possibly regulatory elements in the rad51 promoter region. These data provide useful information for following functional studying of HsRad51 gene.

P017. A novel germline *BRCA1* mutation identified in a Chinese patient with breast and ovarian cancer

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Hereditary breast and ovarian cancer related to *BRCA1* mutations has been reported in 7 to 9% of breast cancer patients from Singapore. Age of cancer onset may not be the sole criteria in identifying at-risk women. In our risk evaluation and prevention program, a 40 year-old Chinese Singaporean woman presented with ovarian cancer at age 37 and breast cancer at 39 years. She had a family history of breast cancer, and a high prior probability of being a mutation carrier based on the BRCAPRO risk prediction model. The entire *BRCA1* and *BRCA2* coding regions were analyzed using direct sequencing and the protein truncation test. No mutations

were detected in the *BRCA2* gene. We identified a novel germline nonsense mutation A4920T (K1601X) in exon 16 of *BRCA1* in this patient and the patient's unaffected sister, but not in 50 normal individuals. Sequence comparison with *Mus musculus* and *Canis familiaris* showed that the base change affected a conserved residue. In addition, common polymorphisms and several variants of unknown clinical significance were also identified. The *BRCA1* gene is large and in individuals with high prior risks, careful evaluation for mutations that span the entire gene continues to be standard practice.

P018. Motivation to attend familial cancer genetics centres for advice about a family history of cancer: the PACT psychosocial study

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Family cancer genetics centres in the United Kingdom are experiencing a surge in referrals. There is a pressing need to understand what motivates interest in seeking advice about a family history of cancer in order to plan for future service development. The primary motivation in unaffected women, at moderate to high risk of breast cancer, has been studied previously. The PACT study aims to provide information on the broader remit of patients currently being referred to familial cancer clinics, including affected and unaffected men and women at all levels of risk for different types of cancer, at several clinics across the country. One hundred and sixty-two patients took part in this descriptive multi-centre study, run at five regional genetics centres (Leeds, London, Newcastle, Oxford and Southampton). The study questionnaire assessed a range of psychosocial factors that are believed to be involved in motivating interest. In addition, sociodemographic characteristics were assessed in order to evaluate potential consumer-related barriers in access to care. Data analysis is scheduled for completion at the end of March 2003. The study findings will be presented at the meeting.

P019. Family stories and the use of heuristics: women from suspected hereditaty breast and ovarian cancer (HBOC) families

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The practice of medicine will increasingly be medicine of the family especially where a genetic condition is involved. This study explores how clients from suspected hereditary breast and ovarian cancer (HBOC) families seeking cancer genetics risk counselling are influenced by family stories and the use of heuristics (inferential shortcuts used to make sense of complicated information) in interpreting and applying genetic information they receive, and suggests ways in which genetic counsellors can integrate family context into their traditional counselling practices. We conducted an exploratory, qualitative study at a major clinical and research cancer centre in the United Kingdom from January - June 2000 which was reviewed by the hospital clinical research and ethics committees. Twenty-one semi-structured, in-depth interviews were conducted using a purposive sample of women coming to the cancer genetics clinic for the first time, supplemented by five months of clinical observation at weekly clinics. In addition to many family stories based on the number and outcomes of the cancers in their families, there were: fragments of stories, ¨secret¨ stories, emerging explanations and misconceptions. The women used three main heuristics in interpreting their breast/ovarian cancer risk: representativeness, availability and illusion of control, as well as what Kahneman refers to as the Peak and End rule. Recent psychological research indicates that illusions of control may have positive affects on physical and mental health. This may pose future ethical issues for genetic counsellors in determining how to balance the benefit of positive illusions with the delivery of statistical probabilities of risk.

P020. The British Familial Cancer Record (BFCR): A Model For Clinical Research Into Familial Cancer

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Cancer family clinics are now widely developed. Large amounts of clinical and genetic information have been routinely collected. This constitutes a tremendous potential resource for epidemiological and clinical research. Exploitation of this resource is constrained by, the wide dissolution of relevant medical information, the limited numbers of persons and families attending even the largest family cancer clinics and the accessibility of the patient population. To resolve these issues, we have developed the BFCR, a database of patients' medical information, family history and risk factor information. Those attending cancer family clinics (currently: Leeds, Guy's (London), Newcastle, Oxford, St. Mark's (London) and Southampton) are invited to participate in the BFCR. Multilevel informed consent is obtained to (a) allow abstraction of all medical records, genetic information and family history, (b) permit this information to be stored on a named database held by the local clinical genetics service and on an anonymized central database held by Cancer Research UK consisting of information from all clinics, (c) complete a questionnaire of epidemiological risk factors and (d) be recontacted for further relevant, ethically approved studies. As proof of principle, we have focused on family histories of breast cancer. 2000 women have been recruited; participation rates are high. The initial study focuses on monitoring screening surveillance for women with such a family history. Software has been developed for the local centres and the centralised database. We have shown that this approach is practicable and acceptable and can play an important role in research into familial cancer.

P021. BRCA1 and BRCA2 Germline mutations in Breast-Ovarian Cancer (BOC) from a Genetic Counseling Unit (GCU) in Catalonia.

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To provide genetic counseling to families at high risk of Hereditary BOC we need estimates of mutation frequencies in our population. In 1998 we started a GCU in Catalonia and here we present the analysis of our series of 161 high-risk families.

Patients and Methods:

511 high-risk families were referred. 247 of them met our selection criteria and 187 (77%) agreed to participate. Selection criteria: A) ≥ 3 family members with BOC; B) 2 family members with at least one high-risk criteria; C) single affected individual with bilateral breast cancer < 40 years, or proband with both breast and ovarian cancer or diagnosis age < 35 years. BRCA1/2 genes were analyzed by full sequencing in 140 patients and by denaturing high performance liquid chromatography in the others.

Results:

Selection Criteria	Number of Patients	Detection Rate (DR)	Gene
A	79	22 (28%)	8 BRCA1 (36%) 14 BRCA2 (64%)
B	48	11 (23%)	5 BRCA1 (45%) 6 BRCA2 (55%)
C	34	4 (12%)	2 BRCA1 (50%) 2 BRCA2 (50%)
Total	161	37 (23%)	15 BRCA1 (40%) 22 BRCA2 (60%)

DR in site-specific breast cancer families was 19% (22/113), (16 BRCA2/ 6 BRCA1). DR in families with ovarian cancer cases was higher, 43% (15/35), (9 BRCA1/ 6 BRCA2). Direct testing was done in 98 relatives at risk: 38 were positive and 51 negative.

Conclusions:

Our DR is similar to that previously reported in Spain, but with a higher number of BRCA2 mutations than expected. Direct testing enables us to focus our efforts in surveillance and prevention on the 42% of healthy carriers. An optimal surveillance has to be determined for high-risk patients where a mutation could not be identified.

P022. A survey regarding the policy of the unclassified variants in the BRCA1 and BRCA2 gene in the Netherlands

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The aim of this study was to document current practices in the genetic testing and counseling of hereditary breast cancer, and identify areas of variability for patients with an unclassified variant (UV) in the BRCA1 or BRCA2 gene. All clinical molecular geneticists (eight laboratories) and ten clinical geneticists involved in hereditary breast cancer diagnostics and counseling in the Netherlands were sent an invitation to participate in a questionnaire. The response rate was high (>85%).

Although the mutation detection rate between the DNA diagnostic laboratories is comparable (BRCA1 5.5-8.5%, BRCA2 1.0-6.0%), the detection rate of UV's varies considerably (BRCA1 1-11.5%, BRCA2 9.5-17%). Except one, all laboratories report the UV's found to the clinical geneticists and almost all offer further analysis to elucidate the variants' clinical significance. All laboratories but one replied that they normally do not offer presymptomatic testing for an UV.

All responding clinical geneticists have received one or more test results with an UV and almost all inform their patient about the variant found. Half of the clinical geneticists already mention this test result as a possibility in their intake. Most of them test more affected family members to look for segregation, but no one offers presymptomatic testing of an UV routinely.

The policy in the Netherlands regarding the UV's found in the BRCA1 and BRCA2 gene is comparable in most centers, but differs sometimes at some critical aspects.

P023. Rapid throughput sequence-based mutation scanning of BRCA2: Development of an improved method based on Meta-PCR

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The challenge for a comprehensive mutation scan in a large gene such as BRCA2 is to maximise accuracy while reducing cost and turn-round time. Our previous strategy for identifying mutations in BRCA2 involved the labour intensive methods of PTT for exon 11 (3 fragments) and SSCP/heteroduplex analysis of the remaining coding regions (38 fragments) followed by bi-directional sequencing of subsequent shifts. We report a cost-effective high-throughput direct sequence analysis for BRCA2. Exon 11, comprising ~50% of BRCA2, is sequenced in 6 overlapping fragments. Exons 10, 27 and 22-24 are sequenced as single fragments. Remaining exons are linked in a further 6 fragments of approximately 900bp by Meta-PCR. Common tails allow sequencing with universal primers ensuring consistent and high quality sequence from the end of the primer. All PCR amplification, purification and sequencing reactions are performed in 96-well plates increasing throughput, reducing hands-on time and improving internal quality control. Sequencing reactions are analysed on a 16 capillary ABI3100 automated sequencer and scanned for mutations using trace subtraction software in the Staden Package. Currently exons 10, 11, 22-24, 27 and three out of the six Meta-PCR fragments have been successfully developed. This diagnostic system will allow panels of 15 patients to be run and analysed for all fragments in a matter of weeks rather than months. A similar strategy is in routine use in the laboratory for mutation scanning of BRCA1. Currently this scan does not detect whole exon deletions, although this may be incorporated in the future.

P024. Analysis of genetic heterogeneity breast cancer families by BRCA1/2 gene mutations and single nucleotide polymorphisms

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BRCA1/2 gene mutations and SNPs were analyzed in a sample of female breast cancer families. 63% of families had four or more breast/ovarian cases. Sixteen (33%) *BRCA1* and only two (4.1%) *BRCA2* deleterious mutations were found among 49 probands. There was no difference in the *BRCA1/2* mutation frequencies between families with at least four and two/threecases (P = 0.71). The *BRCA1/2* mutation frequency in families with four or more cases of breast cancer only was significantly less than in all the rest of the sample (P < 0.03), and less than in families with four or more cases that include ovarian cancer (P < 0.005). Families without *BRCA1/2* mutations predominantly had breast cancer only (77%). Conversely, the majority of families with mutations included cases of ovarian cancer (61%). These results characterize the families with *BRCA1/2* mutations in Russia and show the type of family without mutations.

SNP analysis has defined two major haplotypes of *BRCA1*: a consensus sequence (haplotype A) and a sequence with a set of SNPs in strong linkage disequilibrium (haplotype B). Proband groups with and without *BRCA1/2* gene mutations showed different haplotype frequencies (P < 0.03). Moreover, in the latter group the AA genotype was decreased relative to the control individuals (P < 0.03), with odds ratio 0.29. The data may suggest a connection of the *BRCA1* gene variations with familial breast cancer without *BRCA1/2* gene mutations, possibly in association with another gene or genes.

P025. Evaluation of mouse susceptible loci for human breast cancer

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Genes that confer resistance or susceptibility to cancer development are amenable to study in rodents, where mapping and cloning of quantitative trait loci (QTL) for cancer susceptibility could help to identify low penetrance genes in human cancers. Moreover, in mice, alleles involved in susceptibility or resistance to tumours, are often preferentially amplified (or deleted) in the tumours themselves, which allows us to narrow down the regions to a reasonable number of candidate genes to test.

Thus, in an attempt to validate selected QTL in human breast cancer, 4 candidate genes, FasL (1q23), CSTF1 and STK15 (20q13.2), and CCND1 (11q13), were investigated using association study on 2300 East Anglia Breast Cancer cases matched to EPIC controls.

Five potential SNPs in the 1q23 region, 5 in 20q13.2, and 2 in 11q13 were not found to be statistically associated with breast cancer.

A 3'UTR SNP in the CCND1 gene was found to be statistically associated with breast cancer (OR 1.26, 95% CI 1.03-1.53). Furthermore, women with a common haplotype of CCND1 (29%) were found to have a significant increased risk (14%) of breast cancer.

In conclusion, the 1q23 region was extensively studied and did not appeared to alter the risk of breast cancer in our study population, however the 20q13.2 and 11q13 regions need to be further investigated.

P026. Clustering of breast cancer and other malignancies within families assisted at a Brazilian Cancer Center

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Approximately 36,090 persons will be diagnosed with breast cancer this year in Brazil and 9,115 will die. This type of tumor may occur in the same family associated with other cancers in 30% of cases. About 5-10% may be due to inherited germline mutations in autosomal highly penetrant susceptibility genes corresponding to hereditary breast cancer syndromes (HER) and 20% corresponding to familial clustering (FAM), where there is interplay between genetic and environmental factors. The principal goal from this research is to evaluate the correlation between breast cancer and other malignancies to establish risk categories. For this purpose, 858 records from patients seen between 10/1999 and 02/2002 by our Oncogenetics Department were studied. We selected 332 cases with breast cancer with first and/or second degree relatives affected by malignancies. Details such as the site of the primary tumor and the age of onset was considered. The association with 24 tumors was observed and family history of cancer was classified in FAM (271) or HER (58 cases). In the FAM the more prevalent associations were with breast cancer (176), stomach (90), colorectal (87), lung (63), uterus (55), prostate (50), head/cervical (49), lymphoproliferatives (32), thyroid (15), urinary bladder (12), ovary (9), sarcomas (7), and others. Some rare aggregations were also observed as melanoma (21) and pancreas (13). All patients at high risk were identified and received oncogenetics counseling. This study emphasizes that some familial clustering are suspected of HER and must be considered as high-risk, with implications on the follow-up.
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P027. The complete mutation analysis of the *CHEK2* gene in German *BRCA1/2* negative families

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CHEK2 is a cell-cycle-checkpoint protein kinase that can phosphorylate p53 and BRCA1 in response to DNA damage and mutations in this gene have been associated with cancer suggesting that it may act as a tumor suppressor gene (TSG). Recently, the *CHEK2*-Breast Cancer Consortium (2002) reported that an 1861delC mutation (previously known as 1100delC) confers a low-penetrance susceptibility of breast cancer in BRCA1/2 negative families. This low-penetrance susceptibility was confirmed in the Finnish population by Vahteristo et al. (2002).
Also published in 2002, the German Consortium for Heredity Breast and Ovarian Cancer (GCHBOC) reported the mutation profile and frequencies for the *BRCA1* and *BRCA2* genes in the German population and as an extension of this work the German *CHEK2*-Consortium has investigated the mutation spectra for the entire coding region of the *CHEK2* gene, which consists of 14 exons. Based on our results of over 500 probands that are negative for mutations in both the *BRCA1* and *BRCA2* genes, the low-penetrance susceptibility previously reported by others for the 1861delC mutation, located in exon 13, holds true for the German population with the exception that the observed frequencies are lower. Interestingly, a novel mutation in exon five that causes a severely truncated protein could not be associated with hereditary breast cancer. This result and the low overall frequency of mutations discovered during the complete screening of the *CHEK2* gene indicate that aberrations in the CHEK2 protein have a minor impact for the development of familial breast cancer in the German population.

P028. A recurrent mutation in BRCA1 is frequent among North-African Jews with familial breast/ovarian cancer

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The spectrum of germline mutations in the breast/ovarian cancer susceptibility genes BRCA1 and BRCA2 in Jewish individuals is limited. Of the 25 mutations ever described, only 5 have been described more than once, and three mutations [185delAG 5382InsC (BRCA1) 6174delT (BRCA2)] account for the majority of familial breast/ovarian cancer in Ashkenazi Jews. Since the Non-Ashkenazi Jewish way of life in the diaspora was essentially similar to that of the Ashkenazi Jews, it seems plausible that a few founder mutations would also be detected in that ethnic subset of Jewish individuals. A single mutation in BRCA1 (1100delAT) was initially detected in a Lybian family with breast/ovarian cancer. Subsequent analysis of the rate of this mutation in 135 seemingly unrelated high risk Jewish families of North-African origin, revealed that 6 additional unrelated patients harbored the same mutation (4.4%). Haplotype analysis showed that all mutation carriers had an identical BRCA1 intragenic haplotype. The rate of this mutation in unselected Jewish individuals of the same ethnic origin with breast and ovarian cancer as well as the occurrence of this mutation in the population at large are ongoing. In conclusion, a single BRCA1 mutation is a founder mutation in North African Jews and the mutational reality of BRCA1 in Ashkenazi Jews seems to hold for the Non-Ashkenazim.

P029. Bowel Cancer referral to Regional Genetic Service

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Introduction: The Manchester Regional Genetics Service has conducted an audit of individuals with a family history of bowel cancer referred over the past 10 years. The purpose of the study was to determine the number of referrals being seen annually, their gender distribution and the proportion with high risk of developing bowel cancer.
Method: Hospital computer records and/or department referral books were used to identify cases between the years 1992 to 2001.
Result: A total of 1088 bowel cancer files were reviewed (Familial Adenomatous Polyposis families were in a separate register). The numbers of referral showed a 9-fold increase over the 10 years. There was a 67% to 33% female to male ratio and a 23% to 77% affected to non-affected (relatives) individuals. The majority of referrals were from Hospital Surgical departments (40%) and General Practitioners (35.2%). 18% of families met the Amsterdam or Modified Amsterdam criteria, 65% families were classified as moderate risk and 17% as low risk. 42 families have been identified with mismatch repair mutations. 60% of referred individuals were recommended to have regular colonoscopy ranging from 18 monthly to 5 yearly depending on their risks.
Conclusion: There has been near 10 fold increase in individuals with suspected hereditary bowel cancer referred to the regional genetics service in the last ten years. After verification of family history, 60% of individuals were recommended to undergo screening colonoscopy. The increase referral numbers is mostly likely to be due to cancer genetic awareness among healthcare professionals.

P030. MLH1 and MSH2 rearrangements in UK HNPCC patients.

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The importance of genomic rearrangements of MSH2 and MLH1

in the aetiology of HNPCC is becoming increasingly apparent. We have screened a total of 215 UK patients referred with a strong family history of colon cancer by Clinical Genetics departments. Point mutation screening by FSSCP and DHPLC is ongoing in this panel; results to date are presented. To assess the level of genomic rearrangement, we have used a newly described technique, multiplex ligation-dependent probe amplification (MLPA) [1,2].
Our data show that rearrangements of MSH2 and MLH1 account for a significant proportion of the total HNPCC mutations identified to date in our population. The frequency of rearrangements in our patient panel is approximately 5 % (12/215). In accordance with published studies, we find that deletion of one or more exons accounts for the overwhelming majority of rearrangements identified. Finally, in contrast to the published literature, in which MSH2 rearrangements represent more than 70% of the total, we have observed an equal distribution of rearrangements between MLH1 and MSH2.

1 Gille et al., 2002 British Journal of Cancer 87 892
2 Schouten et al., 2002 Nucleic Acids Research 30 e57

	MSH2	MLH1
Point mutations identified to date	15	21
Of which missense (known pathogenic, or novel, non-conservative changes)	2	8
Rearrangements	6	6

P031. Evaluation of referral criteria and screening procedures in the identification of HNPCC patients

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Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominantly inherited cancer predisposition leading mainly to the development of colorectal carcinoma (CRC) at an average age of 45 years. In 60-70% of HNPCC kindreds the disease is caused by germline mutations in one of the DNA mismatch repair (MMR) genes, *hMSH2, hMLH1, hMSH6, hPMS2, hPMS1*. Inactivation of the MMR system through mutation of one of its components consequently leads to genomic instability.
This study investigates 98 unrelated Swiss patients referred to the Medical Genetics department due to an observed familial clustering of CRC or young age at diagnosis of CRC.
The aims are a) assess the diagnostic value of different referral criteria, b) evaluate sensitivity/specificity of various mutation detection methods, c) compare diagnostic accuracy of microsatellite instability (MSI) testing and immunohistochemistry (IHC) and d) determine pheno-/genotypic differences between MMR mutation-positive and -negative HNPCC patients.
Of the referred patients, 34.7% fulfilled the Amsterdam Criteria (AC), 37.7% the Bethesda Guidelines (BG) and 27.6 neither criteria (NC). MSI status, using 10 microsatellite markers, was established in 89% of the tumours; 35.5% MSI-High, 6% MSI-Low,58.5% MS-Stable. Fifty-two percent of MSI-High tumours stemmed from the AC, 39% from the BG and 9% from the NC group ($p<0.01$). IHC identified 83% of tumours negative for either *hMLH1* or *hMSH2* whilst in 17% of the tumours all MMR proteins were present.
We present here an extensive and critical evaluation of HNPCC prescreening and mutation analysis procedures, aswell as genotype/phenotype comparisons in relation to MMR gene mutation status.

P032. Detection of a novel MLH1 gene mutation in a large Bulgarian pedigree with HNPCC

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Hereditary nonpolyposis colorectal cancer is a dominantly inherited cancer syndrome caused by germline defects of mismatch repair (MMR) genes, in particular *MLH1*, *MSH2* and *MSH6*.
We screened for mutations in the *MLH1* gene in our collection of 22 patients with previously detected microsatellite instability (MSI). The exons of the *MLH1* gene with their corresponding exon- intron boundaries were analysed by PCR/SSCP methods. Whenever an abnormal SSCP pattern was observed, direct sequencing was performed with an ABI Prism 310.
We found one novel frameshift mutation in codon 11 (delC at 31) in exon 1 of the *MLH1* gene in a large Bulgarian pedigree with colorectal and endometrial manifestations of cancer. Our clinical findings and the early age of diagnosis (in the fourth generation between the age of 20 and 27) suggest the severity of this defect or the possible role of a second hit in these cases.

P033. A simple method to screen for exonic deletions and duplications in HNPCC genes

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Hereditary nonpolyposis colorectal cancer (HNPCC) is the major familial syndrome predisposing to colorectal cancer (CRC).
The mutations identified in HNPCC cases are important in our understanding of inherited CRC. Mutations in the mismatch repair genes (MMR) result in microsatellite instability (MSI) and development of CRC. At least five genes have been detected as MMR genes and mutations in the MLH1 and MSH2 genes account for the majority of HNPCC cases. An increasing number of deletions in different exons of these genes is reported to result in HNPCC but conventional methods usually miss large genomic deletions or duplications. Multiplex amplifiable probe hybridisation (MAPH) is a simple DNA-based method to measure copy number alterations. HNPCC MAPH assay uses a set of 38 probes (16 from MSH2, 19 from MLH1, 3 controls from X, Y chromosomes and a non-human probe). Unaffected controls (n= 73) showed reproducible results approximating to a normal distribution. Genomic DNA from 54 HNPCC samples was screened in a blind test. We identified an exon 13 deletion of the MLH1 gene in 4 related patients and independent deletions of exon 1, 3, 8 and 9-16 of the MSH2 gene. The HNPCC MAPH assay can be used to screen genomic DNA from MSI+ HNPCC patients without a known point mutation for any exonic copy number alteration.

P034. The multiple colorectal adenoma phenotype, familial adenomatous polyposis and germline mutations in MYH

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Germline mutations in the base excision repair gene MYH are associated with autosomal recessive inheritance of multiple colorectal adenomas displaying excess somatic G:C to T:A transversions in the APC gene.
We screened for germline MYH mutations in 152 patients with multiple (3-100) colorectal adenomas and 107 probands with classical familial adenomatous polyposis (>100 adenomas).
Six multiple adenoma and eight polyposis patients harboured bi-allelic germline MYH variants, missense and protein-truncating, of which Y165C and G382D were the most frequent; mutation spectra were similar in both groups. In bi-allelic mutation carriers, all somatic APC mutations detected in tumours were G:C to T:A transversions.

Approximately one-third of patients with more than 15 adenomas had bi-allelic MYH mutations. None of these patients with mutations had severe polyposis (>1000 adenomas); three had extra-colonic disease (upper-gastrointestinal tumours and congenital hypertrophy of the retinal pigment epithelium).
This has significant clinical importance, and management of cases should take account of the high risk of multiple colonic adenomas and cancer risk.

P035. Pathogenicity of the HNPCC mutation *hMLH1* del616 linked to shortage of the functional protein

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Hereditary nonpolyposis colorectal cancer (HNPCC) is associated with mismatch repair (MMR) deficiency. Most predisposing mutations prevent the production of functional MMR protein. Thus, when the wild type copy, too, is inactivated, the cell becomes MMR deficient leading to high degree of microsatellite instability (MSI) in tumors. However, tumors linked to nontruncating mutations may display positive or partly positive immunohistochemical staining of the mutated protein and low or atypical MSI status, suggesting impaired functional activity but not total lack of MMR. We describe here expression and functional analyses of *hMLH1* del616, one of the most widespread recurring mutations in HNPCC, which was found to segregate in a large HNPCC family with some atypical molecular characteristics. Genetic and immunohistochemical evidence supported *hMLH1*-linked cancer predisposition in the family. Remarkably, mononucleotide repeats, but not dinucleotide repeats were unstable, and the hMLH1 protein was still partly detectable in tumor tissue. Whereas similar optimal amounts of mutated hMLH1 del616 and wild type hMLH1 proteins were equally functional in an *in vitro* MMR assay, the amount of *in vitro* and *in vivo* expressed hMLH1 del616 protein was much lower than the amount of wild type protein, suggesting that the deletion imparts instability on the mutant protein. Our results suggest that pathogenicity of *hMLH1* del616 is not linked to nonfunctionality but shortage of the functional protein.

P036. HNPCC mutation *MLH1* P648S makes the functional protein unstable and predisposes to neurofibromatosis type 1 as a homozygote

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Hereditary nonpolyposis colorectal cancer (HNPCC) is characterized by a dominantly inherited predisposition to early onset cancer and is linked with malfunction of postreplicative DNA mismatch repair (MMR). An inherited mutation in one MMR gene allele and an acquired defect in the other allele lead to hypermutability, which accelerates tumor progression. Rarely, a family member can also inherit the same MMR gene mutation from both parents with the result that MMR is deficient in all cells. To date, four HNPCC families have been reported to carry such a homozygous child. Remarkably, the children who have inherited MMR deficiency are diagnosed with features of neurofibromatosis type 1 (NF1) syndrome, although, their families have no previous history of NF1. This suggests that *NF1* is a target gene in MMR deficient cells. We describe here a novel MMR gene mutation, *MLH1* P648S, which segregates with HNPCC syndrome in a big family with one homozygous child for this mutation. Also this child was diagnosed to have features of NF1 syndrome. To evaluate the pathogenicity of an amino acid substitution P648S, the expression and functionality of the mutated protein was studied. Our results show that the mutated MLH1-P648S protein is unstable but functional in an *in vitro* MMR assay suggesting that cancer susceptibility in this family is linked to shortage of the functional protein.

P037. Is Prostate cancer part of the Lynch syndrome (HNPCC) tumor spectrum?

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The spectrum of tumors classically observed in Lynch syndrome (HNPCC, or Hereditary Non-Polyposis Colorectal Cancer) includes colorectal, uterine, ovarian and urological tumors (ureter, renal pelvis). We report here a case which suggests that prostate cancer should be included in the HNPCC tumor spectrum.
The proband, who had survived three metachronous adenocarcinomas of the colon and rectum, succumbed to an adenocarcinoma of the prostate, diagnosed at age 61. Immunohistochemical staining of colonic, rectal and prostatic tumor tissues demonstrated lack of expression of MSH2 and MSH6 proteins, and microsatellite instability (MSI-H) was observed in all three tumors.
Molecular investigations of this family were initiated when the proband's son presented with an adenocarcinoma of the colon at age 35; at this point the family met the Amsterdam criteria for HNPCC. Southern blotting analysis of genomic DNA led to the identification of a novel genomic deletion encompassing exon 5 of the hMSH2 gene. Although prostate cancer has anecdotally been described in families with Lynch syndrome, this appears to be the first report where the MSI and IHC analysis of the prostatic tumor clearly link its etiology to the germline mismatch repair mutation.

P038. hMSH2 gene dosage analysis by quantitative real-time PCR

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Hereditary non-polyposis colon cancer (HNPCC) results from mutations within mismatch repair genes responsible for the maintaining DNA sequence fidelity. Six genes conferring susceptibility to HNPCC have been identified with the majority of disease causing mutations found within hMSH2 and hMLH1. Up to 50% of hMSH2 mutations are thought to be hemizygous where single or multiple exons are present in only one copy. Such dosage DNA deletions are undetectable using routinely employed screening methods.
A fluorescent based quantitative real-time PCR assay has been developed to determine the exon copy number for hMSH2 and hMLH1. Exon copy number is determined by comparing the fluorescence generated during the exponential phase of amplification of individual exons to that of the control standard β-globin. PCR amplification conditions for all hMSH2 and hMLH1 exons have been optimised for the use of SYBR green quantification. To compensate for differing PCR efficiencies amplification co-efficients were established to enable accurate quantification relative to β-globin. Analysis of four DNA samples from HNPCC patients negative by routine mutation screening identified partial hMSH2 deletions. This simple technique rapidly identifies dosage DNA gene deletions responsible for HNPCC.

P039. Determination of APC mutations of young-age colorectal cancer patients and their first-degree relatives

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The APC gene encodes a large protein with multiple cellular functions

and interactions, including roles in signal transduction in the wnt-signaling patway, mediation of the intercellular adhesion, stabilization of the cytoskeleton and possibly regulation of the cell cycle and apoptosis. The APC gene which is a tumor suppressor gene plays a crucial role in both FAP formation and sporadic or familial colorectal carcinogenesis.
In this study, we evaluated 40 different Turkish families which have an early age colorectal cancer history. For this reason, genomic DNAs were isolated from pheripheral blood sample of 145 subjects including 27 affected from colorectal cancer at under 45 years old and 118 uneffected first-degree relatives of young-age colorectal cancer patients. Both mutation cluster region (MCR) and D,E, F segment of exon 15 of APC gene were studied by heterodublex analysis (HDA) and non-radioactive silver staining method.
We determined mutation points in three of these families (7.5%). These points were localized at F segment instead of MCR region of exon 15 of APC gene.
Our results suggest that these type studies will be beneficial for both determination of new mutation point of APC gene in different nations and genetic susceptibility studies related with colorectal cancer in these populations.

P040. Partial and whole gene deletions of *APC* represent 15 % of all pathogenic germline mutations in patients with Familial Adenomatous Polyposis

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Germline mutations of the tumour suppressor gene *APC* are responsible for Familial Adenomatous Polyposis (FAP). Most mutations are truncating and spread over the coding region. Rare whole gene deletions or exonic deletions have been described. From a series of 96 patients clinically diagnosed with FAP or attenuated FAP in our Center, 29 (30 %) were found to have truncating or missense mutations. We have now screened the remaining 67 patients for exonic deletions or duplications by semi-quantitative PCR. The whole coding region and promoter were screened in 3 multiplex reactions. Four whole gene deletions and one exon 14 deletion were found (5 %). The whole gene deletions were confirmed by FISH analysis. Fine mapping was performed using extragenic polymorphic markers and/or semi-quantitative PCR. The deletions encompassing *APC* are large (400 to 700 kb) and were not described previously. For two of them, the 3' breakpoint lies in the *MCC* gene. Interestingly, the smallest of these deletions is associated with attenuated FAP, while the largest is associated with classical FAP with mild mental retardation. The deletion of exon 14 in one family with classical polyposis has been confirmed by long-range PCR; the deletion encompasses the entire exon and approximately 2 kb of flanking sequences. Recombination of Alu elements could be involved in the origin of the deletion. In summary, these newly identified defects represent 15 % (5 out of 34) of all pathogenic mutations of the *APC* gene. Thus, screening for genomic rearrangements should complement routine screening for truncating mutations.

P041. Cronkhite-Canada syndrome-like habitus and APC gene codon 1309 germ-line mutation

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Familial adenomatous polyposis (FAP) is an autosomal dominant inherited disease, affecting 1 in 2000 people. 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 progress to cancer if left without surgical treatment. FAP is caused by mutations in the tumour suppressor gene APC. Cronkhite-Canada syndrome is not inherited disease and with the juvenile-type of polyposis have no malignant potential. Here we report one patient (18-year-old female) affected by FAP with Cronkhite-Canada syndrome features (alopecia, cutaneous hyperpigmentations, arachnodactylia, dystrophic changes in the fingernails). We also examined the molecular genetic changes of APC, K-ras and p53 genes in the epithelium of patient's tubular adenoma.
DNAs were isolated from peripheral blood of patient and from the microdissections of deparaffinized tissue sections. Each microdissection was first preamplified by using primer extension preamplification (PEP) method. PCR was performed using specific pairs of primers. PCR products were analysed by heteroduplex, RFLP, LOH analysis and sequencing.
The genetic analysis confirmed the APC gene codon 1309 germ-line mutation and the diagnosis of FAP in this patient as a first case in her family. By using microdissection and PEP-PCR we were able to prove germline mutation and LOH of the APC gene. K-ras gene codon 12 and p53 gene exon 7 mutation were detected as well.
This results show the importance of genetic analysis in the differentiation of polyposis syndromes and evaluation of the risk for development of colon cancer.

P042. APC gene mutations and haplotypes interconnection in Russian patients with familial adenomatous polyposis

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All exons of the *APC* gene were screened in a sample of 37 patients with familial adenomatous polyposis (FAP) and 22 control individuals. There were 24 mutations among patients (65%). Twelve mutations were revealed for the first time. Such a high proportion of new mutations may be suggestive of a *de novo* origin of a significant part of *APC* mutations. Only the frequent European mutations 1309del5 and 1061del5 were found recurrent in Russia. By SNP analysis we showed that polymorphisms 1458C/T, 1635A/G and 4479G/A are in linkage disequilibrium and characterize a haplotype (named B). The consensus sequence defines the most frequent haplotype A. There were three other haplotypes. Four of five 1309del5 mutations were found on haplotype A and one on haplotype B. The presence of haplotype A in genomes with non-recurrent mutations was significantly less than in genomes with recurrent mutations ($P < 0.05$). The proportion of haplotype A in genomes with non-recurrent mutations was decreased nearly twofold in comparison with the control group. These results may suggest a linkage of different mutation types (recurrent and non-recurrent) with different haplotypes and, possibly, an origin of non-recurrent mutations predominantly on haplotype B. Alternatively, the observed difference of the haplotype frequencies may be connected with heterogeneity of the sample with the same clinical characteristics. It should be noted that the haplotype distribution in the group of patients with FAP but without mutations was the same as in the control group.

P043. Variant D1822V of the APC gene modifies the protective effect of NSAID consumption on colon cancer development

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Regular NSAIDs intake reduces CRC risk. COX-2 inhibitors may prevent adenoma formation in FAP. Germline variants in the APC or COX-2 genes may influence NSAIDs effect. **Aim:** To assess whether the APC gene D1822V variant and two novel COX-2 variants influence NSAIDs effect in CRC development. **Population:** 302 cases and 297 controls belonging to a hospital-based case-control study performed between 1996 and 1998 at Hospital of Bellvitge. Epidemiological and dietary questionnaire was delivered. **Methods:** Genotype was assessed using the LightCycler. Heterozygous (AT) and homozygous (TT) allele were analysed as a single group. COX2 (D28235-nt1629 and D28235-nt5209) and NFKB (4098-RS59) variants were assessed. Multivariate methods based on logistic regression analyses were used. Alleles were in Hardy-Weinberg

equilibrium in cases and controls. **Results:** No increased risk was observed between the variant allele and cancer risk. The protective effect of NSAIDs consumption decreased with allele T of D1822V (OR=0.84 [95%CI 0.33-2.14] vs allele A OR=0.28 [0.13-0.59]).
We found a complex interaction between polymorphism in COX-2 (D28235-1629; D28235-5209), NFKB1 (4098-RS59) and D1822V APC variant. Individuals with allele G of D28235-1629 in combination with 1822V, and individuals with allele A of NFKB plus 1822V have higher risk of development CRC. However individuals homozygous GG to D28235-5209 with 1822V are protected.
Conclusion: Variant D1822V at the APC gene is a significant modifier of the protective effect of NSAIDs in colon cancer development. This interaction is further modified by genetic variants of prostaglandin-mediated pathways.

P044. Germline TP53 mutations in families of childhood cancer patients

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Germline TP53 mutations have been found in families with high incidence of cancer diagnosed as the Li-Fraumeni syndrome (LFS). Only about two hundred families suffering from LFS have been described in the world, with germline TP53 mutations found in about 70% of them. Members of LFS families are affected mainly by soft tissue sarcomas, osteosarcomas, breast cancer and brain tumours. The frequency of childhood cancer is also very high. The possibility of reliable presymptomatic molecular genetic diagnostics in the absence of efficient tumour prevention and therapy is a serious ethical problem.
In our presentation we demonstrate several pedigrees of childhood cancer patients suffering from typical tumours and germline TP53 mutations found in these families. Generally, mutations in the TP53 gene are predominantly missense and very heterogeneous. The families have therefore been analysed by automated DNA sequencing of the TP53 gene and/or by the Affymetrix DNA chip technology. We also discuss some problems of genetic counselling and subsequent health care in the affected families. Based on the analysis of our web database of published germline TP53 mutations (www.lf2.cuni.cz/projects/germline_mut_p53.htm) we point to specific tumour types in childhood carriers of germline TP53 mutations and to the possibility of anticipation in this disorder. In addition to adrenocortical tumours, choroid plexus tumours seem to be particularly strong indicators of a germline TP53 mutation. Supported by IGA MZ ČR NC/6513-3.

P045. Clinical characteristics and genetic screening of two Greek Cypriot families with a germinal RET proto-oncogene mutation.

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Multiple Endocrine Neoplasia type 2A (MEN 2A) and familial medullary thyroid carcinoma (FMTC) are two closely related cancer syndromes inherited in an autosomal dominant manner. Mutations in the RET proto-oncogene are responsible for causing both MEN 2A and FMTC. In this study, two patients from two unrelated Greek Cypriot families were respectively diagnosed with MEN 2A and FMTC. The clinical diagnosis of the two patients was based on the calcitonin levels and the CEA tumor marker. We have screened the RET gene by direct sequencing of exons 10, 11, and 16 in the two probands. In both patients we identified the C618R germline mutation of exon 10. As an alternative method to direct sequencing we also developed the amplification refractory mutation system (ARMS) for the detection of TGC to CGC of codon 618. By using ARMS we also performed genetic screening in a total of 18 at risk additional individuals from both families. Two members of the MEN 2A family and five members of the FMTC family were found positive for the C618R missense mutation. These are the first RET proto-oncogene cases identified in Cyprus by molecular testing. Presymptomatic diagnosis of young members has useful implication regarding their future management.

P046. DNA Testing for the Mutations Causing Multiple Endocrine Neoplasia Type 2.

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Medullary thyroid carcinoma (MTC), a neoplasm of the calcitonin-secreting parafollicular thyroid C cells, may occur sporadically or as a component of the inherited cancer syndrome multiple endocrine neoplasia (MEN) type 2. All three clinical subtypes of the MEN 2 syndromes (MEN 2A, MEN 2B and familial MTC (FMTC)) are caused by germline mutations in the RET proto-oncogene which encodes a tyrosine kinase receptor expressed in tissues and tumors of neural crest origin. Since MTC is associated with high mortality, presymptomatic identification of mutated gene carriers by DNA analysis allows earlier identification of subjects at risk in this familial cancer syndrome and provides the basis for preventive thyroidectomy.
MEN-2A and FMTC commonly result from germline missense mutation in one of six cysteine codons in the cysteine-rich extracellular domain of RET. These codons are as follows: 630, 634, 609, 611 618 and 620. MEN-2B is caused by germline mutation in codon 918 (M918T) in more than 95% of cases.
Our purpose was to perform mutation analysis of the RET protooncogene for MEN2 patients and patients with apparently sporadic MTC to exclude the hereditary forms of this tumor. We have revealed 4 different missense mutations in the codon 634 (C634R, C634Y, C634F, C634S) in 23 patients (13 families) and two changes in the codon 620 (C620R and C620Y) in 5 patients (2 families). In 3 families with MEN2-B syndrome the standard germline mutation M918T was detected. We also report about new germline mutation D631Y in the noncysteine 631 codon.

P047. Diagnosis and follow up of a family with MEN IIB syndrome

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We present a family with MEN IIB (multiple endocrine adenomatosis) to illustrate a rare genetic disorder and to present some particularities.
MEN IIB is a form of multiple endocrine adenomatosis associated with dysmorphic features. Most patients have a marfanoid habitus with thickened lips, nodules on the tongue or lips, nervous fibres in the cornea and different endocrine tumours (medullary thyroid carcinoma, phaeochromocytoma or parathyroid tumours). Many cases have point mutations in RET proto- oncogene.
Our proband is a 10 years old male, first child of a young, unrelated couple. His mother died of thyroid cancer. He has a healthy brother. There are no other cases in the family. Pregnancy and birth: uneventful. Postnatal development: normal. He was admitted in the Nephrology Pediatric Clinic due to acute urine retention. Genetic examination remarked the marfanoid habitus with thickened lips and nodules on the tongue and lips. Ophtalmologic examination revealed nervous cornean fibres. Endocrine investigations diagnosed thyroid cancer, with successive surgery and hormonal replacement. The next 4 years of evolution will be presented
In conclusion, we present this case to illustrate a rare genetic

disorder, to present some particularities in the evolution of this case and to discuss the importance of early diagnosis.

P048. The prevalence of germline mutations in families at risk of melanoma in England.

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In England 5% of melanoma patients give a family history. In a small proportion of these, multiple cases occur, indicative of autosomal dominant pattern of inheritance. These families may or may not have an abnormal naevus phenotype known as the AMS. In England there is little evidence from pedigrees to suggest an increased susceptibility to other cancers in the majority. Very rare families appear to have an increased risk of CNS tumours and this syndrome appears to be linked to deletions of chromosome 9.
We have screened 63 three or more case families and 68 two case families for germline mutations in CDKN2A, p14ARF and CDK4. The majority of families with 4 or more cases have CDKN2A mutations in the UK. The proportion of families with 2 or 3 cases is much lower. CDKN2A therefore appears to be the major high penetrance melanoma susceptibility gene in the UK, but there are others yet to be identified. Germline mutations in p14ARF appear to have a role in susceptibility to melanoma.

Number of germline mutations by cases in the family			
5 or more case families	12/16 germline mutations	75%	11 CDKN2A, 1 putative p14ARF deletion
4 case families	9/18 germline mutations	50%	9 CDKN2A
3 case families	8/28 germline mutations	29%	6 CDKN2A 2 p14ARF
2 case families	9/68 germline mutations	13%	8 CDKN2A 1 probable p14ARF

P049. Familial Melanoma: Absence of exon 15 *BRAF* germline mutations

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Melanoma is the most lethal form of skin cancer with 3-5% of cases arising in a familial setting. 20-30% of these families carry germline *CDKN2A* mutations. Recently Davies et al., 2002 reported for the first time *BRAF* mutations in melanoma. *BRAF*, situated on chromosome 7q34 encodes a serine/threonine kinase. Its normal function is to control proliferation and differentiation through the melanocyte-specific MAP kinase pathway. We have analysed DNA from peripheral blood of 42 cases of familial melanoma from 39 families, for germline mutations in exon 15 of the *BRAF* gene. We were particularly interested to see if mutations were present in our families with no evidence of *CDKN2A* mutations. Sequence analysis was carried out using an ABI3100. No exon 15 *BRAF* mutations were found in *CDKN2A* mutation positive families or in *CDKN2A* negative families. DNA from 2 samples of secondary melanoma from these families also failed to show exon 15 *BRAF* mutations. In addition, we have searched for exon 15 *BRAF* mutations in 24 samples of secondary melanoma from sporadic cases and detected the mutation 1796T>A in 6/22 (27%) of the secondary melanomas. 1796T>A leads to a substitution of valine by glutamic acid (V599E) in the kinase activating domain, leading to constitutive kinase activity. Peripheral blood DNA from 2 of these tumour positive cases of sporadic melanoma were however negative for the V599E *BRAF* mutation. We are currently investigating the exon 15 *BRAF* mutation status of primary melanoma tumour samples with a view to comparing with the published data.

P050. Differential gene expression induced by adenovirus-mediated p16 gene transfer into Balb/c nude mouse

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For the safety evaluation of adenovirus-mediated gene transfer, we investigated differential gene expression after transduction of adenoviral vector with $p16^{INK4a}$ tumor suppressor gene (Ad5CMV-p16) into Balb/c nude mouse. We conducted large-scale preparation of adenovirus-based vectors (Ad5CMV-p16, Ad5CMV, Ad5CMV-LacZ) lacking RCA (replication competent adenovirus) and mycopalsma as contaminants. Adenovirus-mediated $p16^{INK4a}$ gene transfer resulted in significant inhibition of tumor growth in Balb/c nude mouse with non-small cell lung cancer. We investigated genes whose expressions were changed after intratumoral injection of Ad5CMV-p16, Ad5CMV (10^{10}pfu) into non-small cell lung cancer (A549) cell xenografted Balb/c nude mouse by cDNA microarray. In comparison with serum-free medium treated group in tumor tissue, Ad5CMV-p16 up regulated 21 genes including cell cycle related genes (cyclin D, cyclin E, p27, Rb), cell adhesion related genes (integrin a4, integrin b1, integrin b3, IGF1), BRCA 1, BRCA 2, c-fos, and c-jun. We are currently confirming several gene expressions by RT-PCR. Taken together, we have to consider the potential effects of the other gene expressions except therapeutic gene on the host cells as a safety concerns.

P051. Analysis of mutations in the p16/CDKN2A gene in cutaneous malignant melanoma/pancreatic cancer families in the Czech population.

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Mutations in CDKN2A gene have been described in cutaneous malignant melanoma, familial malignant melanoma and pancreatic cancer associated with melanoma. The aim of our study was to evaluate the prevalence of mutations in the CDKN2A gene encoding p16 and p14 proteins in cutaneous malignant melanoma/pancreatic cancer families from Western Bohemia region. All individuals enrolled in the study were genetically counseled, three-generation pedigree was constructed and informed consent obtained. Eleven families with at least two first-degree relatives with either cutaneous malignant melanoma or cutaneous malignant melanoma/pancreatic cancer cases were analysed for the presence of CDKN2A germline mutations by TGGE, FRET-PCR based melting curve analysis, and direct DNA sequencing. The results obtained by different methods are discussed, suggesting that all members of cutaneous malignant melanoma/pancreatic cancer families should be counseled and offered screening for p16 mutations. The study was supported by GACR 301/02/1232 and MSMT VZ 111 40005 (6035) grants.

P052. Clinical genetic analysis of familial pancreatic cancer

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Pancreatic cancer (PC) is the fifth leading cause of cancer-related mortality with a very poor prognosis. The only recognized environmental risk factor is cigarette smoking. It is estimated that 3-5% of PC-cases are caused by a genetic predisposition. An 18 to 53-fold risk has been reported among first degree relatives of PC patients in pancreatic cancer families (FPC). To investigate the inherited components in PC we started to enroll families with FPC and with pancreatic cancer-melanoma syndrome (PCMS) in the German Familial Pancreatic Cancer Case Collection (FaPaCa). In a total of 40 families the spectrum of cancers and diseases was analysed. A combination of PC and breast cancer was found in five (12,5%) families. Up to date, BRCA2 germ-line mutations have been identified in two out of 14 FPC-families. PC and malignant melanoma was present in six (18.8%) families. A CDKN2A germline mutation was found in 2 of 5 PCMS and in none of 18 FPC families. We observed the joint occurrence of PC and BCC in three (7,5%) families. A fourth family demonstrated with multiple BCC and PC. A familial accumulation of PC and BCC has not been described as yet. Thus, the joint occurrence of PC and BCC may represent a new

hereditary tumor predisposition syndrome. We conclude that the clinical genetic analysis in pancreas cancer including genetic testing may help to identify high risk family members. These individuals may be subjected to surveillance programs for early detection of PC to reduce the mortality of this cancer.

P053. Multiple deletions and losses of chromosom 11 lead to oncogenesis of sporadic paragangliomas

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Paragangliomas of the head and neck region are usually benign and slow-growing tumours developing from chemoreceptors of paraganglionic origin which are important for sensing and regulation of the gas levels and pH values in the blood. The candidate gene for paragangliomas, *SDHD*, has been mapped to the PGL1 locus in 11q23. It encodes a part of the anchor subunit of the mitochondrial complex II which is involved in respiratory chain as well as in citric cycle reactions. Frequent somatic deletions of the distal part of chromosome 11 including the PGL1 region have been demonstrated for familial cases with paragangliomas. Therefore, we analysed normal and tumour DNA of six patients with sporadic paragangliomas for loss of heterozygosity (LOH), usually connected with oncogenesis of various tumours. LOH experiments were performed applying a set of polymorphic microsatellite markers covering the entire chromosome 11. All examined tumour samples revealed LOH on chromosome 11. To verify the LOH results fluorescence *in situ* hybridisation (FISH) was performed on paraffin-embedded normal and tumour tissues of the same patients using chromosomal centromere-specific DNA as probes. Subsequent FISH analyses confirmed partial or total monosomy for chromosome 11 in all tumour samples. Elucidation of the genetic regions involved in tumour development is a basis for understanding their contribution to normal and pathogenetic cell physiology.

P054. 'Epigenetic inactivation of SDHB by promoter region hypermethylation in phaeochromocytoma and neuroblastoma'.

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Germline mutations in the succinate dehydrogenase subunit B (SDHB) gene cause susceptibility to phaeochromocytoma and / or head and neck paraganglioma. SDHB map to 1p35-36, a common LOH region in phaeochromocytoma and neuroblastoma. We investigated whether (a). SDHB promoter region methylation might cause somatic SDHB inactivation in phaeochromocytoma and (b). whether somatic mutations or epigenetic inactivation of SDHB was a feature in neuroblastoma. LOH analysis in 36 sporadic phaeochromocytoma using 14 polymorphic markers (1p22-36) showed 75% of tumours with LOH and high level of LOH were detected at markers close to SDHB. Using methylation specific PCR (MSP) assay, we detect partial promoter region hypermethylation in two neuroblastoma cell lines and treatment of the cell lines with 5-azacytidine produced a twofold increase in SDHB protein expression. SDHB promoter region hypermethylation was also detected in 31% (9/29) phaeochromocytoma and in 22% (10/46) neuroblastoma tumours. No SDHB mutations were detected in 46 neuroblastoma tumours analysed. This findings suggest that epigenetic inactivation of SDHB is implicated in the pathogenesis of a subset of phaeochromocytoma and neuroblastoma tumours.

P055. CGH reveals multiple chromosomal changes in patients with non-familial paragangliomas

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Paragangliomas of the head and neck region display a group of rare, usually benign, slow growing tumors developing from paraganglionic chemoreceptors in the majority of patients. Recently, mutations in a subunit of the mitochondrial enzyme II complex gene (succinate dehydrogenase, subunit D, SDHD) have shown to be connected with the formation of paragangliomas. In addition, LOH on chromosome 11, mainly on 11q23, was observed by several groups. Screening for SDHD mutations in our patient collective containing 20 non-familial cases indicated that a large proportion of patients did not carry a mutation within this gene. Therefore CGH (comparative genomic hybridization) analysis was performed to determine whether additional genomic regions are affected by chromosomal alterations. These studies revealed multiple changes in tumor samples. In detail, common losses on 1q, 11p, 11q, Xp, and Xq were observed whereas chromosomal regions on 4p, 4q, and 9q frequently displayed gains of DNA. These results suggest the involvement of additional loci besides the already described regions on chromosome 11, which has been demonstrated to play a significant role in the development of paragangliomas.

P056. Molecular analysis of familial cases of renal cell cancer

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To date, eight families have been described in the literature in which constitutional chromosome 3 translocations segregate with a predisposition to the development of renal cell cancer (RCC). Through a multi-center study we have identified several additional families with RCC and chromosome 3 translocations. Previously, we found that loss of the derivative chromosome carrying the 3p arm and subsequent VHL mutations constitute critical steps in the development and progression of these RCCs. In addition, we hypothesized that genes located at or near the translocation breakpoints may be related to tumor initiation (Bodmer et al., 2002). Therefore, we have set out to positionally clone the translocation breakpoints and to characterize them in detail. In one of our RCC families with a t(2;3)(q35;q21) translocation, two novel breakpoint spanning genes were identified. The breakpoint-spanning gene on chromosome 3, named *DIRC2*, encodes a putative membrane-spanning protein belonging to the major facilitator super family (MFS) of transporters. The gene disrupted by the breakpoint on chromosome 2, named *DIRC3*, forms fusion transcripts with *HSPBAP1*, a JmjC-Hsp domain gene that maps proximal to the breakpoint on chromosome 3. Currently, we are characterizing these (fusion) genes in detail at the cell biological and functional level. Together with known and novel genes to be identified by us and others, these studies should lead to a unified model for hereditary RCC development in families with chromosome 3 translocations.

Reference:
Bodmer et al., Human Molecular Genetics 11: 2489-2498 (2002)

P057. Methylation profiling of Wilms' Tumour and Adult Renal Cell Carcinoma

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We determined the methylation profile of Wilms' tumour and RCC using a candidate gene approach. 40 Wilms' tumours and up to 49 adult RCC were analysed by methylation-specific PCR for promoter methylation at *CASP8*, *CDH1*, *CDH13*, *DAPK*, *MGMT*, *p14^{ARF}* and *RARB* in primary Wilms' tumours and *CDH1, CDH13, DAPK, MGMT, p16^{INK4a}* and *RARB* in primary RCC. Wilms' tumours demonstrated a high incidence of methylation at *CASP8* (43%) and *MGMT* (30%), intermediate frequencies at *p14^{ARF}* (15%), ^{p16INK4a} (10%) and *DAPK* (11%), but promoter methylation was rare or absent at *RARB* (0%), *CDH13* (0%) and *CDH1* (3%). No association was detected between methylation of *RASSF1A*, *CASP8* or *MGMT* in individual tumours. The frequency of *MGMT* methylation was higher in stage 1 and 2 tumours (50%) than in later stage tumours (17%), but this did not reach statistical significance (*P=0.06*). RCC were most frequently methylated at *DAPK* (24%), *CDH1* (16%), and *MGMT* (9%) and not or rarely at *RARB* (0%), *p16^{INK4a}* (0%), and *CDH13* (3%). There were no associations between methylation of *RASSF1A*, *DAPK* and *CDH1* in individual tumours. Papillary RCC demonstrated a higher frequency of *DAPK* methylation (43%) than clear cell tumours (19%) (*P=0.14*). We have demonstrated that promoter methylation is frequent in Wilms' tumour and RCC, and this data enables methylation profiles to be constructed for each tumour type. Our results, with data published previously, indicates that promoter methylation occurs frequently (≥20% of primary tumours) at *CASP8* and *RASSF1A* in Wilms' tumour and at *RASSF1A*, *TIMP3*, *DAPK*, and *GSTP1* in RCC.

P058. FIH-1 mutation analysis in sporadic Renal Cell Carcinoma and Gliomas

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Overexpression of HIF-1 and HIF-2 transcription factors, and a consequent upregulation of VEGF expression and other hypoxia-inducible mRNAs, is a feature of many human cancers. HIF upregulation in human cancers may be unrelated to tissue hypoxia. Thus the *VHL* tumour suppressor gene (TSG) regulates HIF-1 and HIF-2 expression in normoxia by targeting the alpha subunits for ubiquitynilation and proteolysis. Inactivation of the *VHL* TSG in VHL tumours and in sporadic clear cell renal cell carcinoma (RCC) results in overexpression of HIF-1 and HIF-2. However, not all RCC have VHL inactivation, and in other cancers with HIF overexpression (e.g. gliomas), somatic *VHL* mutations are not found. Recently FIH-1 (factor inhibiting HIF) was reported to interact with HIF-1a and VHL and to repress HIF-1a transcriptional activity. We hypothesised that mutations in FIH-1 might result in HIF dysregulation. To test this hypothesis, we analysed 35 sporadic gliomas and 15 clear cell RCC without VHL mutations for evidence of FIH-1 mutations. We detected a SNP in exon 1 of both glioma and RCC samples. This SNP was also present in 45% of the normal samples. To date, we have not been able to implicate FIH-1 inactivation in the pathogenesis of RCC or human glioma, however epigenetic inactivation has not been excluded and FIH-1 inactivation may be relevant in other types of cancer

P059. Expression of PAX5, p53 immunohistochemistry and p53 mutation analysis in superficial bladder carcinoma tissue

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Clinical prognostic factors contributing to recurrence and progression of bladder transitional cell carcinoma (TCC) have been widely investigated. These factors however are unable to predict the individual course of the disease. For these reasons, new methods are necessary to identify those patients who are at risk for development of recurrent and invasive carcinoma. Therefore the expression pattern of the PAX5 gene in the superficial TCC, its prognostic value and its correlation with p53 immunohistochemistry and mutation analysis were evaluated. Study comprised 61 patients with histologically confirmed TCC. PAX5 expression was evaluated using the RT-PCR and determined semiquantitatively. The presence of p53 mutations was determined by the SSCP and confirmed by direct sequencing. The p53 immunohistochemistry was performed with DO1 antibody and evaluated using HSCORE (HS) method. PAX5 overexpression was found in 50 patients with superficial TCC. Its quantity however correlated neither with the stage nor with the grade of the tumour. p53 mutation was confirmed only in 1 patient with pTaG2 tumour in exon 5 (deletion of proline 128). On the contrary, positive immunohistochemical staining of p53 was detected in most patients. Quantity of p53 immunochistochemical positivity did not correlate with the quantity of PAX5 expression. Using the cutoff values, 7 of 8 patients with future progression had p53 and 4 had PAX5 overexpression, respectively. In conclusion, our data indicate that the expression of the PAX5 gene is a frequent event in superficial bladder TCC and might be used as diagnostic tool. Supported by the grant IGA MZ NC/5961-3.

P060. FibroTest™: A Novel Primer Extension Assay For The Detection Of Bladder Cancer Asociated Mutations In The FGFR3 Gene.

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Activating point mutations in the FGFR3 gene have been demonstrated as the causative agent of several genetic skeletal malformations and are highly prevalent in several types of cancer. The expression of a constitutively activated FGFR3 in bladder and cervix tumors provieds the first evidence of an oncogenic role for FGFR3 in carcinomas. Currently, FGFR3 appears to be the most frequently mutated oncogene in bladder cancer. It is mutated in almost 50% of all cases and in >70% of recurrent superficial bladder tumors cases. Detection of FGFR3 mutations in this patient poulation could be fundamental in determining treatment follow up and prognosis of bladder cancer.

The four point mutations in the FGFR3 gene that are most often associated with bladder cancer are: S248C, S249C, S371C and Y373C. To detect these mutations simultaneously, we have developed the FibroTest™ kit, which utilizes the Pronto™ technology, a single nucleotide primer extension reaction, followed by ELISA. This post amplification mutation detection system, benefits from the high specificity in which DNA polymerase incorporates nucleotides into the elongating strand. Results are determined by a standard ELISA reader.

More than 110 bladder carcinoma samples, taken from either fresh biopsies or paraffin embedded blocks were screened for mutations in the FGFR3 gene. Comparison of FibroTest™ with a standard SSCP method on the same set of samples provided identical results. FibroTest™ allows fast and precise testing for these mutations in tumor-derived and body fluid samples and may become an integral part of follow-up, evaluation and treatment of bladder cancer patients.

P061. Detection of genetic alterations in the tumor and tumor border tissues of Lung, using FISH method.

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Genetic alterations on the primary tumor tissues of 24 patients with lung cancer on whom radiotherapy and chemotherapy treatments had not been performed, and surgical borders adjacent to these tumor tissues, were analyzed by FISH. Fresh lung tumor samples were studied from the patients who had undergone surgery. Locus-specific probes for the p53 tumor suppressor gene and c-myc oncogene and centromere-specific probes for chromosome 17 and chromosome 8

on which these genes are located were used. All of the patients had Non Small Cell Lung Cancer (NSCLC) according to histopathological examinations.
Of these patients with NSCLC, p53 deletions were detected in 3 patients, c-myc amplification in 2 patients, monosomy 17 in one patient and trisomy 8 in two patients. A high level of polyploidy was seen in the tumor tissues of 4 patients. When considering total chromosomal losses of chromosome 17 and gains of chromosome 8 and polyploidies, genetical alterations of lung tumors are heterogenous qualitatively and quantitatively. P53 deletion and c-myc amplication were found at low frequency in surgical border tissues. One of these cases had metastasis and the other one had metastasis with recurrence; their life spans are short, suggesting that genetic alterations of surgical border tissues have significance for prognosis of the disease.

P062. Gene expression profiles in leiomyoma and myometrium

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Uterine leiomyomas are benign tumours of the female genital tract that originate, during the reproductive period, from myometrial smooth muscle cells. Leiomyomas have been reported to grow under the influence of ovarian steroids, however their etiopathogenetic mechanisms are still poorly understood. In this study, we employed cDNA microarrays (22.215 human cDNA Affymetrix) to evaluate the possible differences in gene expression patterns of smooth muscle cells derived from leiomyoma and myometrium. Total RNA was extracted from both cell types obtained from three patients. Direct comparison between tumour and normal cell gene expression showed that the three specimens of leiomyoma cells share 24 up-regulated genes and 38 down-regulated genes. Six of the 24 up-regulated genes and 15 of the 38 down-regulated genes are uncharacterised expressed sequence tag (EST). The protein products of the other genes demonstrate a broad range of functional activities. Indeed, their are either component of the extracellular matrix or molecules involved in signal transduction, inflammation processes, cell cycle regulation, apoptosis, energy metabolism, cell migration, cell adhesion and transcription processes. The presence among the up-regulated genes, of both caveolins, CAV1 and 2, that are involved in the modulation of many signal protein including several oncogenes, and the presence, among the down-regulated genes, of GAS1 (Growth Arrest-Specific Gene) that is an integral plasma membrane protein whose expression is linked to growth arrest, are noteworthy. Due to the important functional activities of these three proteins, it seems worth to further investigate their possible role in the pathogenesis of leiomyoma.

P063. Methylation of *MLH1, HIC1, CDKN2A, RB1* genes and microsatellite instability as molecular markers of epithelial dysplasia.

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It has been demonstrated that squamous carcinomas are often associated with human papillomavirus (HPV). However, several lines of evidence suggest that HPV infection alone is insufficient to generate the malignant phenotype and other additional events are required. In this study we investigated whether the aberrant promoter methylation of some tumor suppressor genes is involved in the genesis of cervical squamous carcinomas. To determine the role played by DNA promoter methylation in squamous carcinoma susceptibility, we analyzed 25 samples of cervical epithelial dysplasia for frequency of *CDKN2A, RB1, MLH1, MGMT, HIC1, N33, CDH1*genes aberrant methylation. Morphologically normal cervical mucosa was used as a control. The presence of methylation was determined by multiplex methyl-sensitive PCR. The results of research are shown in the table. Statistical analysis was performed using Fisher's exact test. Since the frequency of *CDKN2A, RB1, MLH1, HIC1* genes methylation in dysplasia samples was significantly different to that in control samples, we conclude that detection of these genes promoter methylation could be used as a molecular marker for early diagnostic of cervical squamous carcinomas.
Using set of three microsatellite markers (D5S107, D5S406, D13S153) we investigated the frequency of microsatellite instability (MSI) as well. MSI is associated with *MLH1* gene inactivation and has been reported as a molecular hallmark of the malignancy and has been reveled in 5 cases.
The follow-up study of the patients has shown that methylation of the *CDKN2A, RB1, MLH1, HIC1* genes in addition to presence of MSI and HPV infection was correlated with squamous carcinoma development.

The frequency of CDKN2A, RB1, MLH1, MGMT, HIC1, N33, CDH1 genes methylation in samples of dysplasia

The tumor suppressor genes	Cervical epithelial dysplasia (%)	Morphologically normal cervical mucosa (%)	P
RB1	70	24	<0.05
CDKN2A	52	19	<0.05
MLH1	61	14	<0.05
MGMT	9	0	>0.05
HIC1	96	71	<0.05
N33	22	5	>0.05
CDH1	13	5	>0.05

P064. Cytogenetic abnormalities in serous papillary adenocarcinoma of the ovary

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Epithelial ovarian tumors are usually mucinous or serous type, affecting nearly 1% of women during their lifetime. They may be regarded as benign, borderline or invasive according to pathological examination. Karyotype of the tumor provide critical information about both the genetic predisposition and the stage of the tumor. Fifteen serous papillary adenocarcinoma samples of different stages were examined cytogenetically. Most common chromosome abnormalities included both numerical and structural abnormalities of chromosomes 1, 3, 6, 7, 8, 11, 21, 22 and X. Karyotypes became more complex, as expected, with the later stages.

P065. Epigenetic Changes of Tumor-Related Genes in Colo-Rectal Cancer

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Methylation of CpG islands on the promoter of tumor suppressor genes inhibits expression of the genes, and may lead to the tumorigenesis. Here we have performed the methylation specific PCR to explore the mechanism of inactivation of tumor suppressor genes-P15, P16, E-cadherin, APC, GSK-3 and SOCS-1 in 200 pairs of sporadic colon cancerous and nearby non-cancerous tissues. In the study, the frequencies of aberrant methylation on the promoter of the P15, P16, E-cadherin, APC, GSK-3 and SOCS-1 genes were 7.5% (15/200), 8.0% (16/200), 6.0% (12/200), 12% (24/200), 2% (4/200) and 3.5% (7/200) for cancerous tissues, respectively, whereas the frequencies were 3.5% (7/200), 6.0% (12/200), 7.0% (14/200), 2.5% (5/200), 0.5% (1/200) and none for nearby non-cancerous tissues, respectively. The methylation status of these genes had no clear relationship with cancer cell types. Our results suggest that inactivation of these tumor-related genes through methylation of CpG islands may play a limited role in the development of colon cancer, and methylation inactivation of these genes except SOCS1 may occur at the pre-cancerous stage. Since simultaneous methylation of two or several genes was observed, we suggest that the development of colon cancer involves multiple methylation pathways.

P066. Molecular characterisation of stage III colorectal carcinomas

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Our study aims at correlating clinical parameters with genetic alterations in primary tumour tissue in stage III colorectal carcinoma (CRC) patients all treated with chemotherapy (5-FU/Levamisole or 5-Fu/Levamisole/Leucovorin). We used archival formalin-fixed paraffin-embedded tissue.
Tumour development is often associated with genomic instability. The best known forms of instability are CIN (chromosomal instability) and MIN (microsatellite instability). In order to distinguish between a CIN and a MIN phenotype, we screened the tumours by denaturing gradient gel electrophoresis (DGGE) and sequencing of TP53 and by microsatellite instability analysis. In addition we screened for KRAS mutations.
Scanning TP53 (exons 4-8) revealed 101 mutations in 165 tumours (61%). Fifty-four KRAS mutations were identified in 174 tumours analysed (31%). Screening of 237 tumours for MSI revealed 35 MSI-H tumours (15%).
These figures are in agreement with published data. In contrast to this we found no clear inverse correlation between MSI-H and TP53 mutations, as ten of the nineteen MSI-H tumours had a TP53 mutation. Our results do not exclude coexistence of MIN and CIN developmental routes in the same tumour.
No significant correlation‘s were found with the molecular parameters, (TP53, KRAS and MSI) and the overall survival and the clinical parameters, (age, site, grade, vascular and perineural infiltration), except for the location of the MSI-H tumours which were located predominantly in the proximal colon (30/35 or 86%). Further multivariate analyses could not be performed, as the number of tumours analysed was too small.

P067. Quantitative analysis of *MLH1* expression following reactivating treatments in Mismatch Repair deficient (MMR-) human colorectal cancer (CRC) cell lines

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About 10% of sporadic CRCs show alterations typical of MMR pathway involvement, and are characterized by hypermethylation of the *MLH1* promoter in tumor cells.
In order to establish the level of *MLH1* reactivation, HCA7 (MMR-, due to *MLH1* promoter hypermethylation) and SW480 (MMR+) cell lines were treated with the demethylating agent 5-azadC alone and in combination with the histone deacetylase inhibitors Na-butyrate (BA) and 4-phenyl-butyrate (PBA), and the probable histone acetylator acetyl-L-carnitine (ALC).
By qualitative and quantitative RT-PCR we observed that a 0.2 µM dose of 5-azadC resulted in reactivation of transcription of *MLH1* within 3 days in HCA7. This expression increased with increasing concentrations of the substance and with time of exposure, and persisted for at least 2 weeks following suspension of treatment.
By Methylation-Specific PCR, we observed partial promoter demethylation in HCA7 treated with 5-azadC. The combined 5-azadC/BA resulted in decreased level of expression of *MLH1*, whilst preliminary results with 5-azadC/PBA showed a synergistic effect on *MLH1* expression after 16 h compared to the little or no effect seen after 4, 8 or 24 h. 5-azadC/ALC gave little or no extra reactivation compared to treatment with 5-azadC only.
These data show that the expression of *MLH1* can be overcome following treatments with demethylating agents and enhanced following combined treatments with histone deacetylase inhibitors. These effects are also persistent in time, hence providing a working period to study the cellular response of reactivated cells to drugs that are normally tolerated by MMR-deficient cell lines.

P068. Identification Of Gains And Losses In Lung Cancer Samples Obtained With Fnab Using Cgh

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Lung cancer is a common and highly aggressive neoplasm in the world.It has both chromosomal and molecular pathologies detectable by different methods.In this study, we used comparative genomic hybridisation (CGH) technique to identify the DNA copy number changes in 15 lung cancer samples obtained by fine needle aspiration biopsy.The minimal common regions were found as gains at the chromosomal loci of 18p (6/15), 12p(5/15), 11p (5/15), 3p (4/15), and as losses 19 (3/15) and Y chromosome (4/14). Also, polyploidy and normal profiles were detected in one and two cases, respectively. Sixty three chromosomal aberrations were found per tumor ranged from 2 to 11.As a result, although we have few cases, the combination of CGH,FNAB and DOP-PCR(Degenerated oligonucleotide primer-PCR) has showed a high performance to identify the chromosomal aberrations in patients with lung cancer without need to operation.

P069. Evalution Of Aneuploidy Frequency For Chromosomes 6 And 17 In Extraocular Tumors: By Fish Technique

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Basal cell carcinoma (BCC) of the eyelid, accounting for over 90% of all cancerous lid lesions, and squamous cell carcinoma (SCC) characterized by basement membrane invasion in the conjunctiva, are the most common malignancies in extraocular tumours. This study included 7 patients with BCC of the eyelid and 3 with SCC of conjunctiva. In all patients, total surgical excision was performed under the operating microscope. Pathological analysis was done on one piece of each sample. Chromosomes 6 and 17 aneuploidy were evaluated using FISH with chromosome specific alpha satellite DNA probes in interphase nuclei of the other part of tumour. The distribution of hybridization signals for chromosome 6 was wide ranged, indicating heterogenity of cell populations having aneuploidy from patient to patient. The frequency of aneuploidy was higher in 6 patients with BCC than in controls. However, the frequency of aneuploidy for chromosome 6 was in the normal range in 2 patients with SCC, but one patient with SCC had a high percentage of aneuploidy. As for chromosome 17, the distribution of cells with hybridization signals for chromosome 17 were also wide ranging. In 4 patients with BCC, the incidence of aneuploidy was higher than in controls while the remaining cases had normal distribution. In patients with SCC, the frequency of chromosome 17 aneuploidy was similar to control samples in 2 cases but higher in one case. In conclusion, interphase FISH analysis can be efficiently use for detection of genetic alterations in BCCs and SCCs.

P070. Cytogenetic and CGH findings in 4 cases of breast cancer after neoadjuvant therapy

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We analyzed four tumors from breast cancer patients after neoadjuvant therapy by CGH and classic chromosome banding analysis in order to assess a potential common pattern of genetic alterations in chemotherapy resistant tumors. All patients showed structural aberrations involving chromosomes 1, 5, 11 and 16 and 17. Patients 1-3 showed highly abnormal karyotypes involving more than 10 structural changes, whereas in the „more benign and less proliferating“ tumor of patient 4, only five structural anomalies were seen. In CGH analysis, the patients showed typical imbalances for ductal breast cancer, as gains of 1q (3 patients), 5q (2 patients) 8q (3 patients), X (4 patients), losses of 1p33 approximately p36(3 patients), 16q(3 patients), 17p (3patients), 19 (4 patients), 22q (4patients), and other recurrent imbalances that do not belong to the typical pattern of ductal breast cancer as gain of 4q21q32(2 patients),

20q21q22 (2patients), gain of 21 (2 patients) and loss of 20p (3 patients)
Noteworthy, three patients showed involvement, of several regions involved in drug resistance as 7q11-q22 (mdr1)(2patients), 4q22(BCRP) (2patients), 16p13(MRP1) (1 patient), 21q22(RFC1)(one patient), whereas the fourth patient displayed a deletion of 17p13 associated with an aberration involving 5q21 and an amplification in the region of c-myc, thus providing at the level of the light microscope an explanatory background for the ability of their tumors to survive anthracycline-, taxane- and cyclophosphamide-based chemotherapy. Conventional cytogenetic analysis and CGH displayed highly coincidental findings in the tumors of four patients after neoadjuvant chemotherapy for breast cancer.

P071. Physical and transcript map of the minimally deleted region III on 17p implicated in the early development of Barrett's oesophageal adenocarcinoma

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Allelic imbalance (AI) studies on chromosome 17 (C17) in Barrett's oesophageal adenocarcinoma (BOA) tumours strongly suggest that a minimally deleted region on C17p harbours a BOA- associated gene with tumour suppressor function. This deleted region, designated minimal region III (MRIII), lies between the two microsatellite markers D17S1852 and D17S954. Computational sequence analysis techniques BLAST and NIX were used to assemble a physical map of MRIII, consisting of three overlapping bacteria artificial chromosome (BAC) clones, 297N7, 963H4 and 795F17 from the RPCI-11 library. The 270kb genomic sequence of MRIII was analysed using the computational gene prediction methods NIX and TAP to identify putative BOA genes. A transcript map of MRIII has been generated and contains 25 candidate BOA genes, four of which are named genes: MYH3, SCO1, x006 and MAGOH-LIKE. The other candidates consist of seven genes predicted by TAP with associated ESTs identified by NIX, two genes predicted by TAP alone, and twelve genes/ ESTs (or pairs of ESTs) identified by NIX alone. No disease-specific mutations were identified in x006 or MAGOH-LIKE, although expression analysis of these genes suggests that they may be altered epigenetically or in regulatory regions in oesophageal cancer.

P072. Identification of Novel Genes with Somatic Frameshift Mutations within Coding Mononucleotide Repeats in Colorectal Tumors with High Microsatellite Instability

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We have systematically retrieved genes with coding mononucleotide repeats from sequence databases and analysed them for mutations in tumors with high levels of microsatellite instability (MSI-H). We found somatic frameshift mutations in 7/13 genes previously not analysed in MSI-H tumors. According to the frequency of mutations in MSI-H tumors, these genes could be divided into genes with high coding mononucleotide repeat instability (CMRI-H) and genes with low coding mononucleotide instability (CMRI-L). CMR-H genes were mutated in more than 9/38 and CMRI-L in less than 4/38 of MSI-H tumors. Four genes in our study were CMRI-H and could thus possibly play a role in the development of MSI-H tumors: TFE3 (9/38), TEF4 (12/38), RGS12 (11/38) and TCF1 (12/38). Our results suggest that systematic identification of genes with CMR in the sequence databases and determination of mutation frequency in MSI-H tumors might be a powerful tool for identification of new molecular targets in the development of MSI-H tumors.

P073. C-myc Amplification In Oral Squamous Cell Carcinomas

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In clinical practice it became clear that conventional prognostic parameters of oral squamous cell carcinomas (OSCC) are of limited value for the prediction of a disease-free survival. To gain of better understanding of tumour behaviour, additional prognostic markers are included such as molecular changes in cancer genes. Mutation (amplification) in one of these genes, c-myc protooncogene, was evaluated using double-differential polymerase chain reaction and could be correlated with clinical outcome. In our study, we tested 70 paraffin embedded OSCC specimens and compared the rate of amplification with histopathological parameters. Interestingly, 12 of 70 (17%) samples have higher level of c-myc amplification and belong to T1/T2 tumours. The rest of the samples with different tumour grades did not show amplification. In the analysed T1/T2 tumours, c-myc amplification might specify a subgroup of high-risk patients with adverse tumour behaviour.

P074. Cytogenetic characterization of thyroid cancer cell lines by using comparative genomic hybridization and conventional Q-banding.

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Thyroid carcinoma (TC) comprises approximately 1% of all malignancies. The only clearly identified exogenous factor that may lead to TC is radiation.
The aim of this study was to analyse cytogenetic alterations in 14 thyroid cancer cell lines, including 8 anaplastic (AC) and 6 papillary cancers (PC). Twelve cell lines were analyzed by using both comparative genomic hybridization (CGH) and conventional Q-banding, two were analysed only by conventional Q-banding (one AC and one PC).
CGH analysis in PC revealed six aberrations in three cases out of five. Gains involved chromosomal regions 4q34-35 and 17q25; losses were observed in 1q21, 9q13 and 6q27.
Q banding cytogenetic analysis revealed aneuploidies in all the cases. The number of chromosomes in cells was in a range between 66 and 100. Recurrent structural rearrangements were t (7;11) (p11; q11), t (10;14) (q11.2;q11), t (1;5) (q32;q11.2), 1p and 1q deletions.
CGH analysis in A.C. detected 57 aberrations in six out of seven cases, with an average of 9.5 anomalies. Q banding cytogenetic analysis revealed aneuploidias in all the cases. The average chromosome number ranged between 42 and 196. Recurrent structural rearrangements were t (7;11) (p11; q11), t(1;15)(p12;q11), 1p, 1q and 9q deletions, iso 20p iso 1p, iso 22p.
The combination of classical and molecular cytogenetics shows distinct types of numerical and structural chromosomal aberrations that cannot be detected by a single method.

P075. STAP: A candidate gene for Tylosis with Oesophageal Cancer (TOC)

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The association between autosomal dominant tylosis (focal palmoplantar keratoderma), oral leukokeratosis, and oesophageal squamous cell carcinoma has been recognised in three families, two of which are extensive. Linkage and haplotype analysis has mapped the causative locus, designated TOC, to a 42.5kb minimal region on chromosome 17q25.3. The minimal region contains one complete gene, and partial coding sequences of two other genes. The gene that is contained entirely within the minimal region has recently

been identified as cytoglobin (CYGB), or Stellate-cell Activation-associated Protein (STAP). Experiments in rat have shown that STAP is upregulated in active hepatic stellate cells, which co-ordinate the fibrotic response to hepatic injury, possibly acting as a negative regulator of hepatic fibrosis.
Using RT-PCR, we have shown that STAP is ubiquitously expressed, suggesting that its function is not merely a hepatic one. We present a hypothesis for the involvement of STAP in TOC. Partial loss of function of STAP, a peroxidase, might lead to a higher concentration of free radicals at sites of injury (skin and oesophagus being particularly susceptible due to their constant exposure to frictional and thermal damage). Free radicals are known to activate hepatic stellate cells, and a similar process at other sites could lead to fibrosis. Activated stellate cells are known to produce the ZF9 transcription factor, which can transactivate the keratin K4 promoter in certain oesophageal cancer cell lines. Although no TOC-specific sequence changes are evident in the coding region of STAP, the regulatory and intronic regions are candidate sites for causative mutations.

P076. Clinical meaning and prognostic value of cytogenetic aberrations in a Yugoslav serie of patients with Neuroblastoma

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We have analysed cytogenetic aberrations in a series of 34 Yugoslav neuroblastoma patients, in order to establish their clinical meaning and prognostic value in our population.
Among 34 patients investigated cytogenetically, 12 were younger than 1 year at diagnosis. Of these, 2 presented with stage IV, 2 with stage III, 5 with stage I and 3 with stage IVs disease. The remaining 22 patients were over 1 year of age (1 at stage I, 2 at stage II, 3 at stage III, 16 at stage IV).
Cytogenetic analysis was performed on bone marrow cells without stimulation and chromosomes were identified by standard banding techniques and FISH for deletion 1p36 (2 cases). Normal karyotypes were found in 22 patients: 6 at stage I, 1 at stage II, 4 at stage III, 7 at stage IV and 4 at stage IVs. An abnormal karyotype was found in 12 patients: 5 cases of of mosaic hypertetraploidy (4 at stage IV and 1 at stage III), 2 cases of mosaic tetraploidy (1 at stage II and 1 at stage IV), 1 case of near diploidy in mosaic (at stage IV), 2 cases of complex karyotypes (stage IV) and 2 cases of homogeneously staining regions (HSRs) and double minute chromosomes (DMs). The cytogenetic results, together with the age of the patient and stage of tumor were compared with survival time of NB patients.
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P077. Frequent epigenetic inactivation of the *SLIT2* gene in gliomas

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The *SLIT* family of genes consists of large extracellular matrix secreted and membrane associated glycoproteins. The *Slits* (*Slit1-3*) are ligands for the *ROBO* family of axon guidance receptors. In a recent report we demonstrated that promoter region of human *SLIT2* was frequently hypermethylated in several tumours and the silenced gene transcript suppressed the malignant phenotype in *in vitro* assays.
In this report we undertook epigenetic, genetic and expression analysis of *SLIT2* gene in a large series of gliomas and glioma cell lines. Promoter region of *SLIT2* was found to be methylated in 71% (5/7) of glioma cell lines and was unmethylated in five DNA samples from normal brain tissues. The hypermethylation of the *SLIT2* promoter region in glioma cell lines correlated with loss of expression and treatment with the demethylating agent 5-aza-2'-deoxycytidine reactivated *SLIT2* gene expression. In primary gliomas *SLIT2* was methylated in 59% (37/63) of tumours analysed. In addition, *SLIT2* expression was down-regulated in methylated gliomas relative to unmethylated tumour samples as demonstrated by quantitative real-time RT-PCR. Loss of heterozygosity analysis revealed that *SLIT2* methylated gliomas retained both alleles of a microsatellite marker within 100kb of the *SLIT2* gene at 4p15.2. Our data indicates that *SLIT2* is frequently inactivated by promoter region hypermethylation in gliomas and may be a good candidate for a glioma tumour suppressor gene (TSG) located at 4p15.2. Furthermore, our data suggests that a detailed analysis of both the cancer genome and epigenome will be required to identify key tumour suppressor genes involved in glioma development.

P078. Chromosome 11 abnormalities at the cytogenetic level in neuroblastoma

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Neuroblastoma (NB) is a common malignancy of childhood affecting approximately 1 in 10,000 individuals and has a number of recognised non-random genetic features including aberrations of chromosomes 1p and 17q, and NMYC amplification. In a consecutive series of 51 tumours an abnormal clone was established by conventional karyotyping, and refined by FISH or SKY, in 45 (88%) tumours. Abnormalities of chromosome 11 are a recognised non-random event in NB, and in this series 22 (48%) cases were found to have either full loss (9 cases) and/or an unbalanced rearrangement (16 cases), one case had a constitutional deletion. Breakpoints spanned the whole chromosome but were primarily located on the q arm. In 8 (36%) cases the abnormality was shown to have occurred as a secondary event, with 11q23 involvement in 4/5 structurally rearranged cases. There was no difference between the incidence of an abnormality in disease stages 1 – 4, but no case with disease stage 4S was found with an aberration. Cases with a structural 11q aberration were more likely to be tumours without a 1p deletion or NMYC amplification, however, cases were seen with all three abnormalities. A previously described group, with an 11q and 3p abnormality and without del(1p) or NMYC amplification only occurred 3 cases. The results show that the involvement of chromosome 11 in NB is complex and indicate that a combination of multiple regions and the timing of the mutational events may be important in the disease pathogenesis.

P079. Characterisation of the somatic mutational spectrum of the neurofibromatosis type 1 (NF1) gene in NF1 patients with benign and malignant tumours

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Neurofibromatosis type 1 (NF1) is caused by mutations of the NF1 gene. One of its main clinical features is benign neurofibromas, 10-20% of which become malignant peripheral nerve sheath tumours (MPNSTs). The molecular mechanisms underlying this transformation are however unclear. Although the NF1 germ-line mutational spectrum is well characterised, similar data on tumour-associated somatic mutations of the gene are lacking. Somatic inactivation may involve gross chromosomal rearrangement, subtle mutations, genomic instability at nucleotide/chromosomal level, or methylation-mediated promoter inactivation.
DNAs from 91 tumours from 33 NF1 patients (including 7 MPNSTs). were screened for gross changes in the gene using microsatellite/RFLP markers; loss of heterozygosity (LOH) was found in 15/91 (16%) tumours. Denaturing high performance liquid chromatography (dHPLC) was then employed to screen LOH-negative tumours for microlesions at both RNA and DNA levels. 12 germ-line and 12 somatic mutations were identified in the NF1 gene; 3 germ-line (V325A, 3731delT, 6117delG) and 8 somatic mutations (1888delG, 4374-4375delCC, R2129S, 2088delG, 2341del18, IVS27b-5,C>T, 4083insT, Q519P) were novel. A mosaic mutation (R2429X)was identified in a benign tumour both by dHPLC analysis and cloning/sequencing. Tumours with no apparent mutations are being screened for hyper-methylation. Microsatellite instability analysis using 4 different markers has revealed a low level of instability. Screening

of the 7 MPNSTs for mutations in the CDKN2A and TP53 genes proved negative. Although our study demonstrates strong similarities between the somatic and germ-line mutational spectra, it is clear that NF1 tumorigenesis is a complex multistep process that involves genes other than NF1.

P080. Mapping of candidate region for chordoma development to 1p36.2 by LOH analysis of 27 tumors

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Several cytogenetic and molecular findings point to 1p36 loss as a consistent change in sporadic and inherited chordoma, a rare embryogenetic neoplasm arising from notochord remnants. We studied 27 sporadic chordomas by LOH of 31 microsatellites localized at 1p36.32-36.11 region and restricted to 1p36.2 the minimal LOH interval shared by 85% of the tumors. To investigate the role of candidate genes, selected by bioinformatic tools according to the physical mapping of the LOH region and their plausible oncosuppressor function, we performed RT-PCR analysis of CASP9, EPH2A, PAX7, DAN and DVL1 genes.
RT-PCR on 8 chordomas evidenced the presence of DAN and PAX7 transcript fragments of the expected size in all samples. Conversely the CASP9 specific-fragment was observed only in three tumors, while EPH2A was observed with one exception. Peculiar DVL1 transcripts with a size smaller than expected, were observed in four tumors, but also in the normal counterpart, nucleus polposus, which however also showed the transcript with the typical size. Following sequencing, the smallest DVL1 fragment showed the skipping of three exons leading to frameshift and predicting a truncated DVL1 gene product. The study, describing the most numerous cohort of chordoma patients so far recruited, points to a common molecular lesion at 1p36.2, suggesting a putative role for CASP9, EPH2A and DVL1 genes as oncosuppressor, possibly implicated in chordoma development.

P081. Chromosomal imbalances in recurrent primary nervous system neoplasms, detected by comparative genomic hybridization

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Primary central nervous system neoplasms include a broad spectrum of diseases with a common intracranial location. These tumors are usually dissected carefully with maximal effort not to affect the surrounding tissues, but still they have a tendency to recurr after resection.
In order to determine whether the recurrent tumors are of the same origin, we employed CGH for the detection of genomic imbalances. We tested 9 cases, each with 2-3 recurrent brain tumors. In 78% (7/9) of cases, we observed similar changes in each of the recurrent tumors per case. Two of the cases showed different patterns of genomic changes.
The recurrent changes are summarized in the table below.
These findings indicate that most of the recurrent tumors are of the same monoclonal origin and that genomic alterations, that are common to all recurrent tumors may be early aberrations that play an important role in tumorogenesis.
Cases showing different chromosomal changes can suggest that the tumors have separated at an early stage of the cancer process, creating areas consisting of different genomic alterations, or that the tumors are not of the same origin.

Tumor number	Total changes in first tumor	Recurrent changes
1	22	5
2	15	14
3	15	10
4	15	8
5	6	5
6	4	3
7	1	1

P082. An unusually malignant bilateral multifocal retinoblastoma - a case report.

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We report a case of unusually malignant bilateral multifocal retinoblastoma. The patient was born on June 7th 2002. Approximately two months after birth the mother noticed leucocoria of the right eye. The ophthalmic examination revealed the right eyeball filled with tumour tissue, after eye enucleation the histological diagnosis was retinoblastoma. No pathological findings were present in the left eye at this timepoint. On September, 24th, upon prophylactic opththalmic examination, the paracentral retinoblastomic lesion was discovered in the left eye with prominence of 1.2 mm, in addition two more lesions were seen peripherally. On October 7th 2002, 7 more lesions of different sizes were localized. Brachytherapy with Ruthenium 106 was applied to the central tumour, combined with cryotherapy for the smaller tumours. Systemic chemotherapy was applied afterwards. Upon last examination January, 15th 2003 tumour lesions were either in remission (the bigger irradiated one) or have disappeared (smaller tumours). Molecular analysis showed a characteristic RB1 mutation in the child. This mutation was not present in either of the parents. This mutation alone does not give a clue as to the unusual course of the disease.

P083. RB1 molecular pathology and methylation anomalies of p16, p15, p14, N33, MGMT, HIC1, RBCC1 and CDH1 genes in retinoblastoma tumors.

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Retinoblastoma is an embryonic malignant tumor of retina which is found together with structural and functional abnormalities in RB1 tumor-suppressor gene. We have studied 60 retinoblastoma families. Complex testing for RB1 mutations and functional inactivation of the RB1 and p16 genes revealed a molecular defect in at least one allele in 95% tumors. Analysis of PCR products mobility by SSCP and heteroduplex analyses revealed 47 small mutations in different RB1 gene exons and introns. All familial and sporadic bilateral cases of retinoblastoma had germinal mutations. Loss of heterozygosity (RBint2, RBint20, D13S262, D13S284) of at least one of intragenic markers was found in 71% of analyzed tumors. Complete deletion of RB1 was revealed in two sporadic cases. Aberrant methylation inactivating RB1 was detected in 27% tumors. Having not found any molecular defects of RB1 gene in several retinoblastoma samples we undertook a study of methylation status of p16/INK4a and RBCC1 promoter regions as these genes function as upstream regulators of pRB activity, as well as of promoter regions of such tumor related genes as p15/INK4b, p14/ARF, N33, MGMT, HIC1 and CDH1/E-cadherin. Methylation status was evaluated by multiplex methylation-sensitive PCR. We found abnormal methylation of p16 promoter region in 17% cases, HIC1 promoter region in 41 % and CDH1 promoter - in 60%. Low levels of methylation were detected for MGMT (1%) and RBCC1 (2%) genes, and no methylation at all was shown for p15, p14 and N33 promoter regions.

P084. Molecular cytogenetic characterisation of glioblastoma multiforme by fluorescence in situ hybridization (FISH), comparative genomic hybridization (CGH) and spectral karyotyping (SKY)

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Glioblastoma multiforme (GBM) is the most common primary neoplasm occurring in the central nervous system of adults. Chromosome analysis of glial tumours can provide important pathobiological data; however, conventional cytogenetics has been unable to identify consistent chromosomal aberrations in this group of tumours. Thus, more advanced molecular cytogenetic approaches are required to study the relationship between chromosomal instability and patient prognosis.
In this study, chromosomal abnormalities in 15 cases of GBM were analysed. In all patients, trisomy 7, monosomy 10, p53 deletions and EGFR gene amplification using interphase FISH analyses were investigated. In 5 cases, comparative genomic hybridization (CGH) to identify genomic imbalances (losses or gains of chromosomes) across the entire tumour genome was performed.
Three cultivated glioma cell lines were analyzed by spectral karyotyping (SKY) to determine complex structural rearrangements not resolved by the G-banding analyses.
The results of interphase FISH showed clonal monosomy of chromosome 10 and gains of chromosome 7 in all of 15 tumour samples (100%). EGFR gene amplification was present in 3 cases (20%). In addition, CGH showed the incidence of gains affecting chromosomes 1, 6, 9, 20 and 22. Losses/deletions involved chromosomes 3, 11, and 17.
Detailed spectral karyotype analyses on GBM lines identified hyperdiploidy with high level of numerical and structural changes. Our data confirmed that application of molecular cytogenetic methods may led to improved genetic subtyping and classification of GBM.

P085. A resequencing primer set for 3,000 genes implicated in cancer genetics

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The completion of a reference sequence for the human genome and improvements in high-throughput sequencing technology, including the Applied Biosystems 3730xl DNA analyzer and the BigDye® Terminators v3.1 Cycle Sequencing Kit, have motivated the development of easier solutions for quickly resequencing human genes. We report here work towards the development of a complete and validated resequencing workflow for high-throughput resequencing of the promoter regions, exon regions, and flanking intronic regions for 3,000 genes implicated in cancer. This workflow includes pre-designed primer sequences for amplicons covering these regions, protocols for PCR amplification and cycle sequencing, and software analysis tools specifically tailored to resequencing. The resequencing workflow takes advantage of the latest capillary electrophoresis technology for DNA sequencing. With this development, sequencing remains the most accurate method for rapid SNP discovery and comprehensive SNP screenin

P086. Determination of chromosome aberrations by cytogenetic, fluorescence *in situ* hybridisation, and molecular (RT-PCR) analysis in childhood haematological malignancies

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Direct and 24 hour-short term tissue cultures were set up for bone marrow aspiration materials of 143 cases who had haematological malignancies at the Department of Paediatric Haematology and Oncology at Akdeniz University Hospital, between 1990 and 2002 years. Conventional cytogenetic techniques were applied for 76 cases (53%), while 67 cases (47%) could not be analysed due to lack of mitosis. Simple chromosomal abnormalities were observed in 4 (44%) of 9 chronic myeloid leukaemia (CML) patients. Among 21 acute myeloid leukaemia (AML) cases, 12 (57%) were found to have chromosomal aberrations (1 case having a complex karyotype and the rest having simple rearrangements). In 13 (38%) of 34 acute lymphoblastic leukemia (ALL) cases chromosomal aberrations (one of the cases having a complex karyotype) were detected. Chromosomal rearrangements were observed in 3 of 8 patients with myelodysplastic syndromes (MDS). In our fluorescence *in situ* hybridisation (FISH) studies, translocation t(9;22) was not observed in 2 CML patients, while in 4 ALL cases this translocation was found to be positive. The presence of translocation t(15;17) was shown in 4 AML cases and in a case with AML-M3. In a case with MDS, FISH analysis for chromosomes 5 and 7, were performed, and revealed normal results. In 4 ALL patients, whose karyotypes could not be determined by conventional cytogenetics and FISH analysis, the presence of translocation t(9;22) was shown by standardized RT-PCR protocole. We compare our findings with the recent literature in terms of clinical diagnosis, prognosis and treatment.

P087. Application of gel-based microarrays for genodiagnostics of cancer

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The microarrays, developed in our laboratory consist of an array of gel pads attached to a hydrophobic glass surface. Such gel-based microchips have been used for the analysis of different genetic changes: chromosomal translocations and point mutations. Leukemia is often caused by nonrandom karyotype abnormalities that define subgroups of patients with unique biological and clinical features. The multiplex reverse transcription-polymerase chain reaction (RT-PCR) in combination with hybridization on microarrays was used for the detection of eight clinically important translocations: t(9;22)p190 and p210, t(4;11), t(12;21), t(1;19),(8;21), t(15;17) and inv16, typical for acute and chronic leukemia in children. To demonstrate the potential clinical application of the method, more than 200 cases of childhood leukemia were screened and the above-mentioned gene rearrangements were found in 30% of cases. The sensitivity and specificity of the assay is comparable with RT-PCR technique, so that it can be used to follow minimal residual disease (MRD). A further refinement of the method, on-chip- multiplex PCR has been developed for the analysis of a common translocation t(9;22).
To demonstrate the possibilities of gel-based biochip technology in analysis of point mutations, the BRCA1 gene was used as a model. Five different mutations, small insertions, deletions and missense mutations in 2, 5, 11 and 20 exons of the BRCA1 gene can be identified simultaneously in one multiplex PCR reaction following by multiplex hybridization on the biochip. Our data suggest that gel-based microarrays is a powerful tool in analysis of molecular pathological changes in human genome, leading to different malignancies.

P088. The assessment of MMP-2 and MT1-MMP protein expression in LS180 cell-line treated with different doses of Sulindac.

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Metalloproteinases (MMPs) comprise a family of enzymes that play a key role in basement membranes and stromal extracellular

matrix degradation, thus they are necessary for all physiologic and pathologic processes needing tissue remodeling; neoplastic growth, tumor invasion and metastases. All MMPs are synthesized as latent proenzymes. One regulatory drug, with unknown mechanism, which may be involved in the regulation of MMP expression and activation is Sulindac, which is administred orally to colorectal cancer patients. The aim of this study was to investigate MMP-2 and MT1-MMP expression in LS180 cell-line treated with different doses of Sulindac. Cultured LS180 cells were treated with Sulindac in final concentrations of 200, 500, and 800 µM for 72 hours. An untreated LS180 cell-line was incorporated as a negative control. The MMP-2 and MT1-MMP expression was assessed by fluorescent immunocytochemistry. Results : There were no differences in MMP-2 expression in the LS180 cell-line treated with Sulindac at 200 µM. Incubation of these cells with Sulindac at 500 and 800 µM resulted in slight deregulation of MMP-2 protein expression. MT1-MMP protein expression assessed in LS180 cell-line treated with all doses of Sulindac was downregulated, but in cells co-cultured with Sulindac at a dose of 800 µM for 72 hours, more than 90% of treated cells were MT1-MMP negative.
We therefore conclude that downregulation of MT1-MMP protein expression resulting from Sulindac stimulation can modulate cell invasiveness, and in such cases, MMP-2 can be activated via an alternative pathway.

P089. *WWOX* – the gene from common chromosomal fragile site FRA16D. Aberrant transcripts in cancer.

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The *WWOX* gene, a candidate tumor suppressor gene, is located at 16q23.3-24.1. This region of chromosome 16 was identified as the common fragile site FRA16D. The loss of heterozygosity frequently observed in cancer has been traditionally associated with the existence of a tumor suppressor gene in an affected area. However, chromosomal rearrangement associated with common fragile sites has been also observed in various tumors. Therefore, there is an open question if genomic alternations affecting genes which reside in common fragile sites are a cause or just a consequence of the cancer. It was previously reported that *WWOX* behaves as a tumor suppressor gene when ectopically expressed in breast cancer cell lines. Additionally, aberrant transcripts with deletion of exons 5-8 and 6-8 were detected in various cancers but not in normal tissues. Those alternatively spliced *WWOX* mRNAs were found in tumor cell lines which do not have genomic deletions of appropriate exons. Our current studies are focused on *WWOXΔ6-8* aberrant transcript. We observed that *WWOXΔ6-8* mRNA is frequently present in breast cancer, at about 30% examined tumors. Our preliminary studies suggest that at least in some fraction of tumors expressing *WWOXΔ6-8*, presence of this transcript can be associated with more aggressive phenotype.
Therefore, *WWOX* may acts in a dual fashion, as a tumor suppressor gene or as an oncogene through alternatively spliced transcripts.

P090. Quantitative analysis of methylation status at CpG islands using PyrosequencingTM technology

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Methylation of cytosines at CpG dinucleotides is an epigenetic modification of DNA that has profound effects in the mammalian genome. The methylated status of CpG islands present in the promoter region of a gene has a significant influence on its expression. DNA methylation has been shown to play important roles during embryonic development and X chromosome inactivation. In addition, tumorigenesis is characterized by aberrant methylation patterns resulting in, for example, silenced expression of tumor suppressor genes.
Methods for analysis of methylation patterns usually rely on bisulfite treatment of denatured DNA, which converts non-methylated cytosines to uracils. Since methylated cytosines are resistant to this treatment, differentially methylated CpGs can, by this procedure, be analysed as artificial C/T single nucleotide polymorphisms (SNPs). PyrosequencingTM technology is a rapid and accurate real-time sequencing method for analysis of short to medium length DNA sequences. Simultaneous genotyping of multiple SNPs can be accurately performed and the allele frequencies of several SNPs in a pooled sample correctly quantified. In this study, we show that Pyrosequencing technology is a fast and reliable method for the detection and quantification of methylation differences at specific CpG sites. Multiple closely positioned CpG sites are analysed separately in one reaction and the degree of methylation at individual CpG sites determined.

P091. Identification of the origin of drug-resistant genes in leukemia cells using chromosome microdissection technology

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The use of anticancer drugs in appropriate combinations has led to major improvements in the treatment of malignant disease. Unfortunately, in many cases such success is limited by the development of resistance to the chemotherapeutic drugs. Studies with model cell lines have revealed that simultaneous resistance to unrelated drugs, or multidrug resistance (MDR), can readily develop in mammalian cells. This raises the possibility that similar MDR tumor cells may also arise *in vivo* in human cancers, limiting a patient's response to chemotherapy. Our laboratory became interested in studying MDR cell sub-lines developed by treatment of the T-cell leukemia cell line CCRF-CEM with increasing levels of the anthracycline, epirubicin. The MDR phenotype is associated with an expanded chromosomal region (ECR). We have been interested in the nature of this extra DNA and the molecular events that give rise to amplification. As part of these studies, we have compared the content of the amplified unit in different derived E sub-lines. We microdissected this expanded region and PCR amplified the chromosomal DNA in order to produce sufficient DNA to determine its chromosomal origin and for use as a source of material to investigate candidate drug resistance genes. We demonstrated that the selection against epirubicin actually elevated the level of the multidrug resistance associated protein (mrp) gene. The probes prepared by chromosome microdissection should be useful for studying the organisation and function of DNA sequences within the ECR. We have also demonstarted a useful role for chromosome microdissection in this field of research.

P092. Study of ETV6 gene implication in leukaemia cases with 12p13 rearrangements using fluorescence in situ hybridisation.

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The ETV6 / TEL gene located at 12p13 encodes for a member of the ETS family of transcription factors. ETV6 is frequently involved in chromosomal rearrangements in human haematological malignancies. In this study, fluorescent in situ hybridisation (FISH) was performed to detect rearrangements of ETV6 gene in 10 patients with leukaemia (acute myeloid leukaemia: 5 cases, acute lymphoblastic leukaemia: 4 cases and chronic myeloid leukaemia: 1 case), which had a rearrangement of 12p13 band in cytogenetic diagnosis. Among them, we detect a deletion of one copy of ETV6 gene in 5 cases and rearrangement of ETV6 between 12p13 and derivative chromosome partner in 4 cases. In 1 case the ETV6 gene was not involved. Spectral karyotyping (SKY) was also used to identify chromosome partner for one patient with a new translocation involving ETV6 gene: t(11;12)(q22;p13). we discuss the importance of fluorescent in situ hybridisation in the detection of genes rearrangements in leukaemia.

P093. Computer aided analysis of additional chromosome aberrations in Philadelphia chromosome positive acute lymphoblastic leukemia using a simplified computer readable cytogenetic notation

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Background: The analysis of complex cytogenetic databases of distinct leukemia entities may help to detect rare recurring chromosome aberrations, minimal common regions of gains and losses, and also hot spots of genomic rearrangements. The patterns of the karyotype alterations may provide insights into the genetic pathways of disease progression.
Results: We developed a simplified computer readable cytogenetic notation (SCCN) by which chromosome findings are normalized at a resolution of 400 bands. Lossed or gained chromosomes or chromosome segments are specified in detail, and ranges of chromosome breakpoint assignments are recorded. Software modules were written to summarize the recorded chromosome changes with regard to the respective chromosome involvement. To assess the degree of karyotype alterations the ploidy levels and numbers of numerical and structural changes were recorded separately, and summarized in a complex karyotype aberration score (CKAS). The SCCN and CKAS were used to analyse the extend and the spectrum of additional chromosome aberrations in 94 patients with Philadelphia chromosome positive acute lymphoblastic leukemia (ALL) and secondary chromosome anomalies. Dosage changes of chromosomal material represented 92.1% of all additional events. Recurring regions of chromosome losses were identified. Structural rearrangements affecting (peri)centromeric chromosome regions were recorded in 24.6% of the cases.
Conclusion: SCCN and CKAS provide unifying elements between karyotypes and computer processable data formats. They proved to be useful in the investigation of additional chromosome aberrations in Ph-positive ALL, and may represent a step towards full automation of the analysis of large and complex karyotype databases.
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P094. Identification of a novel gene, GEMS, that is fused to FGFR1 in the 8p11 myeloproliferative syndrome

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The 8p11 myeloproliferative syndrome (EMS) is an atypical stem-cell myeloproliferative disorder caused by activation of the transmembrane receptor tyrosine kinase FGFR1. This typically occurs as a result of fusion of the tyrosine kinase domain of FGFR1 to an unrelated partner gene, which promotes dimerisation and ligand-independent activation of the kinase. To date, five FGFR1 partner genes have been identified: ZNF198 at 13q12, FOP at 6q27, CEP110 at 9q33, BCR at 22q11 and HERV-K at 19q13. We describe a patient with a clinical picture typical of EMS, who was found to have an acquired ins(12;8)(p11;?p11p22) by cytogenetics. Southern blotting and FISH confirmed that FGFR1 was disrupted and RT-PCR analysis indicated that the patient did not harbour any of the known FGFR1 fusion mRNAs or BCR-ABL. To identify the partner gene in this patient we employed 5'-RACE PCR, exploiting the fact that all known FGFR1 translocations have the same FGFR1 breakpoint at exon 9. A 629 bp product was obtained which was found to consist of sequence derived from chromosome band 12p11 fused to exon 9 of FGFR1. Analysis of ESTs indicated that the sequence was derived from a novel gene that we have called GEMS (Gene disrupted in Eight p11 Myeloproliferative Syndrome). GEMS is predicted to be translated into a 29KDa protein containing an N-terminal coiled-coil domain but no other recognisable motifs. RT-PCR confirmed the presence of the GEMS-FGFR1 fusion. The reciprocal chimaeric mRNA was not detected. Experiments to determine the transforming ability of this fusion gene are underway.

P095. Translocation (2;9)(q31;q34) in a case of acute myeloblastic leukemia.

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Here, we report a 24-year-old female patient with acute myeloblastic leukemia (AML) (French-American-British [FAB] class M2).
The patient presented no organomegaly. Her peripheral blood count showed pancytopenia without blast cells.
Cytogenetic study of the bone marrow cells revealed the following karyotype: 46,XX,t(2;9)(q31;q34) [14]/46,XX[5].
A complete remission was achieved 3 months after diagnosis by chemotherapy. The patient underwent allogenic bone marrow transplantation four months later, and has been in a complete remission for 9 months.
To our knowledge, this is the first report of t(2;9)(q31;q34) in a patient with AML-M2.

P096. Diverse mRNA expression and protein localization of suppressor genes in B-CLL and LCL cells.

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B-CLL Chronic Lymphocytic leukemia, the most frequent form of leukemia in Western countries, is characterized by progressive accumulation of clonal B lymphocytes in early phases (G0/G1) of the cell cycle. CLL cells can be infected by EBV but cannot proliferate, whereas normal resting B lymphocytes produce immortalized cell lines after infection.
We have investigated expression of cell cycle genes (p130, pRb, p27, p21,) at the mRNA and protein levels in cultured B-CLL lymphocytes and in EBV-transformed lymphoblastoid cell lines (LCLs). For estimations of mRNA we have used RNAse Protection Assay; the protein level was studied immunocytochemically.
We observed increased levels of p27 mRNA in normal lymphocytes compared to EBV-infected B cells. B-CLL nonstimulated cells exhibited very high levels of p27 mRNA, decreasing slightly after 72h stimulation. P27 protein had nuclear-cytoplasmic localization for both B-CLL cells and normal lymphocytes. P27 protein in LCL cell was almost undetectable or localized in the cytosol. LCL showed increased levels of p21 mRNA, but very low pRb and p130 mRNA levels.
Our data suggest that the mechanisms involved in EBV transformation may include "silencing" of critical cell cycle genes pRb, p130. A high level of p21 makes it possible to maintain the latency in EBV infected cells. Our and other data imply that p27 mRNA levels may be altered in a cell type-specific manner. Increased expression of p27 in B-CLL cells may be a valuable marker responsible for the inability of B-CLL cells to undergo apoptosis.

P097. Multiplex reverse transcription-Polymerase Chain Reactionfor simultaneous screening of 7 translocations

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We have introduced a multiplex reverse transcription-Polymerase chain reaction (RT-PCR) method for diagnosis and screening of patients with 7 chromosomal translocations including: t(1;19)(q23;p13),t(12;21)(p13;q22),inv(16)(p13;q22),t(15;17)(q21;q22), t(9;22)(q34;q11), t(8;21)(q22;q22), t(4;11)(q21;q23).Many of translocations are related with some acute myeloid and lymphoid leukemia.
The Multiplex RT-PCR detected a hybrid mRNAs resulting from fusion genes, which are produced by translocations. We have studied 30 patients in parallel with cytogenetic analysis. In our analysis a fusion gene was detected in 14 of 30 samples.
We identified patients positive for the following translocations:
9 case for t(9;22), 3 case for t (15; 17), 1 case for t(8;21) and 1 case for inv(16). In one case that cytogenetic analysis failed in detection of t(15;17)translocation, because of insufficient metaphase cells, but RT-PCR analysis revealed a t(15;17) translocation.

P098. Two coexisting translocations in a Burkitt-like lymphoma case: two immunoglobulin chain loci involved

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Many B-cell malignancies bear chromosomal translocations juxtaposing one or another immunoglobulin (IG) gene with oncogene, resulting in deregulated expression of the latter. Translocations affecting the IG heavy chain (IGH) locus in chromosomal region 14q32 are most prevalent. Variant translocations involving the IG kappa (IGK) locus in 2p12 or the IG lambda (IGL) locus in 22q11 occur also recurrently in B-cell neoplasias.
We describe cytogenetic, molecular cytogenetic and clinical data of a case of Burkitt-like lymphoma (BLL) cytogenetically characterized by t(8;14)(q24;q32) and t(7;22)(q21.2;q11). They were found in a complex pseudodiploid karyotype which otherwise showed monosomy 4 and additional isochromosome 7q. FISH analysis confirmed the involvement of both immunoglobulin loci, IGH and IGL, at 14q32 and 22q11 respectively. By immunohistochemistry, the neoplastic cells showed strong CD45 expression, weak CD20 expression and were negative for CD45RO.
The patient, 57 years old male, presented with stage IV disease with high tumor burden as indicated by intestine and omental involvement and dense bone marrow infiltration. Plasma immunoglobulins were normal, EBV and HIV tests negative. Chemotherapeutic treatment did not lead to remission and the patient deceased 6 months after diagnosis.
We discuss prognostic importance of the simultaneous involvement of two immunoglobulin chain loci. Furthermore, the delineation of translocation partner breakpoint 7q21 (reported also previously) is expected to reveal the consequence of its juxtaposing with IGL locus.

P099. Bi-allelic silencing of the Fanconi anaemia gene *FANCF* in acute myeloid leukaemia

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Fanconi anaemia (FA) is an autosomal recessive disorder caused by mutations in eight different genes (*FANCA*, *B*, *C*, *D2*, *E*, *F*, *G* and *BRCA2*). FA homozygotes have a high risk of developing acute myeloid leukaemia (AML) and certain solid tumours. It is possible that acquired FA gene inactivation may predispose to the sporadic development of such malignancies. Methylation is a well-recognised mechanism of gene inactivation in human malignancy. We therefore investigated FA gene methylation in AML patients samples and cell lines by sodium bisulphite sequencing. *FANCF* promoter methylation was detected in an AML M7 cell line (CHRF-288) that was known to have absent FANCF protein expression on Western blot analysis and where sequencing of *FANCF* had not revealed any mutations. *FANCF* was unmethylated in blood from healthy individuals. Investigation of FA gene methylation in sporadic adult AML samples did not reveal further cases of methylation of *FANCF* (36 patients examined), or *FANCA* (40 patients), *FANCD2* (21 patients), *FANCC, -E,* or *-G* (5-7 patients). *FANCF* is localised to chromosome 11p15.1, close to 11p15.5, which harbours a large domain of imprinted growth-regulatory genes important in cancer. Although it is not known how this process is regulated, the finding of *FANCF* methylation raises the possibility that regional silencing in this chromosomal domain may include *FANCF*, leading to gene inactivation, disruption of the FA pathway, genome instability and an increased risk of AML.

P100. Molecular and cytogenetic analysis in chronic myeloid leukemia (CML)

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Chronic Myeloid Leukemia (CML) is a hematopoietic malignancy characterized by the presence of Philadelphia (Ph^1) chromosome resulting from balanced reciprocal translocation between chromosomes 9 and 22 leading to the formation of *bcr/abl* fusion gene. The present study was conducted to evaluate cytogenetic and molecular anomalies in CML patients at presentation and during therapy.
Bone marrow samples of 190 suspected CML patients were analyzed using cytogenetic analysis. Sequential cytogenetic analysis was done in 60 CML patients on IFN-α 2b, STI 571 therapy and following Bone marrow transplantation (BMT). Fluorescence In Situ Hybridization (FISH) analysis was carried out using probes for *bcr* and *abl* genes and centromeric probes for chromosomes X and Y (in cases of sex-mismatched BMT). Varied degrees of cytogenetic response were observed, with complete cytogenetic response in 10 patients. However, *bcr/abl* fusion gene was detected using FISH analysis in some cases. Further analysis of sex-mismatched BMT using FISH for chromosomes X and Y could evaluate minimal residual disease. These findings are of tremendous value in detecting minimal residual disease even in patients with complete cytogenetic remission.
To conclude, results of the present study reiterate that molecular analysis has tremendous importance in accurate diagnosis and management of CML.

P101. Cytogenetic, fluorescence *in situ* hybridisation (FISH), and molecular (RT-PCR) analysis results of adult haematological malignancy patients

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Between 1990 and 2002 years, direct and 24 hours tissue cultures were set up for bone marrow materials of 409 patients having haematological malignancies. We had no result for 200 of 409 cases due to lack of mitosis. Chromosomal abnormalities were observed in 107 (51%) of 209 patients. Of the cytogenetically analysed 62 CML cases, 34 (58%) had translocation t(9;22), 3 cases had other types of simple chromosomal rearrangements and 3 cases had complex karyotypes. Chromosomal rearrangements were shown in 31 (51%) of 61 AML cases. Of these 31 cases, 29 had simple rearrangements while 2 cases had complex karyotypes. Among 16 ALL cases, 10 were found to have chromosomal aberrations (1 case having a complex karyotype and the rest having simple rearrangements). In 15 (39%) of 40 MDS cases simple chromosomal aberrations were detected. In our FISH studies, which began in 2001, the presence of translocation t(9;22) was shown in 1 AML-M2,1 AML-M5, 1 AML-M6, 1 myeloproliferative disease, 1 myeloblastic syndrome and 28 CML cases. By FISH analysis, translocation t(15;17) was detected in 4 AML-M3 and 1 AML-M5 cases and, translocation t(4;11) was observed in an AML patient. Translocation t(9;22) in an ALL case and in 3 CML patients, and translocation t(15;17) in 2 AML-M3 patients were absent. By using RT-PCR, which was started to be performed in our department during year 2002, presence of translocation t(9;22) was shown in 7 patients for whom cytogenetic analysis either could not be carried out or revealed normal karyotypes.

P102. Detection of an AML patient with 11q23 (MLL) amplification using M-FISH

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Translocations associated with 11q23 including Mixed Lineage Leukemia (MLL) gene have been frequently observing in AML patients. Gene amplification is a rare phenomenon in AML and recently amplification of the some chromosome bands associated with chimeric gene formation is shown only in a few cases. We observed complex karyotype in the bone marrow cells of a 57 years old male patient with AML-M4 during routine cytogenetic analysis. His karyotype was designated as: 48, XY, del(11)(q22), der(16)t(1;16)(q10;p10), + ring 1, + ring 2. Both of the C-band negative ring chromosomes were found to be originated from chromosome 11 using Multicolor Fluorescence *In Situ* Hybridization (M-FISH). Multiple signals were observed on both of the ring

chromosomes with the FISH study by using 11q23 (MLL) specific probe. The final karyotype was designated as: 48, XY, del (11)(q22), der (16)t(1;16)(q10;p10), + ring 1, + ring 2. r 1 ish der (11)(q32-qter)(Multiple Copies of MLL), r 2 ish der (11)(q32-qter)(Multiple Copies of MLL). Our case was did not respond to the treatment and died during analyses. Amplification of MLL gene (11q23) has been reported in only a few cases to date. Our findings and previous limited reports indicate that amplification of 11q23 affects especially elderly patients, often associated with complex karyotypic abnormalities and poor prognosis. Our results also have been shown that application of M-FISH on complex karyotypes observed in haematological malignancies is very important to designate the exact karyotype and to detect the genes responsible for development of the malignancy.

P103. Analysis of activation of the p53 DNA damage response pathway and apoptosis by ionising radiation in paediatric B-precursor and T-cell acute lymphoblastic leukaemia (ALL)

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We hypothesised that the DNA damage responses of presenting paediatric ALL tumours can identify those with a defect in a pathway that determines blast killing by cytotoxic treatment. We irradiated 33 (24 diagnostic and 9 relapse) paediatric ALLs with ionising radiation (IR) and assessed p53 and p21 protein induction at 4 and 8hrs post-IR. In addition, we evaluated cleavage of the PARP1 protein, also involved in the DNA damage response, which is cleaved early during apoptosis. 10/24 (41.7%) diagnostic leukaemias showed a normal p53 response and cleaved PARP1 by 8hrs post-IR. 8/24 (33.3%) showed defective PARP1 cleavage and normal p53 transcriptional activation except one (4.2%), which showed defective p53 and p21 upregulation, suggesting an upstream defect, e.g. ATM. 3/24 (12.5%) tumours exhibited normal PARP1 cleavage but reduced p21 induction after IR. 3/24 (12.5%) tumours had low levels of PARP1 with subsequent cleavage after IR. These also exhibited reduced p21 induction, possibly due to low PARP1 protein, which post-translationally modifies p53 after DNA damage. Of these, one (4.2%) carried a heterozygous TP53 G818C mutation. 3/9 (33.4%) relapse tumours had an abundant p53 DNA damage response with normal PARP1 cleavage. 5/9 (55.6%) had defective PARP1 cleavage after IR, one (11.1%) with reduced p53 and p21 induction, suggesting an upstream defect. Finally, 1/9 (11.1%) tumours had low levels of PARP1 and p21 proteins. Defective cleavage of PARP1 suggests that damage-induced apoptosis is impaired in a subset of paediatric ALL. As p53 typically appeared fully functional, downstream factors might be more commonly inactivated.

P104. Are cytogenetic aberrations relevant in younger adults with B-cell chronic lymphocytic leukemia?A study of 50 patients

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Various genetic abnormalities are often found in B-CLL, but their relative importance in the pathogenesis and evolution of the disease has not been adequately clarified.
The most frequently observed are deletions in bands 13q14, 11q23, 17p13, 14q32 and trisomy of chromosome 12. We selected bone marrow samples from 50 B-CLL younger adult untreated patients at diagnosis (mean age 51) to look for correlation between these genetic abnormalities and clinico-biological features by I-FISH analysis. 32 patients were followed-up after diagnosis and 17 of them showed a rapid disease progression.
The coexistence of aneuploid subclones in the same leukemic population for all the five chromosomes suggest that all the abnormalities observed in our B-CLL patients are secondary events occuring during disease progression.
Statistical correlations were evidenced between 11q23, 13q14 and 14q32 deletions and patients' clinical characteristics: 14q32 deletion showed a significant association with the presentation age (p=0.008); while 11q23 deletion was associated with the presence of lymphoadenopathy (p=0.04), the need of chemotherapic treatment (p=0.0002) and a shorter treatment free interval (p<0.0001). Interestingly, the presence of 11q23 deletion was found to predict rapid disease progression even in the patients with low-risk stage at diagnosis. Also the presence of 13q14 deletion is associated with a shorter treatment free interval (p=0.0003).
By contrast, no clinical findings were associated with trisomy 12 and 17p13 deletion.
Our findings confirm that 11q23 and 14q32 deletions are the most important prognostic factors identifiying a subgroup of patients with rapid disease progression, thus giving the opportunity for a risk-adapted management.

P105. Transcriptional response to Interferonα2a treatment

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Interferon-α2a (IFN-α2a) is widely used in the treatment of several human diseases including chronic viral hepatitis and some tumors. In order to unravel the molecular basis of a lack of (or a reduced) response, we decided to study the modification of cytokine induced expression profiles. We employed a chip spotted with 15,000 human genes and ESTs (Incyte Genomics, USA). The analyses were performed on three different cell lines before and after treatment with IFN-α2a. The cellular model systems employed were: HepG2, RH30 and K562. These cell lines were chosen since they represent the major targets of IFN-α2a.
The cells were cultured in a medium (DMEM or RPMI plus 10% foetal bovine serum) with or without 1000 U/ml of IFN-α2a. The medium also contained cycloheximide to hamper synthesis of new proteins. After 5 hrs cells were collected, washed and used to prepare total RNA. The expression profile of each sample was then determined. After statistical analysis the genes up- or down-regulated in all the three populations were characterized. Several of the genes identified are already known as IFN-α2a-modulated genes. Moreover, we noticed a remarkably activation of STAT1 expression which appeared essential for the amplification loop of IFN-α2a response. Finally, we identify at least two new genes transcriptionally up-modulated by IFN-α2a. In conclusion, our results represent the first study aimed at identifying genes directly regulated by the cytokine and furnishing new insights into the initial steps of the cell's response to IFN-α2a.

P106. The promyelocytic leukemia protein functions as a negative regulator of DHFR-mediated transcriptional activity

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The promyelocytic leukemia protein, PML, functions as a transcriptional repressor. It was found to directly interact with the Sp1 transcription factor abrogating its activation of the epidermal growth factor receptor (EGFR) gene promoter. Here, we have investigated the effects of PML on the dihydrofolate reuductase (DHFR) promoter, which is mainly regulated by Sp1. On functional analysis, transient transfection of PML into mammalian cells, U2Os and HeLa, resulted in a significant repression of the DHFR promoter. The DHFR promoter also contains DNA-binding sites for the E2F transcription factor. Transient transfections into the Sp1-negative *D. Melanogaster* SL2 Cells, indicated that the repression of the DHFR promoter activity by PML is achieved through the Sp1, but not the E2F DNA-binding sites of the promoter. When the Sp1 DNA-binding sites were replaced with an unrelated DNA-binding site (GAL4), the repressive effects of PML were lost, confirming the necessity of the Sp1 DNA-binding sites for PML's repressive effects. These data demonstrated that PML could function as a negative regulator of the DHFR promoter, which may represent a novel mechanism for the known repressive effects of PML on cellular growth.

P107. Evaluation of Clonality in Iranian Children with B-Precursor Acute Lymphoblastic Leukemia Using IgH and TCR- Deltal Gene Rearrangement

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Background:
The purpose of this investigation is to analyze the pattern of IgH chain & TCR-δ gene rearrangement by polymerase chain reaction as a marker of clonality.
Patients & Methods:
Mononuclear cells of bone marrow aspirates of 29 children with B- precursor ALL were collected at diagnosis (by density gradient). Deoxyribonucleic acid were extracted by proteinase K method. Using consensus primers for CD3 & whole VNDNJ of IgH chain gene, Vδ2-Dδ3 & Dδ2-Dδ3 regions of TCR- δ, clonality were analyzed by heteroduplex analysis.
Results:
The majority of patient show monoclonal pattern according to CD3,VNDNJ, Vδ2-Dδ3 & Dδ2-Dδ3 gene rearrangements.
Twenty five percent of patients show bi/oligoclonal pattern by CD3 gene rearrangement.
Conclusion:
These preliminary data suggest that pattern of clonality is similar to the other population & can be used as a marker of minimal residual disease.

P108. P27, Rb, p130, p107 and p53 protein and mRNA in human acute leukaemia cells

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The cyclin-dependent kinase inhibitor p27(Kip1), Rb family genes (pRb, p130, p107) and p53 are important tumor suppressor genes. They play pivotal
roles in controlling cell proliferation during normal development and tumorigenesis. Some reports show that decreased expression of these genes or inactivation of the proteins correlate with poor prognosis in some malignancies. We have studied expression of these genes in acute leukaemia.
We examined the levels of p27, Rb, p130, p107 and p53 mRNA and protein in 32 bone marrow samples obtained from patients with acute leukaemia before treatment, and as controls we used normal lymphocytes.
For detection and evaluation of mRNA levels we used the Multi Probe RNase Protection Assay System with Multi-Probe Template Sets hCC-2 and hTS-1. Protein levels were assessed by imunocytochemistry.
In all examined leukaemic blasts and normal lymphocytes we observed relatively high levels of p27 and p53 mRNA, while in leukaemic cells there was a lack of pRb mRNA and low levels of p130 and p107 mRNA in comparison to normal lymphocytes. For p27 and p53 leukaemic and normal cells differed in the amount and distribution of protein. In leukaemic cells p27 protein was localized in the cytoplasm in the majority of cells, while in the lymphocytes p27 protein was in the nucleus and cytoplasm. In 83% of leukaemic cells examined immunocytochemistry did not reveal p53 expression .
Our results may indicate important roles of p27, the Rb family and p53 genes in the pathogenesis of acute leukaemia.

P109. Activation of homeobox genes at 5q35 in pediatric T-ALL cell lines via juxtaposition with 3´-BCL11B involves short insertions

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A novel cryptic translocation, t(5:14)(q35.1;q32.2), has been recently described in pediatric T-ALL which may account for a substantial proportion of cytogenetically silent cases (Bernard et al., Leukemia 15:1495, 2001). It seems a homeodomain gene, HOX11L2 at 5q35, undergoes ectopic expression after juxtaposition with enhancers posited in the far downstream region of a zinc finger gene, BCL11B active in normal T-cell development. However, this rearrangement has yet to be documented clinically. We describe the karyotypes of 4 pediatric T-ALL cell lines analysed using panels of BAC clones covering the 5q35.1 and 14q32.2 regions revealing two homeobox targets at 5q35 (NKX2E as well as HOX11L2). In all 4 cell lines juxtaposition of homeobox genes with 3`-BCL11B was effected by submicroscopic insertions (<1Mb) rather than straightforward translocations. Although a human retroviral element (HERV-HD1) was present close to breakpoints at 14q32.2, these were too widely scattered to involve retroviral insertion. Interestingly, 2/4 cell lines showed downregulation of BCL11B indicating that this gene is not required to support the malignant phenotype unlike its close homologue, BCL11A which is involved in B-cell tumors. We believe these 4 cell lines will be useful both as models for this important new class of pediatric tumor and as resources for investigating their hitherto elusive biological basis.

P110. Is FISH technique adequate on its own in detection of chromosomal alterations in haematological malignancies ?

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Detection of clonal chromosomal alterations, in haematological malignancies, is important for diagnosis and evaluation of prognosis. FISH technique is a fast, easy and trusty method in determination of such changes. In this study, cytogenetic and molecular genetic analyses for the detection of secondary chromosomal abnormalities were compared. In 6 of 23 haematological malignancy patients, additional chromosomal aberrations to FISH findings were found by conventional cytogenetic technique. For the rest of the patients, cytogenetic and FISH analysis results were consistent. Translocation t(9;22) in 2 CML, 1 AML-M6, 1 AML-M5, and 1 Myelofibrosis patient; and translocation t(15;17) in a child clinically diagnosed as AML-M3, was observed by FISH analysis. Conventional cytogenetic analysis in patients with translocation t(9;22) revealed also trisomy 11 and isochromosome (17)(q10) in two CML patients, translocation t(2;22) in an AML-M6 patient, and translocation t(3;10) and trisomy 9 in an AML-M5 and in a Myelofibrosis patient, respectively. A 14 year-old patient with translocation t(15;17), displayed also translocation t(3;8) in all metaphases in cytogenetic analysis. It can be concluded that although FISH technique is essential in revealing specific chromosomal alterations important in diagnosis, conventional cytogenetic analysis maintains its role as a gold standard in detection of secondary chromosomal alterations that are closely related with the prognosis and management of haematological malignancies.

P111. Frequency of the heterozygous germline mutation 657del5 within the NBS1 gene in Polish pediatric patients with lymphoid malignancies

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Nijmegen breakage syndrome (NBS) is an autosomal recessive DNA repair disease. All Polish NBS patients carry a homozygous founder mutation, 657del5, within the NBS1 gene. The very high risk of developing lymphoid malignancy among these patients at an early age and relatively low frequency of that type of tumor by the age of 20 years in the general population in Poland, imply that heterozygous carriers of the 657del5 NBS mutation should be more frequent among cancer patients. In addition, the observation that close relatives of NBS patients also have an elevated cancer risk suggests a potential pathogenic role of NBS1 mutations in heterozygotes as well. In order to investigate the role of the common founder mutation 657del5 in the pathogenesis of lymphoid malignancies of childhood and adolescence we screened blood samples of 268 patients from 11 Polish pediatric hemato-onclogy centers. Four heterozygous carriers were found vs. approximately 1.5 expected. Germline 657del5 mutation was found in 1/125 patients with non-Hodgkin lymphomas (NHL), and in 3/143 patients with childhood acute lymphoblastic leukaemias (ALL). In addition, 4 new NBS patients were identified within that group. Our results, in contrast to earlier German studies, clearly show a higher prevalence of the major NBS1 gene mutation, 657del5, among pediatric patients with lymphoid malignancies (~1/67) than in the general population in Poland (~1/190). Further studies on possible involvement of the NBS1 gene in that type of cancer are warranted.

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P112. No correlation between temolere length and telomerase activity and expression in leukemic cells

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The aim of the work was to investigate the expression of three components of telomerase complex : telomerase reverse transcriptase (hTERT), telomerase proteins (TP) and RNA template for telomeric DNA synthesis (telomerase RNA-TR) along with telomerase activity and telomere length in leukemic cells. Cells have been isolated from peripheral blood and/or bone marrow of children with acute lymphoblastic (ALL) and nonlymphoblastic (ANLL) leukemia. Expression of three components of telomerase as well as telomerase activity has been found in all leukemic cells. Chemiluminescent detection of terminal restriction fragments (TRF) from DNA isolated from ALL cells showed variable patterns expressing considerable heterogeneity of telomere length. The ALL cells appeared to have both long and short telomere lengths, in contrast to normal peripheral lymphocytes, which produced limited pattern of TRF. The ANLL cells produced predominantly short telomere' pattern despite high telomerase activity and expression. It can be concluded that high telomerase activity and expression in leukemic cells is not always correlated with long telomeres (TRF pattern).

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P113. Human telomerase in normal and malignant cells in gastric and colon cancer patients

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Reactivation of telomerase is believed to play an important role in immortalization and carcinogenesis. We investigated the expression of three components of telomerase complex (hTR, hTERT, TP1) along with telomerase activity in malignant cells isolated from gastric and colon cancer patients, and normal stomach and colon mucosa from the same patients. Expression of hTERT, hTR and TP1 was studied by RT-PCR. The telomerase repeat amplification protocol-TRAP and PCR-ELISA were used for analysis of telomerase activity. All telomerase components were consistently expressed in colon and gastric cancer cells. Neoplastic RNA produced consistently very strong amplification signals either for hTR hTERT and TP1. The expression of hTR was observed in RNA isolated from all normal mucosa samples and from peripheral blood lymphocytes. Expression of TP1 and hTERT was found in the majority of normal cells, however the amplification signals were usually much weaker than in malignant cells. Limiting dilution experiments indicated that the cancer cells have at least 100-fold higher telomerase activity and at least 25-fold higher TP1 and hTERT expression compared to normal cells. We conclude that all cancer cells tested have higher telomerase expression and activity, as compared to normal cells. Therefore telomerase can be a good cancer marker provided quantitative analysis is carried out.

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P114. Methylation of a number of cancer related genes in various tumors

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Aberrant methylation of normally unmethlated CpG-rich areas, also known as CpG islands, which are located in or near the promotor regions of many genes, has been associated with transcriptional inactivation of defined tumor suppressor genes in human cancer. Thus, abnormal methylation serves as an alternative to the genetic loss of a tumor suppressor gene function by deletion or mutation. We investigated the frequency of aberrant methylation of $p16^{INK4a}$ (cyclin-dependet kinase inhibitor 4), $p15^{INK4b}$ (cyclin-dependet kinase inhibitor 6), $p14^{ARF}$ (cell cycle inhibitor), *Rb1* (retinoblastoma), *HIC1* (hypermethylated in cancer), *MGMT* (O^6-methylguanine-DNA-methyltransferase), *N33* (oligo-saccharyl-transferase) and *CDH1* (E-cadherin) genes in different cancers, such as breast cancer (100 samples), non-small cell lung cancer (55 samples) and acute lymphoblastic leukemia (80 samples). The methylation status of investigated genes was determined by the method of methyl-sensitive PCR (MS-PCR).

We determined that the investigated genes were differentially methylated in various cancers. *HIC1* and *CDH1* genes were more often methylated in BC and NSLC samples, than in ALL. Level of the $p16^{INK4a}$ gene methylation was considerable in all tumors samples. *Rb1*, *MGMT* and *N33* genes were equally methylated in all tumors samples. Methylation of *p14* gene was not detected in our samples. Our data demonstrated that BC and NSLC are "strongly-methylated" tumors, and ALL is a "weakly-methylated" tumor.

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.

	BR1	p16ex1	p14ex1	p15ex1	CDH1	MGMT	HIC1	N33
NSLC	20%	72%	0%	1%	73%	1%	77%	14%
BC	17%	56%	0%	2%	37%	8%	79%	9%
ALL	13%	70%	0%	1%	13%	1%	7%	1%

P115. High levels of chromosomal imbalances in typical and small-cell variant of t-cell prolymphocytic leukemia.

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T-cell prolymphocytic leukemia (T-PLL) is a rare post-thymic T-cell disorder that may show different morphological variants and a very aggressive clinical behavior. The disease is genetically characterized by the presence of complex karyotypes with recurrent alterations involving chromosomes 8, 14 and 11. However, the possible relationship between genetic alterations, morphological variants, and the clinical course of the disease is not well known. Comparative genomic hybridization (CGH) was used to detect chromosomal imbalances in eight patients with a diagnosis of T-PLL, including three cases of small cell variant with an indolent clinical evolution. Abnormal profiles were detected in all cases (100%). The chromosomal regions most often over-represented were 8q (75%), 5p (62%), 14q (37%), 6p and 21 (25%). The chromosomal regions most often under-represented were 8p and 11q (75%), 13q (37%), and 6q, 7q, 16q, 17p, 17q (25%). CGH analysis revealed alterations in fifteen chromosomal regions not detected by conventional cytogenetics.The number of chromosomal imbalances in the three small cell variants was relatively similar to that of cases with typical morphology. These results indicate that T-PLL carry a high number of chromosomal alterations that are not related to the morphological variants or the clinical behavior of the disease.

P116. High incidence of additional chromosomal changes in childhood acute lymphoblastic leukaemia with TEL/AML1 gene fusion

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TEL/*AML1* gene fusion, the result of a cryptic translocation t(12;21), is the most common genetic aberration in childhood B-lineage acute lymphoblastic leukemia (ALL). We investigated 21 cases of ALL with *TEL*/*AML1* gene fusion by cytogenetic and molecular cytogenetic methods and revealed additional chromosomal changes in 20 (95%) patients. Eighteen patients were examined at the time of diagnosis and three in relapse.

11 cases (52%0 had deletion of the normal *TEL* allele. In two this was the only secondary change; in 18 cases 2-6 other secondary changes were detected. In three cases complex translocations involving chromosomes 2, 8 and 13 were revealed and confirmed by FISH. In another 4 cases a double fusion signal appeared in FISH. In one of these both fusion signals were located on two der(21)t(12;21), in another on 12q and 20q and in the third on two isochromosomes 21; the fourth patient was evaluated only on interphase cells.

In another patient, the expected fusion signal of t(12;21) was not observed on der(21)t(12;21) but on 8q. In three patients the reciprocal *CBFA2* signal was observed on 2p, 12q and 21q and in one case it was deleted. Finally, in 13 (62%) of 21 patients with t(12;21), complex rearrangements were revealed using FISH, CGH and/or M-FISH. Our findings indicate the importance of using all cytogenetic and molecular cytogenetic methods and of analysing metaphase chromosomes to allow precise determination of all rearrangements.

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P117. Interphase analysis of del(13)(q14.3) and trisomy 15 in Smoldering Multiple Myeloma patients

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Multiple Myeloma (MM) is a haematological neoplasm characterised by uncontrolled plasma cell accumulation in bone marrow, increased M-protein serum levels and lytic lesions. Partial or total deletions of chromosome 13 and trisomy 15 have been described by conventional cytogenetic and/or CGH studies, and related to prognosis. Smouldering Multiple Myeloma (SMM) patients have diagnostic criteria of MM, but they remain stable and asymptomatic during five or more years. However no data about chromosome abnormalities have been reported. Simultaneous interphase analysis with LSI D13S25 SO and CEP15 (D15Z1) SG, and dual LSI PW/AS probes (Vysis) was performed in 10 cases, to elucidate possible 13q14.3 and 15 aneuploidies. In seven cases CGH studies were also done and metaphase and interphase analysis with both probes of chromosome 15 was performed in ten healthy controls. The LSI D13S25 probe showed del(13)(q14.3) in 40% of samples. Only 10% of trisomy 15 was detected with D15Z1 probe, whereas LSI PW/AS probe detected 70% of cases. FISH results were concordant with CGH studies in SMM patients in all cases where both techniques were used. On the other hand, discrepancies between results of both chromosome 15 probes, CGH studies and FISH controls suggest the presence of a polymorphism in 15p11.2 (D15Z1) region, dissuading their use to evaluate chromosome 15 trisomy in interphase.

P118. Cytogenetic Analysis in Hematological Disorders (Results of Cytogenetic Study of 1200 Bone Marrow Sample)

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Precise therapeutic management of hematological disorders depends on clinical, hematological, as well as cytological studies. During the last five years, more than 1200 bone marrow samples have been analyzed in our center, 734 (61%) were referred for malignant disorders and 171 (14%) for non-malignant disease. 295 cases (25%) didn't have primary diagnosis at sampling, 169 belonged to the first 500 samples. The probable clinical diagnoses for malignant cases were as follow: 237 (32%) for ALL, 226 (31%) AML, 159 (22%) CML, 49 (7%) MDS, 29 (4%) Lymphoma and 29 (4%) for other malignant disorders such as Multiple Myeloma, MPD and CLL.

Chromosomal aberrations were detected in 98 (52%) out of the 190 successful cultures of patients without any diagnosis, 103 (53%) of 196 cultures with diagnosis of ALL, 123 (58%) of 212 AML patients, and 107 (75%) of 142 patients suspected for CML. Among 51 conclusive MDS cases, 28 (55%) patients had some chromosomal changes.

Overall 176 (<15%) cultures failed or the spreads were inappropriate for chromosomal analysis, 77 from among the first 200 samples, 10% failure rate for the remaining 1000 samples. 102 (58%)of the failed cultures had no diagnosis, 39(22%) were referred for ALL, 15(9%) CML, 12(7%) AML, and 8(5%) for other reasons.

Our data indicate that communication between the clinician and the laboratory plays a key role in better cytogenetic results. Lack of proper information on the part of the patient and poor morphology of the bone marrow spreads are constant limitations.

P119. Cytogenetic response to STI571 treatment in Chronic Myeloid leukemia(CML) patients

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Imatinib mesylate (Gleevec, STI571) is commonly used in CML chemotherapy. STI571 is a specific inhibitor of the BCR-ABL tyrosine kinase that can induce hematologic and cytogenetic response.

We report cytogenetic results of 15 chronic phase CML (Chronic myeloid leukemia) patients. A major cytogenetic response was detected in 33% of patients. Loss of Y chromosome (13%), trisomy 8(20%),10 (7%) and 19(13%) were observed in patients showing partial or minor cytogenetic response in addition to Ph chromosome. Numerical chromosomal abnormalities were detected in CML patients analyzed: hypodiploidy (59%), hypotetraploidy and tetraploidy(13%), hyperdiploidy(18%), hypertriploidy(5%) and near-hexaploidy (5%). Double Ph chromosomes, trisomy 8 and 19 were observed in four patients(26%). The most frequent abnormalities were with chromosomes 3, 17 and 7. In addition to these abnormalities dup(3)(p14p24), del(5)(q33), del(7)(p22) , inv(13)(q12q34),del(13)(q 34) , inv(14)(q11.2q22), del(17)(q23), add(17)(q25), inv(17)(q21q25), del(20)(q11),del(20)(q12) were observed in patients who were treated with STI571.

P120. Chromosomal imbalances: a hallmark of tumour relapses in primary cutaneous CD30+ large T-cell lymphoma

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Primary cutaneous CD30+ large T-cell lymphoma (CD30+ CTCL) is a well-defined subset of cutaneous lymphoma usually characterised at presentation by solitary or localised skin lesions and good prognosis. Although frequent spontaneous regression may be observed, skin relapses occur frequently. The genetic changes involved in the pathogenesis of CD30+ CTCL are largely unknown. Moreover, no predictive marker for the risk of cutaneous relapse has yet been identified. From this point of view, a systematic investigation for chromosomal aberrations correlated with clinical outcome of neoplasm is warranted.
The purpose of our work was to investigate the cytogenetic abnormalities involved in a series of CD30+ CTCL samples fulfilling both the EORTC and WHO criteria, by the use of comparative genomic hybridisation (CGH).
CGH analysis revealed a non-random distribution of chromosomal imbalances between relapsing and non-relapsing tumours. The mean number of changes in non-relapsing tumours was 0.33 (range, 0-1), compared with 6.29 (range, 1-16) in relapsing tumours. The recurrent chromosomes involved in relapsing cases were chromosomes 6 (86%), 9 (86%) and 18 (43%). While chromosome 9 was mostly affected by gain, chromosomes 6 and 18 mainly contained regions of loss, exclusively on 6q and 18p arms.
Although further studies, such as microsatellite analysis, are required to delimit the minimal deleted region, for the first time our data pinpoint small chromosomal regions where putative tumour suppressor gene(s) involved in the pathogenesis and the clinical outcome of CD30+ CTCL may be located.

P121. Genetic fingerprinting of patients after allogeneic bone marrow transplantation using recipient mouthwash samples.

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Serial monitoring of chimerism after allogeneic hematopoietic stem cell transplantation (HSCT) can be performed rapidly using PCR-based assays analyzing informative tandem repeat genetic markers. Prerequisite for this type of analysis is knowledge of donor and recipient pretransplantation genotypes. In some cases, recipient cells prior to BMT are not available for genotyping of recipient's VNTRs before transplantation. Our study was to evaluate the feasibility of mouthwash samples that contains mouth epithelial cells as BM recipients only after their genotype in blood cells. Of 19 patients who had undergone BMT, DNA was isolated from mouthwash cell pellets obtained from mouthwashes. PCR analysis of six STR loci on six chromosomes was performed. Even though the mouthwash cell pellets contained about 75% epithelial cells (presumably of recipient origin)and only about 25% leukocytes (presumably of donor origin), three of nineteen patients showed recipient genotype and the rest exhibited chimeric DNA patterns from 5.0% to 60.0%. It means that this DNA contained donor and recipient material in different ranges. From our results it appears that blood cells serve as preferential DNA source in mouthwash samples which can be obtain before BMT.

P122. Follow-up of bone marrow transplantation success by FISH

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Bone marrow transplantation (BMT) have been used in therapy of various disorders in recent years, and is successfully applied in hematological disorders. FISH technique was applied in the follow-up of 10 thalassemia, 2 fanconia anemia and 3 acute lymphoblastic leukemia (ALL) patients subsequent to BMT performed. The rate of chimerism was detected as 100% in two of 15 patients with thalassemia and 94%, 93%, 97% in three patients by this technique, which has a high confidence range in cases with different sex from the donor. The first FISH applied in two cases revealed 98% chimerism, while 100% chimerism was detected in the second FISH performed. One case displayed almost the same amount of chimerism (96% and 98%) in FISH analysis applied two times. Three FISH applications in two patients with thalassemia revealed 86% chimerism in one patient and nearly the same amount of recipient and donor cells in the other case. Another case with thalassemia in a period follow-up displayed 79% chimeric cell rate in the first and 69% chimeric cell rate in the eighth application of FISH technique. FISH was performed also eight times to a patient with thalassemia and 72% chimerism was detected in the first and 27% in the eighth application of FISH. Two patients with thalassemia revealed no donor cells. Interestingly, one patient with Fanconia anemia who developed AML after BMT revealed 4% donor cells only. In conclusion, FISH technique which we perform in the follow-up of hematological disorders subsequent to BMT is a trusty technique.

P123. Uncommon cytogenetic markers at the onset of chronic myeloid leukemia and acute lymphoblastic leukemia

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We present two uncommon chromosomal rearrangements which were identified at first presentation in chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL).
First case: one patient out of 61 with CML at diagnosis exhibited in all bone marrow cells, beside Ph-chromosome, an extralong marker originating from a translocation t(1;1)(p31;p36). The marker was absent in peripheral blood cells, so that it is not a constitutional change, but acquired, and possibly involved in leukaemogenesis.
Second case: a 4 year old boy with B-cell ALL showed in bone marrow cells a derivative chromosome resulting from a translocation t(7;9). Trisomy 7p and monosomy 9p resulted as a consequence of this translocation. Usually, chromosome 7p anomalies are described in T-cell ALL. The translocation t(7;9)(pter-p11;q11), generating 7p trisomy, has not been reported by date, either in childhood or adult ALL. We suppose that this chromosomal rearrangement does not indicate a bad prognosis, because complete remission was rapidly achieved after induction chemotherapy (FRALLE protocol).
These two uncommon markers identified at diagnosis prove that every patient may exhibit, apart from classical cytogenetic rearrangements already assigned to one or another type of leukemia, new chromosomal changes which have to be characterized to find their involvement in the pathogenesis of lymphoid and myeloid disorders.
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P124. An Interphase FISH study of CLL patients.

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Chronic Lymphocytic Leukaemia (CLL)is the most common type of leukaemia in the USA and Europe, occuring mostly amongst the middle aged and the elderly. Chromosome studies of this disease have been hampered by the low mitotic index of the leukaemic cells. The aim of the present study was to validate our FISH screening for cases of CLL, in view of the fact that the majority of cases previously studied by conventional Cytogenetics either gave a normal result, or failed to give a result at all.

Recent studies using fluorescent in situ hybridisation (FISH) have revealed a high incidence of chromosome abnormalities, yielding valuable prognostic information.
This study therefore aims to ascertain whether FISH analysis of CLL patients for chromosome abnormalities yielded valuable information for clinical management.
As a follow up from data presented at the BSHG meeting (September 2002), we report on the first 87 consecutive CLL patients referred only for FISH studies for deletions of 11q22-23(MLL and more recently ATM probe), 13q14, 17p13, rearrangements of 14q32 and trisomy 12.
Our results found an abnormality rate of 68% in the 87 cases studied. 53% were single abnormalities, 15% had two or more abnormalities. These results show that FISH is an effective tool for studying CLL providing valuable diagnostic and prognostic information.

P125. Retrospective screening of Philadelphia positive Chronic Myeloid Leukaemia (CML) patients treated with Glivec for deletions of 9q in relation to their success rate.

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Chronic Myeloid Leukaemia is a clonal haematological malignancy, characterised by overproduction of granulocytes and accounts for approximately one quarter of all leukaemia cases. In the majority of patients there is replacement of normal bone marrow by cells with an abnormal chromosome - the Philadelphia (Ph) chromosome due to the translocation between chromosomes 9 and 22. A cohort of patients with CML has recently been reported to have deletions of the derivative chromosome 9. These deletions are large, occur adjacent to the translocation breakpoint and take place at the same time as the translocation. They are reported to be associated with a poor prognosis and these patients are said to have a median survival rate of half that of those patients without deletions. The BCR/ABL ES probe along with the LSI 9q34 Argininosuccinate Synthetase (ASS) gene probe were used to identify this subgroup of patients with deletions. Deletions were found in 10/59 (17%) patients with Philadelphia positive CML. In this study it was found that there was no significant association between the presence of a derivative chromosome 9 deletion involving the ASS gene and a poor prognosis, however this maybe due to the patients in this study being treated with GLIVEC.

P126. Prognostic Value Of Complex Chromosomal Changes In Children With Acute Lymphoblastic Leukemia (all) And High Hyperdiploidy.

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Children with ALL and high hyperdiploidy (>50 chromosomes) in bone marrow cells have improved outcome in comparison to other ALL patients. However, good prognosis of these patients could be affected by concurrent structural chromosomal abnormalities in hyperdiploid cells. The aim of the study was to evaluate the prognostic significance of complex chromosomal changes in ALL patients with high hyperdiploidy.
For the assessment of hyperdiploidy consecutive double target interphase FISH with combination of alpha-satellite and/or locus-specific probes for 10 chromosomes most frequently overrepresented in hyperdiploid clones were used (200 interphase nuclei analysed per probe-mix, cut-off level 2.5% tested on controls, standard deviation ≥0.5%). The patients were divided into five prognostic groups according to results of molecular-cytogenetics and level of ploidy. Structural and/or complex chromosomal aberrations in hyperdiploid cells were analysed by mFISH.
During the last four years we examined prospectively or retrospectively 89 children with ALL (58 boys, 31 girls; mean age 7,7 years). Cells with high hyperdiploidy were found in 42 patients (47%) and structural or complex chromosomal rearrangements together with hyperdiploidy were ascertained in bone marrow of 11 of them (26,2%). Analysis of event-free survival (EFS) revealed significantly shorter survival in patients with complex chromosomal rearrangements in high hyperdiploid cells in comparison to those with only numerical changes (p=0,0002). Complex rearrangements were always connected with rather fast progression of the disease and poor response to the therapy, even in patients with primary good pognosis.
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P127. Deletion of 12p in hematological malignancies.

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Deletions and rearrangements of the short arm of chromosome 12 are frequent chromosomal changes in patients with hematological malignancies. The loss of genetic material in tumour cells is usually indicative of the inactivation of tumour suppressor genes. The region of 12p has been largely studied by molecular genetic and molecular cytogenetic methods to identify candidate tumour suppressor genes involved in leukemogenesis. The *TEL*/*ETV6* gene is not a classical tumour suppressor gene but is often deleted or rearranged in a variety of hematological malignancies.
In this study we present 10 patients with deletion del(12)(p) found by conventional cytogenetic analyses in bone marrow cells. Patient´s diagnoses were either lymphoid or myeloid leukemias.
We used FISH with the locus-specific DNA probe LSI TEL/AML1 (Vysis ™) to assess deletion of *TEL*/*ETV6* . To excluderearrangements of 12p we examined two patients by FISH with whole chromosome painting probe for chromosome 12 (Cambio™). Multicolor FISH (24xCyte, MetaSystems) was applied in two patients with complex karyotypes.
Deletion of *TEL*/*ETV6* was found in five of eight patients examined. No translocations of 12p were confirmed.
The region closely linked to the *TEL*/*ETV6* gene probably contains one or more genes (tumour suppressors) which play a role in the progression of oncogenesis. We suppose that in patients where deletion of *TEL*/*ETV6* is not confirmed, other genes with tumour suppressor function were inactivated.
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P128. Complex chromosomal rearrangements in patients with myeloid malignancies studied by mFISH and mBAND.

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Specific translocations and chromosomal aberrations as del(5)(q), monosomy 7, trisomy 8, del (11)(q), del(20)(q) can be found in bone marrow of patients with myeloid malignancies. During the progression of the disease further structural and numerical abnormalities can be ascertained. We describe complex karyotype and clonal evolution in bone marrow cells of five patients with myeloid malignancies (4 patients with myelodysplastic syndrome (MDS) and one with chronic myeloid leukemia (CML)). All cases were studied by classical cytogenetic techniques, by fluorescence in situ hybridization (FISH) with locus specific probes (LSI) and/or whole chromosome painting probes (WCP), by multicolor FISH (mFISH) and by multicolor BAND (mBAND). Molecular-cytogenetics results of four patients with MDS are summarized in the table below. One patient with CML had clonal evolution of karyotype: 46,XY,t(9;22)(q34;q11),der(17)t(3;17) (19) /

48,idem,+der(17)t(3;17),+der(22)t(9;22)(q34;q11) (3).
Details of clonal evolution will be presented and prognostic value of cytogenetic and molecular-cytogenetic analyses will be evaluated in the poster.
This work was supported by grants IGA MZCR NC/6675-3 and GACR 301 01 0200.

Patients with MDS:			
1.	female	52 years	45,XX,t(1;17),-7 (22)
2.	male	50 years	46,XY,del(5)(q13q33),ins(17;21) (5) / 46,XY (5)
3.	female	68 years	46,XX,+der(1)t(1;17),der(3)t(3;5;6),del(5)(q31),der(6)t(3;6;8), der(9)t(9;17),+der(11)t(11;18),der(17)t(6;17),-17,-18
4.	male	78 years	44,XY,-4,-5,der(7)t(5;7;10),der(10)t(4;5;10),der(11)t(4;11) (20)

P129. Combined genetic analysis of bi-lineage non-Hodgkin's lymphoma

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Peripheral T-cell lymphomas (PTCL) including angioimmunoblastic lymphadenopathy (AIL) account for 7% of non-Hodgkin's lymphomas. AIL and rare cases of PTCL are associated with large B-cells reminiscent of Reed-Sternberg (RS) cells scattered among neoplastic T-cells. As with Hodgkin's disease, the RS-like B-cells may be infected with Epstein-Barr virus (EBV). We report on a 70 year old woman presenting with an enlarged right cervical lymph node. The majority of cells within the node comprised T-cells staining positive for CD3. In addition, a population of large pleomorphic cells stained positive for the B-cell marker CD20 and EBV. Clonality testing by PCR identified both IGH and TCR clonal rearrangements. Cytogenetics of cultured lymphocytes further revealed an abnormal female karyotype consisting of two apparently unrelated, abnormal clones (A and B). To determine the origins of these clones, FISH using satellite probes specific to each clone was carried out on paraffin-embedded tissue sections previously immunostained for CD20. 30.5% of cells negative for CD20 belonged to clone A and 0.5% to clone B. 24% of CD20 positive cells belonged to clone B and none to clone A. These findings therefore indicate clone A as T-cell derived and clone B as B-cell derived consistent with morphological and molecular genetic findings. Our report represents the first study confirming a genetic basis for this unusual bi-lineage lymphoma.

P130. Chromosomal abnormalities in chronic myeloid leukemia after treatment with interferon-α and imatinib mesylate: report of four cases

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Chronic myeloid leukemia (CML) is genetically characterized by the presence of the reciprocal translocation t(9;22)(q34;q11). However, during the evolution of the disease, additional cytogenetic and molecular aberrations frequently occur. We present four patients with Ph+CML and additional anomalies, for whom the cytogenetic investigation was performed, after different therapeutic approaches, from bone marrow samples using classical methods.
The first case, received interferon-α (IFN-α) as sole therapy. Alongside t(9;22) there were identified: +8, +6, +16,+17.
The second case was treated with imatinib mesylate for 12 months, in accelerated phase (AP). The cytogenetic investigation revealed hyperdiploidy (47-51 chromosomes) with two Ph chromosomes plus trisomy 8 and 9.
The third case received IFN-α along with chemotherapy and was referred for karyotype investigation, in AP. Beside Ph, a derivative chromosome 1, which seems to result from a complex translocation involving chromosomes 1, 3, 8 and 9, was observed in all cells. We intend to apply FISH in order to elucidate this rearrangement.
The fourth case developed AP after 30 months of IFN-α associated with chemotherapy and was treated with imatinib for 3 month prior to karyotype examination. Apart of Ph, a small supernumerary marker chromosome resembling G group was observed.
Complex structural rearrangements may occur in CML in addition to well-known secondary chromosome changes and form marker structures that require extensive analysis. The cases presented here point out that karyotype investigations during therapy are essential not only for practical reasons but also for revealing new insights.
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P131. Cytogenetic and Morphological Characterization of Acute Lymphoblastic Leukemia: A report of 293 cases from Saudi Arabia

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Cytogenetic and morphological data on 293 patients with acute lymphoblastic leukemia is presented. Out of 293 total cases studied, 217 were pediatric ALL and 76 were adult ALL cases. Males were present in 133 of the 217 pediatric patients (61.2%) and in 54 of the 76 adult patients (76%). FAB subtype classification showed 69.5% of pediatric and 63% of adult ALL as L1; 26.4% of pediatric and 28.3% of adult ALL as L2 and 4.2% of pediatrics and 8.7% of adult ALL as L3. Culture success rate was 72% in pediatric and 95% in adult patients. An abnormal clone was detected in 70% of both pediatric and adult cases. Numerical abnormalities were present in 33% of pediatric and 22% of adult cases. Hyperdiploidy (> 50 chr.) was present in 18% of pediatric and 7.5% of adult patients, whereas hyperdiploidy (47-50 chr.) was seen in 4% of pediatric and 2.7% of adult ALL cases. A pseudodiploid karyotype was observed in 71 pediatric cases (37%) and in 35 adult patients (48.6%). The common recurrent structural chromosome abnormalities seen in pediatric ALL cases : der (1)t(1;19) in 11% (8/71); del (9p) in 10% (7/71); t(8;14) in 8.5% (6/71); t(9;22) in 7% (5/17) and t(4;11) in 7% (5/71). In the adult ALL group: t(9;22) was present in 20% (7/35); del (9p) in 11.5% (4/35); t(4;11) in 5.7% (2/35); and del (6q) in 5.7% (2/35). Rare translocations were seen in 5.7% of pediatrics (4/71) and in 20% of adult patients (7/35). .

P132. Chromosomal Abnormalities Detected In Acute Myeloid Leukemia (aml) Patients

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AML (Acute Myeloid Leukemia) describes malignancies of adults and children affecting myeloid progenitor cells. Between 50% and 80% of cases of AML have a chromosomally abnormal clone present in the bone marrow and/or peripheral blood. Herein we documented the chromosomal abnormalities detected in 328 AML patients applied to our department. The most frequent changes were t(8;21)(q22;q22), t(9;22) (q34;q11), -Y, -X, -7, -8 and +8 as reported in the literature. In addition to these most common chromosomal abnormalities seen in AML, we have detected new chromosomal changes including t(2;21)(q11.2;q11.2),t(3;20)(3qter-> 3q13::3q13-> 3q2?::20qter-> 20pter), t(5;22)(q13;q11), t(7;15) (q32;q26), t(8;12)(q13;p11), t(8;12)(q13;p11), t(8;18)(p23;q21.1), t(9;10) (q21;q26), t(12;18)(p11;q11), t(15;17;2 1)(q24;q23;q21), inv(5)(p11q13), inv(7) (p11q32), inv(7)(p22q32), inv(10)(p11q11), inv(13)(p12q32), inv(13)(q12q22), inv(17)(p11q15), inv(18)(p11.2 q23), del(2)(q33q35), del(4)(q27), del(6)(q26), del(13)(q12) and dup(14)(q24q32). These abnormalities have never been reported before in AML patients.

P133. Mutational analyses of NF1 and NF2 genes.

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In occurence of mutation , NF1 and NF2 are genes responsible for development of neurofibromatosis type 1 and neurofibromatosis type 2, respectively, genetically distinct clinical syndromes with development both benign and malignant tumors predominantly affecting the nervous system. **NF1** gene is located on chromosome 17q11.2 and codes protein neurofibromin which is connected with cell cycle regulation and acts as tumor suppressor. Neurofibromatosis type 1 is inherited by an autosomal dominant mechanism, however, 50% of found mutations arise *de novo*. Wide spectrum of known mutations (about 500) and mutation types (from point mutations to complex of rearrangements), absence of expressive hot spot regions and size of NF1 gene (350 kb, 60 exons) made us change our diagostics strategy

from primary DNA- based screening of 8 exons (6, 12b, 16, 28, 29, 30, 31, and 37) and linkage analysis to cDNA-SSCP based approach which enables us to examine whole NF1 coding region.
Gene **NF2** was identified on chromosome 22q12.2, comprises only 17 exons and codes merlin protein with homology to the ERM protein which are thought to play role in linking cytoskeletal components to cell membrane glycoproteins. Mutations (almost 200) in this gene are also spread through whole gene. We are starting with molecular diagnostics of this disease by capillary SSCP mutational screening of all 17 exons.

P134. Congenital polyonychia in an infant and ectrodactyly-ulnar aplasia in his sister: A new syndrome?

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Congenital polyonychia (CP) is a rare anomaly that implies the presence of an ectopic supernumerary nail on the volar aspects of the tips of the digits or rarely, on toes. In the more than 30 reported cases, most sporadic and from Japanese extraction, CP generally involving the 4th, and most frequently 5th fingers. We report a Mexican family in which the proposita showed CP in both fifth fingers and his sister presented ectrodactyly with ulnar ray digits deficiency and ulnar aplasia. In previous reports, CP has been related to ectrodactyly with involvement of the ulna and ulnar digital rays as well as with postaxial polydactyly. Thus findings observed in our family can be included in the expression spectra of CP. Autosomal dominant inheritance for CP is more likely based in previous informative families with vertical transmission with instances of male-to-male transmission. Present and two previous families with affected sibs only, could be explained by parental gonadal mosaicism, whereas *de novo* mutation or incomplete searching could be an alternative explanation for sporadic cases. This variable expressivity in the CP spectra could be recognized as a new entity with probably autosomal dominant inheritance proposed as congenital polyonychia-postaxial limb defects syndrome.

P135. The preaxial deficiency-postaxial polydactyly-hypospadias syndrome: case-report

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We report on a 35-year old male, who was advised about the excretory infertility (azoospermia). The patient was born with preaxial deficiency (hypoplasia of the thumbs and halluces), postaxial polydactyly of the toes and glandular hypospadias. In addition to the phenotype one can mark clinodactyly, limitation of movement of the interphalangeal joints of the thumbs, one cafe au lait spot (1.5 cms in diameter) in the back's region. The patient has normal level follicle-stimulating and luteinizing hormones. Karyotype 46,XY.
We decided this disorder is distinct from the hand-foot-genital (HFG) syndrome which is resembled in some ways. We suggest that we have reported the rare case of the preaxial deficiency-postaxial polydactyly-hypospadias syndrome (Guttmacher syndrome).

P136. Fibular hypo-/aplasia and brachy-/oligodactyly – clinical presentation of 2 cases

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Deficiency or absence of fibula is a rare congenital anomaly. Its inheritance pattern is either autosomal recessive or dominant. It can be an isolated deformity or part of a malformation syndrome e.g. with brachy-/oligodactyly. We report two families (a sporadic case and a familial one diagnosed in a mother and her child) where fibular hypo-/aplasia was associated with multiple anomalies of hands and feet. In the sporadic case of a 3-month-old girl bilateral oligodactyly with partial syndactyly and shortening of ulnae were also noted.
In the familial case Madelung deformity of forearms, metacarpal shortening, thumb hypoplasia, brachydactyly and significant "ball-like" shortening of toes in a 27-year-old mother and her year-old daughter were observed. In this presentation we provide detailed clinical characteristics of both cases and discuss differential diagnoses including genetic counselling aspects in these families.

P137. Two sibs with bilateral renal agenesis and severe tetramelic oligo/syndactyly; a new autosomal recessive condition?

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We report on a brother and sister with renal agenesis and tetramelic cutaneous syndactyly as well as osseous oligosyndactyly and bilateral radio-ulnar synostosis. The features in the second child were detected prenatally by ultrasound examinations. The X-rays of the feet showed bony fusions in the post-axial rays.
Cutaneous syndactyly and bilateral renal agenesis are features of the Fraser syndrome, the autosomal recessive cryptophthalmos syndrome. However, the combination of normal eyes and the fusions of metatarsal and metacarpal bones were never described before in Fraser syndrome. In addition laryngeal abnormalities were absent. These sibs appear to have a unique acrorenal syndrome with probable autosomal recessive inheritance.
The *formin* gene controls polarising activity in vertebrate limb buds and is also expressed in the ducts and tubules of the developing kidney. Mice homozygous for mutations of *formin* have symmetrical limb malformations consisting of syndactyly, bony fusions, radioulnar and radiotarsal synostosis and in addition renal malformations like hypoplasia or agenesis. The human *formin* gene or one of its downstream targets might be good candidate genes for this acrorenal syndrome.

P138. Three cases with limb anomalies

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We present two patients with Tibial aplasia-Ecterodactyly syndrome. This pattern of malformations includes split-hand/split foot, absence of Arms&Legs. Our patient is an 18 month old male born to unrelated parents that suffered from Hypoplastic /absent tibia, split hands, Hypoplastic/absent carpals, Absent metacarpals & metatarsals, and missing phalanges of the toes. There was bilateral patellar hypoplasia and contracted knee joints. The second case is a two year old male with a reduction deformity of arms, absent hand, hypoplastic/absent carpals, absent finger/toe or oligodactyly, absent metacarpals & metatarsals, hypoplastic or absent tibia.
The second syndrome is Fibular aplasia- oligodactyly-camptomelia originally reported by Hecht& Scott (1981). Our case was a one year old male from unrelated parents that suffered from a reduction deformity of arm, absent hands, oligodactyly, absent lower limb, bowed and hypoplastic femur, hypoplastic/absent fibula and tibia, absent feet and toes, and syndactyly of toes .
Our two cases with tibial aplasia- Ecterodactyly are similar to patients that reported with this syndrome in the literature, but our case with fibular aplasia- oligodactyly- camptomelia may be similar to the femur-fibula- ulna complex (FFU syndrome) although ulnar deficiency was not present in our case. On the other hand femoral deficiency has not been reported in fibular- oligodactyly- camptomelia.

P139. A new cardiomelic malformation pattern: complex tetramelic syndactyly and bradyarrhythmia

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The purpose of this report is to present a new association of congenital anomalies involving an unclassified tetramelic syndactyly

and cardiac arrhythmia. Fetal bradycardia was detected in a healthy 28 years old woman, gesta III para II during her 3rd gestation. Echocardiography was normal with heart rate of 60 beats per minute. Major structural defects were also excluded by morphological ultrasound examination. There was no history of parental consanguinity or exposure to any probable teratogen. Maternal infections as well as antinuclear antibodies were all absents during gestational period. A male child was born at term by cesarean section due to the difficulty to monitor fetal heart rate. Physical examination showed bilateral complete syndactyly between 1st-2nd and 3rd-4th-5th fingers with fusion of distal phalanges by X-ray evaluation. In both feet syndactytly was also complete involving 1st to 4th toes. Echocardiography was normal, electrocardiogram demonstrated atrioventricular block, Mobitz type II, resulting in a heart rate of 55 beats/min and cardiac failure. A pacemaker was implanted and the child had a good clinical evolution. Further neonatal evaluations, hearing and cytogenetic analysis were both normal. The heart-hand syndromes mainly involve limb deficiencies and cardiac structural defects. Syndactyly and cardiac arrhythmias has been described in only six cases in which syndactyly type I was associated with long QT interval. The patient here described represents a new cardiomelic pattern. Therefore, this case prompts us to recommend electrocardiogram examination for all children with apparently isolated syndactyly.

P140. Preaxial Syndactyly - A Case Report.

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The syndactylies are a heterogeneous group of anomalies in which the digits of the hands or feet fail to separate completely. In the fingers these most frequently involve digits 3 and 4, and in the feet digits 2 and 3 or 4 and 5. Rarely digits 1 and 2 alone are involved. In fact, only one such family has been described thus far in the literature, and appears to be inherited in an autosomal dominant manner.
We present a female infant who was born with unilateral preaxial syndactyly of the upper and lower limbs, unilateral fibular agenesis and bilateral choanal atresia. The limb anomalies were diagnosed prenataly at 21 weeks gestation, where the syndactyly was seen as oligodactyly. There were no other anomalies present in this otherwise healthy child. Her development was within normal limits.
We will discuss the natural history of this patient‘s congenital anomalies from her initial diagnosis in utero to today.

P141. Nonsyndromic Hypodontia - Clinical Variability and Genetic Heterogeneity

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Congenital absence of one or a few permanent teeth without any systemic disorders is relatively common and often hereditary. In dental practice may be encountered a vast spectrum of clinical manifestations in patients with isolated hypodontia. It is generally assumed that variation in clinical phenotypes is cause by different mutated genes. Two mutated genes, MSX1 and PAX9, causing a few forms of human hypodontia, were identified. Intraoral and radiographic examinations were performed. We analyzed phenotype and inheritance pattern of the condition in 238 family members, in two or three generations, of 18 probands. The permanent teeth most frequently missing were upper lateral incisors, followed by the maxillary third molars and mandibular second premolars. Less frequently, hypodontia occured unilateral; the left side of the upper arch was more involved. The absence of a maxillary lateral incisor was often associated with a small peg-shaped contralateral tooth. Associated tooth number abnormalities were recorded. For many clinical phenotypes, pedigrees and segregation analysis was sufficent to demonstrate genetic heterogeneity. In ten families the inheritance pattern of the hypodontia confirms the autosomal-dominant transmission with reduced penetrance and variable expressivity. Five families showed an autosomal-recesive manner of inheritance. Three sporadic cases occured. Family studies have showen hypodontia at higher than in general population frequency in relatives of the probands. We concluded that hypodontic phenotypes are determined by different genes involving locus heterogeneity.

P142. Autosomal dominant form of congenital single coronary artery : the first reported family.

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Anomalous origin of the right coronary artery from the left main coronary artery is a very unusual congenital anatomic variation. In 1992, a 64 year old man presented with a recent myocardial infarct. The coronary angiogram revealed a single coronary artery : single left coronary ostium with a left main trunk and the right branch arising from the superior side of the left main coronary. Two significant stenosis located on the right branch were successfully treated by conventional transluminal angioplasty. In 2002, the only son of the patient was admitted for unstable angina and the coronary angiogram demonstrated a similar single coronary artery with significant stenosis of the three branches. He was successfully managed with surgical revascularization. His mother’s coronary distribution, who presented too with coronary disease, was normal, suggesting a paternal transmission with autosomal dominant inheritance.
Several sporadic cases of this malformation have been reported. A maternal transmission of this congenital anomaly had been previously suspected in a family with an affected 12-year-old male whose both the deceased mother and maternal grandmother died suddenly. Nevertheless, the malformation was not proved in both the mother and grandmother.
At our knowledge this is the first reported familial case of single coronary artery, with male to male transmission, highly suggestive of a dominant autosomal disease. In sporadic cases, it could be suggested to do familial investigations, although this raises ethical problems. Diagnosis of other familial cases should be necessary to initiate molecular studies for searching the disease causing gene.

P143. 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 shortened axial skeleton. The main features are short, immobile neck and small thorax with the pathognomonic “crab-like” rib cage associated with multiple vertebral defects. This frequently leads to respiratory problems and death in infancy. Careful 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 other four infants presented features of SCD. These had short neck and trunk, different degree kyphoscoliosis and occasionally spina bifida. All six patients were sporadic, and parental consanguinity was present in 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.

P144. An apparently new syndrome of sensorineural deafness, distinctive facial features, exomphalos, hypoplasia of the corpus callosum, seizures and mental retardation

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Genetic syndromes often have variable expression both regarding the presence or absence and the severity of specific malformations/ traits. When the specific diagnostic test is lacking it is often difficult to decide if a patient presents an atypical case of an already described rare syndrome or a distinct entity.
We report on a three and a half-year-old boy with a multiple congenital anomaly-mental retardation syndrome characterised by profound sensorineural hearing loss, severe hypermetropia, exomphalos, bilateral inguinal hernia, hypoplasia of the corpus callosum, seizures and developmental delay. He had dysmorphic facial features including wide forehead, high arched eyebrows, telecanthus, down-slanting palpebral fissures, high and prominent nasal bridge and submucosal cleft. High resolution karyotype and multicolor subtelomeric chromosome screening by fluorescent in situ hybridisation yield normal results. The patient shows overlap with the two rare autosomal recessive disorders - Malpuech syndrome characterised by growth retardation, hypertelorism, facial clefting, hearing impairment, inguinal hernia, and urogenital abnormalities and the syndrome described by Donnai and Barrow (1993) consisting of diaphragmatic hernia, exomphalos, absent corpus callosum, hyperteorism, myopia and sensorineural deafness. However, the pattern of anomalies in our patient does not support convincingly any of the given diagnostic possibilities which lead us to believe that this constellation of anomalies represents a distinct clinical entity.

P145. Iniencephaly and Klippel-Feil syndrome within the same sibship : further evidence for a continuum of malformations belonging to the same autosomal recessive syndrome

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Iniencephaly is a major defect of the cervical spine. Most of the cases are lethal. It belongs to the NTD (neural tube defects) spectrum of malformations, alongside anencephaly and spina bifida. More than hundred cases have been reported in the medical literature, most of them of sporadic occurrence. Klippel-Feil syndrome is a short-neck syndrome which is invariably associated with fusion of two or more vertebral bodies. We present the unusual observation of an iniencephaly case detected by ultrasound in the second trimester of pregnancy in a 25 years-old caucasian female. There was nos consanguinity between her and her husband. Pregnancy was terminated. The patient became pregnant soon after and miscarried an anencephalic fœtus at twelve weeks. Interestingly, among the three live born boys of the patient, the oldest was referred at seven years for shortness of the neck. Skeletal survey disclosed several vertebral bodies fusions establishing the diagnosis of Klippel-Feil syndrome. Concurrence of these three malformations in the same sibship is compatible with variable expression of a recessive condition.

P146. Two sibs with sensorineural deafness and apparently mild variant of Roberts syndrome

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Roberts syndrome (RS) is a rare autosomal recessive disorder characterized by symmetric limb defects, craniofacial abnormalities including cleft lip/palate, somatic and developmental delay, and a variety of other less common abnormalities. Limb defects vary from the complete tetraphocomelia to mild growth deficiency. There are very few reports on mild clinical presentation, but even mild cases exhibit at least minor limb involvement. Chromosomes of RS patients often show characteristic cytogenetic phenomenon called RS effect, premature centromere separation (PCS) or heterochromatin repulsion, consisting of puffing or splitting apart of the constutive heterochromatin around the centromeres, and splaying of the acrocentrics and distal Yq. Here we present the results of the cytogenetic and clinical investigation in two sibs, brother and sister, with typical RS phenomenon in their cells, the brother having additionally 47, XYY karyotype. The clinical presentation in both sibs is highly atypical. Although they both have prenatal and postnatal growth retardation, microcephaly, and mild developmental delay, no limb involvement, no dysmorphic features, and no abnormalities described in RS patients are observed, including hypoplastic nasal alae and hemangiomata considered to be specifically associated with mild cases of RS. On the other hand, they have a severe sensorineural hearing impairment which has not been described in RS patients. Cytogenetic analysis from both healthy parents revealed normal karyotype with no evidence of PCS. We conclude that our patients present either an unusual mild form of RS or a new syndrome with RS effect consisting of severe sensorineural deafness, growth retardation and mild developmental delay.

P147. Lethal immunodeficiency and heterogeneity of X inactivation phenotype in a large family with a splicing mutation in the *NEMO* (*IKK-gamma)* gene

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Mutations in the NF-κ B essential modulator (*NEMO, IKK-gamma*) gene are the cause of a group of disorders with immunodeficiency and ectodermal dysplasia (EDA-ID). We report a family with seven males with an X-linked immunodeficiency. They were dystrophic at birth, and were either stillborn or died within eight months, except one boy who died at age five years. This boy had cone-shaped teeth and oligoodontia. He had serious bacterial infections and inflammatory bowel disease. Analysis of the *NEMO* gene revealed a nucleotide change in the consensus sequence of the splicing donor site of exon 6 (gtGAG/gtGAA) which has not previously been described in EDA-ID. NEMO protein analysis in fibroblasts from an affected male fetus showed a faint band corresponding to the 48 kD normal NEMO protein. A 32kD protein revealed by NEMO antibody which may represent the truncated version of the NEMO protein was also observed. Analysis of IkBα degradation showed that this was strongly impaired in fibroblasts carrying the mutation. Three female carriers were healthy, and had only subtle teeth anomalies. One carrier had a completely skewed X inactivation with the normal X as the active X chromosome, whereas the other two carriers had a random X inactivation. This indicates that this mutation is not deleterious enough to cause lethality in cells with an active mutant X chromosome. The diagnosis in this family could easily be overlooked, since one male only lived long enough to manifest tooth anomalies. This family may represent a new phenotype within the EDA-ID disorders.

P148. EEC syndrome, R227Q *p63* mutation and micturition difficulties: Is there a genotype-phenotype correlation?

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We report three individuals with EEC syndrome (ectrodactyly, ectodermal dysplasia, cleft lip/palate) aged 2, 28 and 66 years from a three generation kindred in which no individual had ectrodactyly or cleft lip. They presented with micturition difficulties, ectodermal dysplasia affecting nipple and breast development, dacryostenosis, hypoplasia of the perineal body and mild post-axial digital hypoplasia. One individual had anal stenosis and another a cleft uvula. An R227Q *p63* gene mutation, previously reported in EEC syndrome, was identified. Severe micturition difficulties presented in early childhood with biopsy-proven interstitial cystitis in both the proband and her mother. Mucosal atrophy, which has been postulated to underlie the micturition difficulties, was not observed. Ulceration, haemorrhage and urethral stenosis developed in late childhood and for reasons unknown, the symptoms spontaneously remitted in early adulthood. Two of three other families described with an R227Q mutation also had severe micturition difficulties, indicating this may be mutation-specific.

P149. Dominant inheritance of Moya-Moya syndrome

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Moya-Moya is a radiological syndrome consisting of a cigarette smoke-like appearance of cerebral arteries arising from circle of Willis. Neurological signs and symptoms are highly variable. Most of the cases occur as sporadic events but less than ten percent are transmitted in a Mendelian fashion. Among these, a minority have been found to be compatible with autosomal dominant inheritance. We describe a 17-year old girl who suffered transient right hemiparesis with spontaneous recovery and relapse after a few weeks. She was found to harbor a typical Moya-Moya appearance of the middle cerebral artery. Interestingly her mother had a similar episode when aged 24 but didn't recover. She also had Moya-Moya and a family history of mental retardation and seizures in her deceased brother. Linkage studies performed by two Japanese groups in familial cases of Moya-Moya (mostly recessive) yielded positive LOD scores for 17q25 and 3p24.2-p26. In the former, a good candidate gene is fibulin-2.

P150. Exclusion of candidate genes in a family affected with arterial tortuosity syndrome

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Arterial Tortuosity Syndrome (ATS) is a rare hereditary disorder characterised by tortuosity and elongation of the major arteries, often associated with pulmonary artery stenoses (MIM 208050). Some patients also show excessively stretchable skin and joint laxity, suggestive of a connective tissue disorder such as Ehlers-Danlos syndrome (EDS). In the majority of the ATS families described, the analysis of the pedigrees has suggested an autosomal recessive transmission of the disease. We report an inbred Italian family in which 5 patients show signs of ATS; in particular, a severe pulmonary valve stenosis has been observed in one patient, while in all the others arterial tortuosity and elongation of the main arteries were present. In two of these latter patients, peripheral stenoses of the main pulmonary artery have also been detected. In the ATS patients signs typical of EDS were also observed: soft skin with an abundant subcutaneous tissue and/or ecchymoses, joint laxity, hernias, disorganisation of the extracellular matrix of fibronectin and of actin microfilaments in skin fibroblasts. In order to ascertain the possible involvement of genes responsible of EDS or other connective tissue disorders, autozygosity analysis was performed for some of these genes: COL1A1, COL2A1, COL3A1, COL5A1, COL5A2, COL5A3, COL6A2, ADAMTS-2, ELN, FN1, TNXA, TNXB. The segregation analysis excluded all these candidate genes from ATS in this family, indicating that ATS is a distinct entity differing from the so far characterised EDS forms. A genome-wide scan and search for the disease gene are in progress.

P151. A new disorder of brain development affecting grey and white matter-a single gene defect?

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Background: Many cases of primary congenital microcephaly show an autosomal recessive mode of inheritance. Genetic studies in affected families have enabled identification of novel genes important for normal brain development. We describe clinical, neuro-physiological and neuro-radiological findings in three related children from a highly consanguineous pedigree who present with a characteristic phenotype.
Subjects and Results: Three children,(two male, one female), each born of related, consanguineous parents presented with congenital microcephaly and severe failure of postnatal brain growth. Intractable epilepsy started in the neonatal period, characterised by multiple seizure types. EEG showed high amplitude multi-focal epileptic discharges. Failure of pyscho-developmental progress was evident in each child with axial hypotonia, variable limb tone and paucity of spontaneous movements. Muscle bulk was normal with no development of joint contractures. Deep tendon reflexes were variably present.All 3 children had severe visual impairment with evidence of optic nerve atrophy, but no other ophthlamic abnormalities. Ultrasound cerebral scans in the newborn period showed similar features, confirmed later on MRI brain scans: severely attenuated corpus callosum, brainstem and cerebellar hypoplasia, simplified gyral pattern, lateral ventricle dilatation, (particularly posteriorly), white matter changes consisting of subcortical and periventricular cystic areas. Investigations included a normal karyotype with no micro-deletion of 17p13.3, normal CK and viral studies and negative neurometabolic workup.
Conclusion:We propose that the phenotype in this family represent a new, autosomal recessive disorder of brain development. Autozygosity mapping is planned which may facilitate identification of the causative gene.

P152. One more case of the Bardet-Bield syndrome with situs inversus

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To our knowledge, there is only one case of the Bardet-Bield syndrome with situs inversus described in literature (Lorda-Sanchez et al., 2000). It is assumed that this combination takes place in 1:0.8-1:1.5 mln newborns.
That is why we find it interesting to present our own case.This concerns a seven-year old girl with obesity from early infancy (current weight 44 kg), mild mental retardation, postaxial polydactyly of the hands, retinal dystrophy, poor visual acuity, situs inversus, and mild aortic stenosis. In addition she had mild enophthalmos, strabismus, irregular teeth, brachydactyly, simian crease on the left side and muscular hypotonia. Hypothyroidism was documented. A history of repeated bronchitis and pneumonias was noted. The karyotype was 46,XX.

P153. The case of Ohdo syndrome in Ural's region

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We observed an one-year-old girl in the Regional Pediatric Clinical Hospital. The main reason of genetical consultation was the developmental delay. The proband had the following dismorphological

features: microphtalmia, ptosis of eyelids, blepharophimosis, epicanthic folds, simple, dysplastic and low-set ears; microgenia, high palate, abnormally shapped and wide-spaced teeth; flat nasal bridge; second and fourth toes on the feet overlapping third. The congenital heart disease (ventriculoseptal defect) and the hypoplasia of the corpus callosum were revealed. The cytogenetic analysis was normal (46,XX). The parents of the proband were 34-years-old healthy people. The proband had a healthy half-sibling. This pregnancy was complicated with the risk of interrupted and polyhydramnion. The childbirth was normal, however the newborn had severe neurological problems and trombocytopenia. According to the indicated features and symptomes we diagnosed Ohdo syndrome (mental retardation, congenital heart disease, ptosis, hypoplastic teeth). The case was sporadic in the family. So it was impossible to establish the type of inheritance. We report the first case of this syndrome in our region.

P154. Phenotypical variability in Turkish juvenile hyaline fibromatosis families linked to 4q21

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Juvenile hyaline fibromatosis (JHF, MIM 228600) is an autosomal recessive disorder manifesting skin tumors, gingival fibromatosis and flexion contractures of joints. Recently, this disorder was mapped to chromosome 4q21 using Indian, Turkish, Moroccan and European families (Am. J. Hum. Genet 2002; 71: 975). Previously, we described a Turkish family (Family A) with five siblings three of whom are affected with JHF. Molecular analysis confirmed linkage to the JHF locus on chromosome 4q21.We have compared the phenotype of the two Turkish JHF families shown to be linked to chromosome 4q21. Family A was reported by Balci et al (Eur. J. Hum. Genet 2002; 10: 121) and originates from Eastern Turkey. Family B was ascertained independently by Keser et al (Clin Rheumatol 1999; 18: 248) and originates from Western Turkey. The age of onset and severity of the symptoms are different between these two families. One affected member did not develop the disorder until 9 years old in Family B whereas very early onset was observed in Family A. Although scalp tumors and gingival hyperplasia were consistent findings, in both families the severity of locomotor problems was very different. None of the affected siblings in Family A were ever able to walk whereas only one affected case in Family B had locomotor failure and this was not observed until 14 years old. Our analyses confirm the location of a JHF predisposition gene on chromosome 4q21 and demonstrate that the phenotypic expression of JHF gene mutations is likely to be variable.

P155. Aqueductal stenosis in Townes-Brocks syndrome

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Townes-Brocks syndrome is an autosomal dominant trait described in 1972. The gene is on chromosome 16q 12.1 but direct DNA testing is not a part of standard medical care yet. The main clinical manifestations are ear abnormalities, including hearing loss, anorectal and urogenital abnormalities, varied polysyndactyly, and infrequent cardiovascular anomalies. A male proband, ES had the phenotype of the syndrome with additional aqueductal stenosis and XY/XYY mosaicism in peripheral lymphocytes. The hydrocephaly prompted a retrospective clinical analysis of the 11 probands with Townes-Brocks syndrome evaluated between 1/2/82 and 11/30/02 at the University of South Florida genetic clinics. The probands/families were part of the 42,641 families evaluated at the clinics during the same period. Another proband, DH had affected first and second degree maternal relatives. By report one of them, a maternal half aunt had microtia, hearing loss and aqueductal stenosis leading to hydrocephaly and mental retardation. The two patients suggested that aqueductal stenosis might be an infrequent anomaly in Townes-Brocks syndrome. Regarding the aqueductual stenosis of ES, the mosaicism XY/XYY is probably coincidental.

P156. Revisiting the unique survival case of chondrodysplasia-pseudohermaphrodism syndrome first described in 1992.

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In 1992, Nivelon described a new autosomal recessive chondrodysplasia-pseudohermaphrodism syndrome (MIM600092). Both siblings presented severe dwarfism with generalised chondrodysplasia, microcephaly with cerebellar vermis hypoplasia, facial dysmorphism, hypoplastic iris, papillous coloboma and mental retardation. The first sibling had a 46,XY karyotype despite normal female phenotype and SRY gene analysis was normal. Recurrence in the second sibling (46,XX) led to termination of pregnancy. In the literature, no other case was secondarily described. Here, we report on the 16 year follow-up of this unique survival case. She presents with severe growth retardation (length -9SD, OFC -9SD) and moderate mental retardation. She currently has a normal speech and is in a school for special needs. Facial dysmorphism includes up-slanting palpebral fissures, large mouth and mild prognathism. She has a spontaneous attitude with an anteriorly bent thorax with rigid spine, brachydactyly, muscular hypertrophy and myopia. Endocrine studies performed because of total impuberism reveals hypergonadotrophic hypogonadism. X-rays show narrow, bell-shaped thorax with thin ribs and posterior vertebral notches, micromelia with enlarged epiphyses and metaphyses with striations, thin diaphyses, short femoral neck, narrow iliac wings, severe brachydactyly, rounded metacarpals and metatarsals, very short phalanges and a spondylolysthesis. She complains of frequent generalised muscular contractures since 9 years of age, CPK level 695UI/l, electromyography showed spontaneous muscular activity in favour of myotony. Cholesterol metabolism tests, other metabolic studies, high resolution chromosome analysis and cardiac, renal and pelvic ultrasound are normal. Reports of other patients with the same association would be helpful in defining this rare entity.

P157. Meier-Gorlin syndrome: Delineation of the adult phenotype

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Meier-Gorlin syndrome (MGS;MIM224690) is an autosomal recessive disorder, characterised by microtia, patellar a-/hypoplasia, growth retardation, a typical craniofacial appearance, genitourinary tract malformations, and skeletal anomalies. In the absence of a specific molecular marker, the diagnosis is established on the classical triad of clinical features (ear and patellar anomalies and short stature) and a characteristic facial appearance at young age. Previous data on MGS are mainly derived from transversal observations of children and longitudinal studies are lacking. This study aims to delineate the MGS phenotype during ageing in a cohort of thirteen previously described cases and seven new patients, including two pubertal boys, two postmenarcheal girls, and two male and six female adult patients.

The facial characteristics of MGS children, including full lips, beaked nose, and micrognathia, change to a more prominent triangular face and a small high nasal bridge during ageing. Mean female adult height was 145 cm and the height of two male adults was 154 cm

and 155 cm, respectively. Growth hormone tests were normal in most cases and growth hormone therapy revealed no significant benefit. Hypoplasia of labia minora/majora and scrotum was found in young females and males, respectively. Median age at menarche was 13 years with regular cycles. However, mammary hypoplasia was shown in all postmenarcheal and adult females with no effect of oestrogen replacement therapy in three treated patients. In both sexes, axillary hair development was sparse or absent, but pubic hair development was normal. This study might contribute to a recognisable adult MGS phenotype.

P158. Pelizaeus - Merzbacher Disease. Case Report

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The authors present a family with occurrence of Pelizaeus - Merzbacher syndrome - disease X linked recessive inheritance. PLP /proteolipid protein gene/ has been mapped to Xq21.3 - q22. In the family, there have been eleven affected cases in six generations. Six of the affected boys are still living, four of them have a positive DNA diagnosis - PLP gene duplications. All affected boys have a normal karyotyp. Two have not been examined due to incooperation of the family. One of the affected boys and two women carriers have astonishing molecular finding. We consider intephase fluorescence in situ hybridization analysis. At the four boys with positive DNA analysis the classic type of PMD is assumed. In the family, there has been recorded a high occurrence of carriers, which is confirmed by the results of the DNA analysis. The clinical features are variable as well as the symptoms, where neurologic symptomatology - rotary nystagmus, head shaking, hypotonia, choreoathetosis and mental retardation is dominant. Prenatal genetic diagnosis in the observed family is feasible.

P159. Laterality defect, absent corpus callosum and craniosynostosis in female monozygotic twins – a new syndrome?

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We report female monozygotic twins, the first children of consanguinous Somalian parents, with a laterality defect, an absent corpus callosum and craniosynostosis. Both twins have asplenia and pulmonary stenosis. Twin 2 has had surgery for intestinal malrotation, twin 1 has a redundant duodenal loop which is managed conservatively. Twin 1 has also been shown to have left optic nerve aplasia with microphthalmia. Both twins are currently being evaluated for craniosynostosis and facial asymmetry. This combination of features has not been described previously, and may represent a new recessive syndrome.

P160. A homozygous missense mutation in the IGF-I gene leads to severe dwarfism, microcephaly, deafness and mental retardation, while heterozygosity is associated with moderate impairment of statural and cranial growth.

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The proband is an extremely short, totally deaf and mentally retarded man born as the first of 5 children of consanguineous parents, after 8 months of gestation. He was severely growth retarded at birth, with a birth weight of 1420 g (-2.5 SDS) and length of 39 cm (-3 SDS). At the age of 55 years height was 117.8 cm (-9.0 SDS), weight 24.5 kg, BMI 17.9 (-2 SDS), and head circumference 44.2 cm (-8 SDS). Serum IGF-I was 79 nmol/l (+7.3 SDS). DNA analysis revealed a homozygous G>A nucleotide substitution in exon 4 of IGF-I. This mutation changing valine 44 into methionine is located in a receptor binding domain of IGF-I and is expected to result in inactivation of IGF-I.
Of 24 family members, 9 turned out to be carrier of the mutation. Carriers of the mutation tended to be shorter than non-carriers (-0.91 vs –0.40, p=0.09), had a significant lower head circumference (-1.00 vs +0.46, p=0.001) and a significant higher serum IGF-I (0.50 vs –0.29, p=0.04) compared to non-carriers. No significant association with hearing loss could be found.
This is the first case of a homozygous missense mutation of IGF-I, and its similarity with the earlier described case with a deletion of exon 4 and 5 of IGF-I is further proof of the critical role of IGF-I in pre-and postnatal growth and early brain and auditive development. Furthermore, we have shown that a heterozygous inactivating IGF-I mutation may lead to moderate reduction of stature and head circumference.

P161. Characterisation of chromosomal breakpoints in patientswith hearing loss and microcephaly and identification of candidate genes

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Primary microcephaly is a genetic disorder, used to describe patients with head circumference less than 3 standard deviation below mean and without any other malformations. Primary microcephaly causes mental retardation and it is usually inherited as an autosomal recessive condition. Five different primary microcephaly loci have been described. Causative genes have been identified for the MCPH1 locus (microcephalin, 8p23) and for the MCPH5 locus (*ASPM*, 1q31), while genes for three other loci MCPH2 (19q13.1-13.2), MCPH3 (9q34) and MCPH4 (15q15-q21) are yet unknown. We have studied two patients, A and B, with microcephaly and hearing loss, where patient B also had microphthalmia. The karyotype of patient A was 46,XY,t(1;16)(q31;q13) and the karyotype of patient B was 46,XX,t(1;4)(q31;q21),t(3;13)(p13;q34). Since both patients had common breakpoints at 1q31, we have mapped these by FISH analysis. The two breakpoints were 9.5 Mb apart and more than 4 Mb away from the MCPH5 gene *ASPM*. FISH analysis showed that *ASPM* was present in both patients. The *ASPM* gene will be screened for mutations, minor insertions and deletions to reveal whether *ASPM* is involved in the abnormalities. The BAC truncated in patient A contains two RefSeq genes, which may be considered as candidate genes.

P162. Clinical and molecular analysis of 9 families with Adams-Oliver syndrome.

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Adams-Oliver syndrome (AOS) is defined by the combination of limb abnormalities and scalp defects, often accompanied by skull ossification defects. In this study, clinical analysis of 24 patients belonging to 9 different families have been performed. This revealed that in our patients, scalp abnormalities were most often found, followed by limb and skull defects. The most common limb abnormalities appeared to be brachydactyly, syndactyly of toes 2 and 3 and hypoplastic toenails. Additional features observed were cutis marmorata telangiectatica congenita, cryptorchidism and cardiac abnormalities.
In an attempt to identify the disease-causing mutations in our families, we selected two genes, ALX4 and MSX2, which were considered serious candidates based on their known function in skull and limb development. These genes were then evaluated using a combination of linkage-analysis and mutation-analysis. The linkage analysis with intragenic and flanking markers allowed us to exclude the involvement of ALX4 and MSX2 in a number of families. Mutation analysis of both genes in the remaining families, performed by direct sequencing of all coding regions, identified several polymorphisms, but no disease causing mutations. We can therefore conclude that the AOS in our set of patients is not caused by mutations in ALX4

or MSX2. Further studies have to be performed to identify the responsible gene(s) in Adams-Oliver Syndrome in our set of AOS families.

P163. Cervical spine anomalies in Dubowitz syndrome

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Dubowitz syndrome is a rare autosomal recessive disorder characterized by microcephaly, craniofacial abnormalities, eczema, delayed skeletal maturation, and short stature. The orthopedic manifestations of this condition primarily involve the hands and feet with brachy-clinodactyly of the fifth finger and syndactyly of the second and third toes. Thoracic scoliosis requiring surgical correction was described in one patient. Cervical spine anomalies are reported only in two patients in the past, one of which had „mild“ dyplasia and another was found to have „dysplastic“ cervical vertebrae. However, the spectrum of cervical spine anomalies and its clinical significance in patients with Dubowitz syndrome remains unclear.
We report two pediatric patients with Dubowitz syndrome and severe malformation of the cervical spine. Both patients had severe dysplasia of the C1 vertebra with subluxation of C1, narrowing of the spinal canal and bony impingement on the spinal cord. One of patients presented with congenital torticollis, which led to discovery of the cervical anomaly, while the cervical spine abnormality was found in the another patient incidentally. Long term clinical follow-up is needed to determine clinical significance and outcome in these patients. Congenital malformations of cervical spine might be more common in this rare syndromic condition than originally thought and clinical screening for vertebral anomalies is warranted.

P164. Autosomal dominant pterygium colli in a large 5 generation family: Delineation of the phenotype and exclusion of mutations in the PTPN11 gene

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We have analysed the PTPN11 gene in more than 100 patients with clinical diagnosis of Noonan syndrome and found mutations in only about 30% of patients. No mutation was detected in a patient with pterygium colli, chest deformity, and cryptorchidism, who had initially been diagnosed as Noonan syndrome.
Reevaluation of his family revealed a large 5 generation pedigree with 16 affected males and females. Pterygium colli was the most prominent feature, and surgical correction had to be performed in two severely affected family members. Short stature was present in 5 affected females. However, the characteristic facial features of Noonan syndrome, cardiac defects, and mental retardation are missing in affected family members. Chromosome analysis and radiographs of the cervical spine gave normal results. Interestingly, many affected persons complained of recurrent patella luxations, often requiring surgery. The features in this family were not consistent with any other well known pterygium colli syndrome. Isolated pterygium colli with autosomal dominant inheritance has only been described by Graham et al. in 1981, but recurrent patella luxations were not mentioned. We are planning to perform linkage analysis in this family.

P165. Cutis laxa, lipodystrophy and transient progeroid phenotype in mosaic polyploidy

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Wiedemann-Rautenstrauch syndrome is a rare disorder with low birthweight, muscle hypotonia, characteristic, progeroid face, wrinkled skin and abnormal fat distribution. Mental/growth retardation, relative macrocephaly with late closure of the anterior fontanel and congenital heart defects are also present. Progressive course has been described in most children with early lethality.
We report on a female infant with all the characteristic features of Wiedemann-Rautenstrauch syndrome at birth. Chromosomal studies on lymhocytes showed a normal karyotype. By follow up, based on the observed slow psychomotor development and the presence of abnormal lipid distribution, disorder of protein glycosylation (Congenital Dysorders of Glycosilation: CDG) as a possible underlying cause was suspected. Results of metabolic studies including transferrine isoelectric focusing and several enzyme measurements were normal. At the age of 2,5 years the progeroid signs were no longer present, the psychomotor development was better than expected; she was able to walk with support and use some words. Based on the overlaps between the clinical signs of Wiedemann-Rautenstrauch syndrome and mosaic polyploidy, including psychomotor retardation, reduced peripheral muscle bulk, arachnodactyly and lipodystrophy, chromosome analysis of cultured fibroblasts was performed. Mosaic tetraploidy and triploidy were detected in 14% and 60% of the cells, respectively. We recommend a chromosome analysis of cultered fibroblasts in patients with a neonatal presentation of progeroid features and lipodystrophy.

P166. COL5A1 haploinsufficiency in a patient with the classical form of Ehlers-Danlos syndrome

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The classical form of Ehlers-Danlos syndrome (EDS) is a heritable connective-tissue disorder that severely alters the collagen-fibrillar structure of the dermis, joints, eyes and blood vessels. Previous findings indicated that approximately one-third of individuals with classical EDS have mutations of COL5A1 that result in haploinsufficiency. Forty eight patients from 29 Russian families with classical EDS were clinically investigated. Both genomic DNA and cultured fibroblasts were available from 21 patients from different families. Three polymorphic sites in the COL5A1 gene were analyzed in genomic DNA by use of intron-based primers - PstI site in exon 5, StyI site in exon 65 and DpnII site in exon 66. Of 21 patients, 9 were heterozygous for the PstI polymorphism and 10 were heterozygous for the DpnII polymorphism in genomic DNA. We have identified nearly complete loss of an PstI allele in cDNA sample in 1 patient. The clinical features of the patient were typical for the classical EDS (type I “gravis”) and included hyperextensible and velvet skin, easy bruising, wide atrophic scars, hypermobility of the joints. Family history demonstrates autosomal dominant inheritance of the EDS in this case. The patient also has atypical form of diabetes mellitus, insulin-dependent, with late onset and without characteristic immunological markers.

P167. Blepharophimosis-mental retardation syndromes: a proposed clinical classification of so-called Ohdo syndrome, with delineation of a new, recessive form

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We report 2 male sibs with a consistent association of microcephaly, severe blepharophimosis, round face with characteristic nasal shape and narrow mouth, and cleft palate. Both have adducted thumbs and abnormal penile shape. Meningeal and intraparenchymatous cysts were present in one of them, and both developed severe delay and epilepsy. Caryotype an telomere screening were normal. Clinical phenotype is consistent with Ohdo syndrome, but with extra features. We have reviewed the literature on Ohdo syndrome, and suggest a clinical classification of blepharophimosis-mental retardation syndromes in 5 types 1] cryptic del 3p type, 2] Ohdo type (form limited to the original patients), 3] Biesecker type (AR ? - most cases sporadic) : includes most cases reported as “Ohdo syndrome” and patients with Young-Simpson syndrome, who are similar but with hypothyroidism, 4] Mahni type (AD) and 5] this report. (AR or XLR)

P168. A case of Cohen syndrome - problems with the diagnosis

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The Cohen syndrome was first described by Cohen at al. /1973/ and it is characterised by hypotonia, obesity, high nasal bridge, prominent incisors and mental deficiency. Fuhrmann- Rieger at al. /1984/ pointed out the similarities between the Prader-Willi and the Cohen syndromes.
We report a nine-year-old boy, first child of unrelated parents. He was born at forty weeks' gestation by spontaneous delivery after an uneventful pregnancy. His diagnosis at the age of five, based on the phenotype was Preder-Willi syndrome. The molecular diagnosis excluded deletion of 15q11-12 region. The child has been treated for two years with Genotropin, because of the obesity, with a good result. At the age of nine the author examined the child. The following dysmorphic feature was determined - microcephaly, micrognatia, short filtrum, narrow and high arched palate, high nasal bridge, protruding and dysplastic ears, narrow hands and feet, hypotonia, obesity and mental deficiency. On the basis of this clinical picture was decided that Cohen syndrome is present.

P169. Treacher Collins Syndrome: Clinical study over three cases

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Treacher Collins syndrome is an autosomal dominant condition with wide variability in expression. It is a disorder of craniofacial development (first and second pharyngeal arch). Defining features are: symmetrical facial abnormalities consisting of malformed ears, variable degree of malar and mandibular hypoplasia, cleft palate, conductive deafness, down slanting palpebral fissures and defect of the lower lid. Occasional patients have congenital heart defects. New mutations are present in 60% of cases.
We are presenting three cases of Treacher Collins syndrome:
*I.A., female, 14 years old, evaluated for the first time at 3 weeks of age and reevaluated periodically. Clinical exams revealed: dysmorphic face (downslanting palpebral fissures, everted lower lid and lower lid coloboma), assymmetric ears, bilateral preauricular pits, narrowing of the right external ear canal, bilateral deafness; mandibular hypoplasia. She had repeated hospital admissions for chest infections. There are no other similar cases in her family.
*G.R., male, 1 month old, first child of an young, unrelated, healthy couple. He was admitted to the hospital due to an interstitial pneumonia and suspicion of congenital heart defect. Clinical evaluation revealed: dysmorphic face (downslanting palpebral fissures, lower lid coloboma, high palate, micrognatia), malformed asymmetric ears (hearing could not be evaluated due to young age).
*P.G., male, 4 months old, evaluated for dysmorphic face consisting of downslanting palpebral fissures, everted lower lids, small, dysplastic ears and mandibular hypoplasia. Echocardiography revealed patent foramen ovale.
In conclusion, we emphasize the importance of clinical features for diagnosis, leading further to an appropiate management.

P170. Complete and incomplete achromatopsia in the same family

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Achromatopsia, also referred to as rod monochromacy, is mostly an autosomal recessive ocular disorder characterized by total color blindness, low visual acuity, photophobia, and nystagmus. This condition is sometimes referred to as ‚absence of cones'; in fact cones are present but functionally defective. Patients with achromatopsia have nystagmus as infants, which decreases later. Photophobia is striking. Patients squint even in light of ordinary intensity. Vision in ordinary lighting is severely restricted; vision in dim light is relatively better. Colors cannot be distinguished.
Recently, GNAT2 has been demonstrated as the third gene associated with achromatopsia, the other 2 being CNGA3,which is mutant in ACHM2, and CNGB3, which is mutant in ACHM3.
We present here a family with five children; two of them with achromatopsia, day blindness and Bull's eye macular lesion, the other two with incomplete absence of color discrimination (dyschromatopsia) and nightblindness and the remaining one with normal color vision. Their mother presented some abnormalities by Ishihara color discrimination test but she does not complain of reduced vision. The inheritance pattern in this family is discussed.

P171. Novel mutation in the 5'splice site of exon 4 of the *TCOF1* gene in the patient with Treacher Collins syndrome.

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Treacher Collins syndrome (TCS), the most common type of mandibulofacial dysostosis, is an autosomal dominant disorder of craniofacial development. While 40% of TCS cases demonstrated previous family history, the 60% possibly arise from *de novo* mutations of the *TCOF1* gene. This gene encodes a low-complexity, serine/alanine-rich 1411 aminoacid protein, named treacle. Clinical features included malar hypoplasia, micrognathia, coloboma of lower eyelids, macrostomia, auricular defects and cleft palate. To date, 105 mutations of the *TCOF1* gene have been described.
The structure of the *TCOF1* gene was investigated in 20 patients with clinical symptoms of TCS and their 30 relatives. The DNA fragments were amplified by PCR and were subjected to multitemperature single-stranded conformation polymorphism (MSSCP) analysis, followed by real time PCR analysis and direct sequencing. In one patient sequence analysis of the amplified exon 4 revealed on one of the alleles an 18bp deletion (376delAAGGTGAGTGGGACTGCC). Computer analysis of the 18bp deletion demonstrated that the novel *de novo* mutation generated alternative splicing site and stop codon resulting in premature termination of translation and truncation of protein product from 1411 to 133 aminoacids. Amplification using LightCycler system showed that the allele harbouring the 18bp deletion revealed the second peak of melting temperature different from that of the normal allele.
We concluded that the TCS syndrome of our patient was probably due to the haploinsufficiency of treacle. Real time PCR analysis might provide a rapid screening assay for this mutation and might be easily adapted to screen for other deletions in the *TCOF1* gene.

P172. Two sisters with spastic paraparesis and macrocephaly : a new recessive syndrome?

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We describe two sisters, born from non consanguineous parents, with spastic paraparesis, mental retardation, seizures and macrocephaly. The eldest 32 years old had congenital spastic gait with proximal weakness and distal wasting of the lower limbs at examination, associated with urinary incontinence. Dysmorphic features are characterized by macrocephaly (OFC 60 cm) and hypertelorism. Due to spastic gait, hyperlordosis was prominent as well as bilateral deformity of knees and feet. She had a mild mental retardation, but is able to assume daily living activities.
The younger sister was 20 years old. She had a more severe lower limb spasticity and was unable to run. At examination bilateral flexion deformity of the knees were severe, requiring surgical treatment as well as severe scoliosis treated by arthrodesis during the childhood. The facial dysmorphy was similar to her sister's one (OFC 60 cm), but associated with facial muscles weakness and a dysarthria. She also had a mild mental retardation.
The biological findings for the two sisters provided a normal standard karyotype, a negative search for Fragile X syndrome and for CDG syndrome. Very long chain fatty acids were also normal. Cerebral and cervical MRI and skeletal radiographs were normal.
Clinical examination of the mother showed no pyramidal signs at examination with normal OFC. The father was not available for examination.
Our report and the similar clinical descriptions in three cases in the literature report spastic paraparesis with macrocephaly, epileptic seizures and non-progressive mental retardation.

P173. The frequency of clinical features and variable expressivity in Oculo-Auriculo-Vertebral Spectrum Clinical study about 17 patients

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We present the study of 17 patients with Oculo-Auriculo-Vertebral Spectrum (OAVS) in order to appreciate the frequency of different features and their importance for the diagnosis. The groups is formed of 13 boys and 4 girls, aged 0-16y, with different clinical pictures illustrating the variable expressivity of the syndrom.
The diagnosis was : Hemifacial microsomia (7 cases), Goldenhar syndrom (3 cases), Oculo-Auriculo-Vertebral Spectrum (7cases).
The frequency of the defining features will be presented -globally and separately for every category. The results of our study showed that males are more frequently affected than females (13:4), left side is more frequently affected (uni/bilateral) and the most frequent associated abnormality is represented by heart defects.
In conclusion, we present a clinical study of 17 patients with Oculo-Auriculo-Vertebral Spectrum in order to appreciate the most suggestive and important features for this diagnosis.

P174. Hip dysplasia, characteristic face, relative macrocephaly, delayed motor development, failure to thrive and café au lait spots-possible new autosomal dominant syndrome

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We present two families with short statue, frontal bossing, triangular face, blue sclerae, café au lait spots and multiple pigmented nevi, increased body hair and dyslocated hips. Initially the family members were diagnosed to have Silver-Russel syndrome. This syndrome is a well-recognised clinical entity associated with a relatively large head, triangular face, latreal asymmetry, with intrauterin and postnatal growth retardation. Other anomalies include fifth finger clinodactyly, café au lait spots and occasional autosomal dominant inheritance. Skeletal asymmetry is present in about 50% of the patients. Hip dysplasia has been observed, although not characteristic for the syndrome. Among our patients there was no family member with significant intrauterine growth retardatin or clinodactyly. Motor development was delayed in most individuals. Hip dysplasia has occured as a dominantly transmitted severe condition. We performed micrisatellite marker analysis in both families in order to rule out uniparental disomy on chromosome 7, with normal results. Therefor we suggest that our patient may have a previosly undescribed dominant condition.

P175. A Further Case of Frontonasaldysplasia and Dilated Virchow Robin Spaces

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Frontonasal dysplasia (FND) is a sporadic or dominant developmental field defect of midfacial area characterized by a broad nose, hypertelorism, and low anterior hairline. Another similar syndrome is frontofacionasal dysplasia (FFND) which is inherited in an autosomal recessive fassion. Clinical features of FFND are midfacial hypoplasia with specific eye findings and various skeletal abnormalities.
Here we present a case with another similar entity comprising frontonasal dysplasia and dilated Virchow Robin Spaces.
A male was born to consangineous parents at 40 weeks gestation by normal spontaneous delivery after an uneventful pregnancy. His weight, height and routine blood tests and urine analysis were normal. Methabolic tests for mucopolysaccharidosis and mucolipidosis were found to be normal. His karyotype was 46,XY. Cranial MR revealed gray matter heterotopia in the subependymal area around the ventricles, subarachnoid cyst and mildly dilated Virchow Robin Spaces. Bone X ray showed delayed bone age at 3 years of age. Mild mental retardation was observed at a later age. He required special education and speech therapy.
While his facial appearance at different ages was very similar to the features of patients from the literature, we believe that this syndrome has unique features and is different from FND and FFND. Consanguinity between the parents of the patient supports autosomal ressesive inheritance.

P176. A four generation familial Van der Woude syndrome with higlhly variable clinical presentation

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Van der Woude syndrome (VWS) is a dominantly inherited developmental disorder characterized by labial anomaly, lip pits and/or sinuses or lip mounds, cleft lip and/or palate, hypodontia, and represents the most common syndromic form of cleft lip or palate. Recently mutations in the gene encoding interferon regulatory factor-6 (IRF6), mapping to 1q32-q41, have been identified in VWS families demonstrating also that popliteal pterygium syndrome is allelic to VWS. Here we describe a four generation italian family with variable clinical manifestation of the disease. The proband, a female neonate, presented with complete monolateral cleft lip and palate, the mother presented lip mound with profound sinuses and labial salivation related to accessory salival glands,which required surgical excision. Moreover she presents a mid-line 3 mm sinus of the hard palate, considered to represent a mild expression of the palatal devolopmental defect. The maternal aunt presents a submucous palatal cleft with mild velo-palatal insufficiency. The grandmother and the great -grandfather present labial pits and sinuses as the only manifestation of the disease. The molecular analysis of IRF6 gene revealed the presence of two nucleotide variants in DNA from the proband. The first alteration causes a missense mutation while the second is an intron variant likely affecting the normal splicing. We are now analysing the segregation of these two DNA variants in the family collected to establish their causative role and a set of control individuals to define if one of these two alterations represents a polymorphism.

P177. Second case of inherited Laurin-Sandrow Syndrome (LSS)?

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We report a father and son with almost identical features:
· Lower limbs: Bilateral tibial aplasia; duplicated fibula, calcaneus, cuboideum, achilles tendons (shortened), and anterior/posterior arteries of crus; 7/8 metatarsals, 9/10 toes, distal phalangeal splitting, syndactyly.
· Upper limbs: Preaxial/postaxial polydactyly, triphalangeal thumb, malformed middle/distal phalanges, fused distal phalanges, total syndactyly.
· Nose: Nasal stenosis, septal deviation.
· Cranium/cerebrum/psychomotoric development: Normal
Out of 9 LSS cases previously reported all but two were sporadic. Nasal anomalies, total hand syndactyly, mirror foot and interphalangela joint deformity are the most consistent findings. The stylopods are never affected.
Complete syndactyly of fingers is associated with tibial-hemimelia-polysyndactyly-triphalangeal-thumb syndrome (THPTTS) and triphalangeal-thumb-polysyndactyly syndrome (TTPS). LSS has also been reported in THPTTS/TTPS families suggesting a causal relationship. THPTTS/TTPS has been mapped to 7q36.
Additional limbs can be induced by ectopic expression of several genes in the flank mesoderm. Preaxial duplications can be produced by grafting experiments inducing a second anterior polarizing zone, via direct Shh-signalling or by increasing the number of anterior cells migrating into the limb bud. Stylopods/zeugopods/autopods are specified segmentally. Duplicated ulna with mirror-image polydactyly

were induced experimentally in Hoxb8 transgenic mouse. Based on this we hypotesize that the duplicated fibula and mirror foot in LSS may be produced in two ways: 1) By increasing the number of anterior flank cells migrating into the limb bud without affecting the localisation of the polarizing zone, or 2) by misinterpretation of signals specifying the zeugopods, either at the limb bud stage or during outgrowth.

P178. Autosomal recessive congenital cutis laxa and frontal polymicrogyria: an unreported association

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Autosomal recessive cutis laxa is divided in two forms: one with pulmonary emphysema and one with enlarged fontanelles and developmental delay. The former has been associated with disease-causing mutations in the fibulin 5 gene (Loeys et al 2002) and the latter is of unknown cause. We describe a 15 y-old Italian female with the enlarged fontanelles type (or Debré type) which was diagnosed in neonatal period because of her loose skin and failure to thrive. Pregnancy and delivery were uneventfull.In addition to paucity of the elastin network at skin biopsy, she had the typical facial appearance including receading forehead, upslanting palpebral fissures and Ehlers-Danlos-like scarring of the pretibial skin.
Her developmental milestones were delayed. She was oriented in a special education school. At 11 year-old, she developed seizures of the grand mal type which were intractable by most of the antiepileptic drugs. Brain MRI disclosed a marked frontal polymicrogyria. This singular association has not been reported so far and may represent the pleiotropic manifestations of a gene expressed in the skin and CNS.

P179. Oculo- cerebro- renal syndrome - clinic and genetic study of two families

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Löwe syndrome is a form of XLMR with short stature, cataracts, renal tubular dysfunction and hypotonia. We present 2 cases to illustrate this rare entity, to discuss the suggestive facial appearance and to underline the importance of molecular tests for genetic counselling. A comprehensive literature review will be presented.
First child: 6 Mo old male infant, first child of a normal couple, born naturally, at term, after an uneventful pregnancy. Neonatal intensive care was necessary. Postnatal development: delayed, with hypotonia. Physical examination: short stature, dysmorphic face (tall, broad forehead, deepset eyes, large ears), hypotonic muscles, marked developmental delay. Ophtalmologic examination: child- congenital bilateral cataracts; mother- normal. Neurologic examination: generalized hypotonia, abolished osteo- tendineous reflexes. Urine: albumin present. Molecular test: nonsense mutation in exon 23 of OCRL gene (mother and child).
Second child: 13 Mo old male infant, the only child of a normal couple, born by caesarean section, at term, after an uneventful pregnancy. At 3 days he was diagnosed with bilateral congenital cataracts (surgically treated at 1 Mo). Postnatal development: marked delay, hypotonia. Physical examination: mild short stature, dysmorphic face (tall, square forehead, deepset eyes, nistagmus, large ears), umbilical hernia, hypotonic muscles, marked developmental delay. Ophtalmologic examination: horizontal nistagmus, pseudofakia. Neurologic examination: generalized hypotonia, abolished osteo- tendineous reflexes. Urine: albuminuria, aminoaciduria. The mother refused cooperation.
In conclusion, we present these 2 cases to illustrate a rare genetic disorder, to underline the typical dysmorphic face and to discuss the importance of ophthalmologic examination and molecular tests for genetic counseling.

P180. Prenatal and postnatal study of a particular form of Cranio- frontonasal dysplasia

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We present a case of Cranio-frontonasal dysplasia to illustrate a rare form of this genetic disorder.
This condition combines frontonasal dysplasia with craniosynostosis. Main features are: severe hypertelorism, broad bifid nose, frontal bossing, low posterior hairline. Other features are: webbed neck, round shoulders, abnormal clavicles, raised scapulae and longitudinal nail ridging. Most cases are females and have normal intelligence. Inheritance is uncertain.
Our proband is a female newborn infant, first child of a normal couple. There are no similar cases in the family. Pregnancy was normal, but an ultrasound scan (31 weeks gestation) revealed abnormal head conformation and mild dilatation of left lateral ventricle. The child was born by caesarean section and needed intensive care (APGAR score 7). Measurements: Ht, Wt = normal, OFC = -1.72 SD, brachycephaly, marked hypertelorism. Physical examination: prominent forehead, large anterior fontanelle, marked hypertelorism, broad nose, short philtrum with a small hemangioma, high palate, thick ears, short neck with excess skin and left axillary pterigyum. Positive diagnosis of Cranio- frontonasal dysplasia was based on the typical clinical aspect of frontonasal dysplasia associated with microcephaly. Differential diagnosis with Frontonasal dysplasia and other syndromes with marked hypertelorism will be presented. The present case presents with the following unusual features: mild and asymmetric hydrocephaly, thick ears and axillary pterigyum (the last one is cited in the literature as rarely associated).
In conclusion, we present this case to illustrate a rare form of Cranio-frontonasal dysplasia and to present some particularities.

P181. Pyknodysostosis: report of three patients

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Pyknodysostosis (MIM: 265800) is a rare autosomal recessive disease characterized by short stature, distal phalanges acro-osteolysis, increased bone density with fragility, delayed suture closure, clavicle and skull deformities and dental anomalies. Characteristically the mandibular angles are absent.
This disease is caused by cathepsin K deficiency, and treatment is supportive.
We report three patients observed at our outpatient clinic and will describe the clinical and radiological features that allowed the diagnosis.
The molecular studies and genetic counselling are discussed.

P182. A further case of Fryns syndrome without diaphragmatic hernia.

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We report on a child with Fryns syndrome including characteristic coarse hirsute facial appearance, cleft lip and palate, cardiac anomaly, renal anomalies, dilated bowel and distal limb abnormalities. However diaphragmatic hernia, which is considered a cardinal feature in this condition, was absent in our patient. Parents were consanguineous supporting autosomal recessive inheritance. In summary, Fryns syndrome is a malformation syndrome characterised by craniofacial anomalies, distal limb malformations and diaphragmatic defects. However the diaphragmatic defects may not be mandatory and we submit a table comparing our case with other published cases of Fryns syndrome.

P183. Two sisters with a neuromuscular disorder, congenital cardiac malformation and dysmorphism: a new recessive syndrome ?

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We report on two sisters from consanguineous parents with a yet unknown neurodegenerative disorder, congenital cardiac

malformation and facial dysmorphism. Both patients suffered from severe mental retardation, borderline microcephaly, progressive diffuse cerebellar atrophy, hypotonia, cerebellar tremors with reduced spontaneous motility and hyporeflexia, retinitis pigmentosa, severe post-valvular aortic stenosis with very thin aortic arch, secondary hypertension, left ventricular hypertrophy, vescico-ureteral reflux with atonic bladder. The EMG was compatible either with first motoneuron disease or peripheral neuropathy. The brain stem evoked potentials showed unstable and delayed responses compatible with brain stem abnormality. Swallowing function was lost by the end of the first year. Dysmorphism consisted of deep set eyes with absence of orbital fat, prominent forehead and abnormal fat distribution on the cheeks but also on the hands, feet and mons pubis. Both girls died of heart failure. The muscular biopsy was suspected for fat storage. Cockayne sundrome, SCA2, SCA7 (including the form with extreme repeat expansion), several disorders of fat metabolism (peroxisomal, cholesterol-precursors and -esthers, bile acids including cholestanol, mitochondrial fatty acids, sphingolipids), CDG syndromes and lysosomal disorders were excluded. Chromosomes and FISH at 22q11 locus were normal in blood and skin fibroblasts. Routine analysis of point mutations and deletions in mtDNA was normal. Two sibs and both parents are healthy. DNA analysis of SCA2 with extreme repeat expansion is pending. Although the brain anomalies are suggestive of a progressive pontocerebellar hypoplasia, the association with eye abnormalities, dysmorphic features and a rare congenital vascular abnormality rather suggest a separate syndrome.

P184. Genetic malformation syndromes - how useful is information in the Internet for patients and their families

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The Internet has become an effective source of information for large numbers of health consumers and the general public. Health related web sites are among those pages of the Internet with the highest rate of interest.
Since anyone can create his own web site the quality of the information in the web is highly questionable. This may cause serious problems in the realm of medicine.
For 40 malformation syndromes we analyzed the first 20 URLs provided by a search engine in the English and the German part of the web according to predefined criteria, which comprised formal characteristics of the respective web site, and for the syndromes the correctness of clinical description, etiology, diagnosis and diagnostic procedures, prognosis and psychosocial aspects.
We found a high quality of web sites for 19 of the 40 syndromes in the English-speaking part of the web and for 13 syndromes in the German-speaking part. A positive correlation existed between the length of the web site and it‘s quality, while no correlations were found between the prevalence of a syndrome or the number of scientific publications and the number of web pages.
While it is evident, that the Internet shows a high power, velocity, fecundity and efficiency for the processing of information, the problem of reliability remains unresolved up to now, however.

P185. Some bone and teeth dysplasias

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The bone and teeth dysplasias represent a numerous series of growth defects of hereditary origin. Their symptomatology exhibit a large scale of abnormalities which are the aim of this presentation. From the broad scale of diseases we demonstrate following conditions:
- amelogenesis imperfecta
- dentinogenesis and/or osteogenesis imperfecta
- some syndromes with manifestation in orofacial area

In all our observed cases the clinical examination was acompanied by genealogical and laboratory (histological, cytogenetical and molecular biological methods) as well as stomatological examination.
The analysis of our data showed some interesting relationships between bone, hard dental tissues and mesenchymal orofacial structures.
The most promising direction of our further research seems to be the correlation between osteoblasts and odontoblasts in selected cases of osteogenesis imperfecta with inborn defects of collagen I.
The precise etiopathogenetic knowledge promotes the correct genetic diagnostics which is of crucial importance for determining further medical preventive tactics.

P186. A Translocation Breakpoint Disrupts The Aspm Gene In A Patient With Primary Microcephaly

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Primary microcephaly (microcephalia vera) is a developmental abnormality resulting in a small brain, with mental retardation. It is usually transmitted as an autosomal recessive trait, and five loci have been reported to date. We and others reported linkage of primary microcephaly to MCPH5 at 1q25-q31 by homozygosity mapping. We here report the molecular characterization of a translocation breakpoint at 1q25-31 found in a microcephalic patient. A lymphoblastoid cell line was established from a patient‘s relative who bears the same translocation, t(1 ;4)(q31;p15.3). BAC clones spanning the linkage region were purchased from publicly available resources. One of these BACs, RP11-32D17, produced a FISH signal on both chromosomes 1q and 4p, while BACs with more telomeric 1q inserts were translocated to 4p. We then showed that the translocation disrupts a 9 kb BamH1 digestion fragment subcloned from RP11-32D17 and used as a FISH probe. This segment is located within the ASPM gene, which was recently shown to bear point mutations in patients with MCPH5-linked autosomal recessive microcephaly. We conclude that the very rare translocation observed in our patient caused microcephaly by disrupting one allele of ASPM, and presume that his second allele bears a yet unidentified point mutation.

P187. Nasopharyngeal teratoma and diaphragmatic hernia: a non random association?

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Diaphragmatic hernia can occur as an isolated event, or combined with other malformations in syndromes, associations or chromosomal anomalies. Genetic syndromes in which the defect is part of the clinical spectrum or is an associated feature are well known. We report on the association between diaphragmatic hernia and nasopharyngeal teratoma. To the best of our knowledge this is the second report of such an association (Aughton *et al*, 1990).
A 33 year old pregnant woman, gravida 1 para 0, come to our attention for a suspicion of foetal diaphragmatic hernia at 32 weeks. The ultrasound evaluation confirmed the presence of a diaphragmatic hernia with liver and stomach herniation causing dextrocardia and disclosed the presence of depressed nasal bridge, a lower lip protrusion, a suspicion of macro and protruding tongue, abnormal hand bone length and abnormal foot position. Other morphological data were normal. Amniocentesis, performed due to the presence of the malformations, showed a normal foetal karyotype (46,XX).
The baby born at 38 weeks (weight: 2670 gr) by cesarean section for foetal distress, and died soon after birth.
Pathology revealed craniofacial dysmorphisms (short palpebral fissures, hypertelorism, broad nasal bridge), the presence of a voluminous mass protruding from the mouth, abnormal tongue, lumbar vertebral schisis, diaphragmatic hernia and a bicornuate uterus. Histology of the mass confirmed that it was a hairy polyp nasopharyngeal teratoma.
Overlap of the clinical spectrum of our case and the one previously reported suggests the same aetiology. Other possible differential diagnoses are considered.

P188. Family case of acrodermatitis enteropathica (MIM201100)

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Acrodermatitis enteropathica (AE) is a rare autosomal recessive disease, an inborn error of metabolism resulting in zinc malabsorbtion. The main symptoms are dermatitis, diarrhea and zinc deficiency. We report on the family case with AE. Proband parents are not consanguineous. The elder sister was born after the 1st physiological pregnancy by spontaneous normal delivery. Her birth weight was 3,9kg (97th centile), her length was 53cm (75th centile). 3 mo and 8 mo aged she was admitted to hospital with bilateral microfocal pneumonia and bronchopneumonia. At 8 mo and 9 mo of age she had aphthous stomatitis and diffuse progressive erosive crusted rash with accentuation of lesions in skin creases and at mucocutaneous junctions. Her symptoms resolved within 5 days of initiating oral Enteroseptol (1g/day) supplements. At 5.5, 16 and 18 yr of age she had recurrence of AE. When she was 16 yr old ZnO supplements were initiated. At 18yr of age she had low blood zinc levels (0.053 vs 0.1 mg/dl). Before her first pregnancy she had some episodes of skin lesions around mouth and on the heel region. Her blood zinc level was 0.04 mg/dl when she was primigravida. Dose of ZnO was elevated till 0.15 per day. Her pregnancy and labor were successful. Her son has no symptoms of AE and blood zinc level is normal . Healthy younger sister of proband developed sings of AE when she was 4 mo old. She has mild symptoms of AE and uses lesser doses of ZnO.

P189. True mosaicism in a case of Williams syndrome

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Williams syndrome is a neurodevelopmental disorder with multisystemic manifestations. Most patients (>96%) exhibit a common 1,5 Mb de novo heterozygous deletion in 7q11.23 that results from non-allelic homologous recombination between regional segmental duplications during meiosis. To our knowledge, mosaicism has never been reported.
We have studied a 5-year old Chilean patient with characteristic facial features, supravalvular aortic stenosis, gastroesophageal reflux, bilateral inguinal hernia, hoarse voice, severe psychomotor retardation, characteristic autistic features, besides global delay in language development and partial heterochromia of the left iris. Repeated FISH analyses on two independent blood samples showed the coexistence of cells with and without a heterozygous deletion of the ELN probe (deletion of 46% and 41% out of 80 and 39 metaphases, respectively). Typing of short tandem repeats on blood genomic DNA revealed biparental inheritance with a relative dosage reduction of the paternally inherited allele (47-51%) at HSB055XE5, ELN and D7S1870, while normal dosage ratios were found at D7S672 and D7S2518. Our data indicate the presence of somatic mosaicism in lymphocytes for the common WS deletion. Other tissues are currently being analysed. Contrary to expected, the phenotype of the patient is rather severe.

P190. The 3D face of Smith-Magenis syndrome (SMS): a study using dense surface models

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The SMS facial phenotype has been studied previously both subjectively and objectively, the latter using anthropometry. Here, we build a 3D surface model of face shape using images of 30 individuals with SMS and 146 controls, all under 20 years. A dense correspondence of thousands of points on each of the faces is computed and their co-ordinates are subject to a principal component analysis (PCA). This reduces the number of variables from tens of thousands to just 44 PCA modes, giving a compact model that is computationally amenable to further analysis. A linear morph between the average face surfaces of each subgroup gives a dramatic 3D visualisation of dominant shape differences including many identified previously: broad and square face shape; heavy brow; close deep-set eyes; and major nose and upper lip differences. Notable are the upward and backward displacement of the pronasale and outward rotation of the philtrum giving the previously noted „tented appearance". The PCA modes were analysed using pattern-recognition techniques to discriminate between SMS subjects and controls using 10-fold cross validation of training and unseen test sets. The best performing algorithm, nearest mean, gave an average specificity of 96%, a sensitivity of 80% and an overall accuracy of 93%. These results confirm the potential of 3D dense surface models to support both training and clinical practice in dysmorphology. When more SMS data is available, even better results are anticipated.

P191. Seckel phenotype and partial GH deficiency in a patient with Wolf-Hirschhorn syndrome.

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Wolf-Hirschhorn syndrome (WHS) is caused by a variably-sized deletion of chromosome 4 involving band 4p16 whose typical craniofacial features are "Greek warrior helmet appearance" of the nose, microcephaly and prominent glabella. Almost all patients show mental retardation and pre-postntal growth delay.
Patient was born at term, after a pregnancy charachterized by IUGR. Delivery was uneventful. Developmental delay was evident since the first months of life. At 2 years he developed generalized tonic-clonic seizures. Because of short stature, low growth velocity and delayed bone age, at 4 years he underwent growth hormone (GH) evaluation. Peak GH after two provocative tests revealed a partial GH deficiency. Clinical observation at 7 years disclosed a facial appearance resembling Seckel syndrome, with microcephaly, prominent eyes and beaked nose. Brain MRI showed left temporal mesial sclerosis. GTG banded karyotype was normal. Because of mental retardation, subtelomeric FISH analysis was performed, disclosing a large deletion involving 4p16->pter (about 4 Mb), in the proband, not present in the parents.
Seckel syndrome could be confused with WHS because of IUGR, postnatal growth deficiency, microcephaly and beaked nose, but only one patient with striking Seckel-like phenotype has been reported. The smallest deletion detected in WHS includes two candidate genes, WHSC1 and WHSC2, reported in a patient. Interestingly, he did not show shortness of stature, that could be due to the haploinsufficiency of other genes localized in the flanking regions. Contribution of GH alterations and possible GH therapy should be further evaluated in WHS patients.

P192. Normal live born offspring of an Angelman syndrome mother with the Prader-Willi syndrome phenotype

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Angelman Syndrome (AS) and Prader-Willi Syndrome (PWS) are two clinically distinct neurodevelopmental conditions that are caused by the lack of expression of imprinted genes in the PWS/AS critical region on chromosome 15q11-q13. This can result from one of several genetic mechanisms, which include deletions, uniparental disomy, intragenic mutations, imprinting mutations and rare balanced translocations.
We report on a woman who presented with a clinical PWS phenotype, but following molecular testing was diagnosed with atypical AS. Microsatellite analysis in the proband showed biparental inheritance of markers located within and outside of the AS/PWS critical region. The combination of an abnormal methylation pattern and biparental inheritance indicated that the cause of AS in the proband was due to an imprinting mutation carried on her maternal chromosome. Haplotype analysis showed that the proband and her

unaffected brother had inherited the same maternal chromosome, suggesting that the imprinting mutation had arisen *de novo* or that the proband's mother was a gonadal mosaic. A faint maternal band on the proband's blot was suggestive of mosaicism. Further analysis showed that the child of the proband had also inherited the mutation-carrying grand-maternal chromosome. The normal phenotype and methylation of the child provides evidence that the proband was a mosaic. This case report has identified another AS patient with the PWS phenotype, has described the first case of a live-born unaffected offspring of an AS patient and has provided evidence that these mild AS phenotypes are the product of *de novo* imprinting mutations resulting in cellular mosaicism.

P193. Apparent mosaic imprinting defect characteristic of Angelman syndrome in a patient presenting for molecular genetic analysis for Prader Willi syndrome

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A 16 year old patient with delayed development and obesity was referred for cytogenetic and molecular genetic analysis for fragile X and Prader Willi syndromes. Methylation analysis at the SNRPN locus using Southern Blotting revealed a strong paternal band but a faint maternal band, a pattern more consistent with a mosaic Angelman result than a Prader Willi result. Methylation specific PCR analysis and repeat blot analysis on a second sample confirmed this result thereby excluding technical artefact. FISH analysis using a probe for D15S10 excluded a deletion or duplication of this region. Microsatellite analysis using markers across 15q11-q13 and in distal 15q revealed a pattern consistent with biparental inheritance. This supports the proposal that this patient is mosaic for an imprinting defect resulting in the majority of lymphocytes having both chromosome 15s marked with a maternal epigenotype at this locus. This is a further case of a patient with AS presenting with features of PWS caused by a mosaic imprinting defect (as reported by Gillessen-Kaesbach G *et al* (1999). Cases of both atypical and typical AS have been reported with a similar result (K Buiting, personal communication). None of these patients has an IC deletion, however the patient reported here will be analysed for an IC deletion to exclude a raised recurrence risk.
More detailed clinical details regarding this patient will be presented.

P194. Characteristics of Prader-Willi syndrome in neonates and young infants

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Prader-Willi syndrome (PWS) is an imprinting disorder characterized by feeding difficulty, infantile hypotonia, obesity, hypogonadism, and mental retardation. Diagnosis of PWS in young infants is important not only for the avoidance of unnecessary invasive diagnostic procedures but also for the consideration of preventive and therapeutic options.
From May 1, 1999 to December 31, 2002, 259 cases of suspected PWS were collected. Methylation-specific PCR was performed to amplify the CpG island of the *SNRPN* gene. Of the 27 infants less than 3 months of age and suspected of PWS, 16 (59.3%) showed the 174 bp maternal-specific band only and proved to be PWS. The occurrences of the major consensus diagnostic criteria for PWS in the 16 infants were as follows: central hypotonia, 14 patients (87.5%); feeding problems, 15 patients (93.8%); excessive weight gain, 0 patients (0%); characteristic facial features, no less than 3 items present in 8 patients (50%); hypogonadism, 11 patients (68.8%); global developmental delay, 2 patients (12.5%); hyperphagia, 0 patients (0%). In 6 PWS patients, the minor consensus diagnostic criteria for PWS were also recorded, and the occurrences were as follows: hypopigmentation, 4 patients (66.7%); decreased fetal movement, 4 patients (66.7%); infantile lethargy or weak cry, 3 patients (50%); small hands and/or feet, 1 patient (16.7%); narrow hands with straight ulnar border, 1 patient (16.7%). The average score according to diagnostic criteria was 3.
We conclude that methylation-specific PCR is a useful tool for the rapid screening of PWS, especially in neonates or young infants with obscure characteristic features.

P195. Recurrence of Prader-Willi syndrome due to (presumed) paternal germinal mosaicism for an imprinting centre deletion

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Historically, the recurrence risk for Prader-Willi syndrome (PWS) has been considered to be minimal. While true for cytogenetically visible deletions or uniparental disomy (except consequent on maternal translocation), there are now several reported cases with much smaller deletions affecting the imprinting center (IC). Most of these IC deletions are familial mutations, inherited from the father.
We report a couple in their 30's whose first child (LT) has PWS due to IC deletion, identified by FISH and DNA methylation studies, and which spans all exons of the SNRPN gene and at least 35 kb of the IC region proximal to SNRPN. A normal SNRPN dosage blot on parental DNA suggested the deletion had arisen de novo, although cannot exclude germinal mosaicism.
In a second pregnancy, DNA was obtained from an 11-week CVS. Methylation analysis for SNRPN exon 1 region performed by Southern blot, and methylation-specific PCR, showed a pattern consistent with recurrence of PWS, but the FISH probe required previously to demonstrate deletion in LT was no longer commercially available. Confirmation of recurrence came from microsatellite marker PAR-SN, which maps 5 kb distal to SNRPN and lies inside the deletion. This demonstrated 2 normal alleles in peripheral blood DNA in both parents, but only a maternal allele in the CVS sample, identical with LT. The couple chose termination of pregnancy. We assume that the father has germinal mosaicism for the IC deletion, and hope to explore the possibility of single sperm FISH for confirmation.

P196. UBE3A gene mutations in Finnish Angelman syndrome patients detected by conformation-sensitive gel electrophoresis

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Intoduction. Angelman syndrome (AS) is a neurogenetic disorder associated with loss of maternal gene expression in chromosome region 15q11-q13 due to either maternal deletion, paternal uniparental disomy (UPD), imprinting defect, mutation in the *UBE3A* gene or unidentified mechanism(s). *UBE3A* encodes an ubiquitin protein ligase and shows brain-specific imprinting. We have analysed the *UBE3A* coding region in ten AS patients with a normal biparental inheritance and normal DNA methylation pattern at 15q11-q13.
Methods. For screening of mutations both CSGE (conformation-sensitive gel electrophoresis) and sequencing were used.
Results. A disease-causing mutation was identified in five of the patients: two novel missense mutations (902A->C, 975T->C) and three deletions (1930delAG in one and 3093delAAGA in two patients). These deletions have been reported previously in other AS patients, suggesting that both sites may be prone to deletions in the *UBE3A* gene. All present AS cases were sporadic, but a mosaicism

for the mutation 902A->C was found in the patient's mother. **Conclusions**. Screening for *UBE3A* mutations in AS patients was found useful both for the confirmation of the diagnosis and for genetic counselling. CSGE was found to be a sensitive and simple method for mutation analysis of *UBE3A*.

P197. Prevalence of Prader-Willy syndrome and Angelman syndrome in Estonian children

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The purpose of this study was to establish the prevalence of Angelman syndrome (AS) and Prader-Willy syndrome (PWS) in Estonia.
In year 2000 we started a focused search for the patients with AS and PWS. Only 2 patients with AS and 2 patients with PWS were diagnosed before. The target population consisted of children referred to the two Children Hospitals or to genetic counselling due to developmental delay. In addition, we visited all Estonian institutions for disabled children. The selection of investigated patients based on the consensus diagnostic criteria for PWS described by Williams *et al* (1995), and for AS by Holm *et al* (1993).
The DNA methylation test was carried out in 45 patients with suspicion of PWS and in 73 patients with suspicion of AS, this test was positive in 6 and 3 patients, respectively. Patients with positive test results and previously diagnosed patients were analysed by FISH and chromosomal analysis. From 8 identified PWS patients 4 had a deletion in the region 15q11-13, one had a translocation 15;15 and 3 had a matUPD15. From 5 AS patients 4 had a deletion and 1 had a patUPD15. Two patients with AS were included into study based on clinical picture only.
The preliminary prevalence of PWS and AS in Estonia was estimated as 1:40000 and 1:46000, respectively. The search of AS and PWS patients among disabled children gave us 9 new previously undiagnosed cases. UBE3A and MECP2 gene mutational analysis should be done in the patients with atypical AS.

P198. Myelodysplastic findings in patients with chromosome 22q11.2 microdeletion

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Chromosome 22q11.2 deletions are associated with a wide variety of phenotypic abnormalities, and are responsible for the majority of cases with DiGeorge syndrome and velocardiofacial syndrom. The complete spectrum of clinical features associated with this deletion is currently defined and categorized under the heading of ''chromosome 22q11.2 deletion syndrome'' (22qDS). Recent studies demonstrated 25 genes identified in this locus. Myelodysplasia (MD) refers to abnormal bone marrow (BM) and/or peripheral blood smear (PBS) morphology frequently associated with certain genetic defects. We previously found MD findings in BM and PBSs in a patient with 22qDS. For that reason we searched 5 patients with 22qDS [4 with conotruncal congenital heart defects (CTCHD) including index case, 1 with no cardiac abnormality], 3 patients with CTCHD without 22qDS and 8 normal children who only have infection at time of sampling. Patients with 22qDS (5) and patients who have CTCHD without 22qDS (3), had not any infection in this study. We found that MD scores of patients with 22qDS were significantly higher than those who do not carry this deletion. We suggest the gene(s) deleted in 22q11.2 region may be responsible for these findings.

P199. Detection of deletions in chromosome 22q11 by microarray comparative genomic hybridization (microarray-CGH).

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We have constructed a genomic microarray consisting of the chromosome 22 tiling path and used it to study patients with known chromosome 22q11 deletions in order to validate this method for the detection of small chromosomal deletions.
A tiling microarray was constructed using sequenced golden path clones from chromosome 22. DNA from patients with known 22q11 deletions confirmed by FISH analysis was labeled with Cy3 and hybridized to Cy 5-labeled control DNA, either from a lymphoblastoid cell line or from a pooled panel of DNA samples extracted from whole blood. The labeled DNA was then co-hybridized to the chromosome 22 tile-path array. Ratio of intensities of test and control DNA was calculated.
We found that 22q11 deletions were readily detectable by this method. Not all clones within the deleted region gave the expected 1: 2 hybridization ratio and it is likely that this is due to the high repeat content of the region. Hybridizations to the control lymphoblastoid cell line also showed an apparent duplication in the region flanking the telomeric end of the deletion, at the immunoglobulin lambda variable (IGLV) locus, that was not evident on repeat hybridization to the pooled DNA samples. This 'duplication' is likely to be due to deletion at the IGLV locus in the B cell line used for control DNA.
We conclude that microarray-CGH is valid for the detection of chromosomal deletions and, when extended to other chromosomes, will prove useful for diagnosis in patients with a normal G-banded karyotype and negative FISH studies.

P200. Assessment of the incidence and clinical relevance of atypical 22q11.2 deletions

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The clinical variability of the common 3 Mb 22q11.2 microdeletion is well known and led to a broad application of FISH diagnostic with probes for the loci TUPLE1/HIRA or D22S75, although rarely reported atypical deletions would not be discovered by these tests. As virtually all types of 22q11.2 deletions occurred between several low copy repeats (LCR), we assumed that atypical deletions might be more common than reported. Therefore we established a set of 10 DNA probes which would detect all reported and hypothetical types of deletions in between the LCR.
First we analysed 73 retrospective patients with typical congenital heart defects (CHD), which had been tested 22q11.2 normal with the conventional probes. As we did not detect any deletion, we subsequently analysed 104 prospective patients, 78 of whom had a typical CHD and 26 patients who were referred for atypical CHD, cleft palate or developmental delay and who were suspicious of 22q11.2 deletion.In these prospective study group we detected 31 deletions. 84 % of the deletions represented the common 3 Mb deletion, 10 % the proximal 1.5 Mb deletion, and 6 % atypical distal deletions, one within the 3 Mb interval, and one distally to the 3 Mb region. Both of the atypical distal deletions occurred in patients with atypical phenotype. Although it is known from earlier case reports, that the phenotypic spectrum in atypical deletions might be as broad as in the typical ones, our systematic study shows, that atypical deletions occur more common with atypical mild phenotypes.

P201. A search for chromosome 22q11 deletion in non-syndromic Tetralogy of Fallot patients

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Microdeletions of chromosomal region 22q11 are now well recognised as the principle cause of DiGeorge syndrome (DGS), Velocardiofacial syndrome (VCFS) and Conotruncal anomaly face syndrome (CTAF).

It results in a wide clinical spectrum, ranging from neonatal death to developmental or psychiatric problems in later life. Congenital heart defects (CHD) are among the major findings of these syndromes and mainly consist of cardiac outflow tract defects. The most common form of conotruncal cardiac abnormalities is Tetralogy of Fallot (TOF). Because both the DGS and VCFS are commonly associated with abnormalities of cardiac outflow tract, a logical extention of this studies is to investigate the possibility that 22q11 deletions cause sporadic heart disease of similar type. But these studies include patients with different types of congenital heart diseases and have contradictory results. We aimed to reveal the possible association in a isolated group and choose non-syndromic Tetralogy of Fallot cases. 50 patients with isolated non-syndromic Tetralogy of Fallot diagnosed in a pediatric cardiolgy center were included in this study. Dysmorphological, neurological and immunological examinations of all patients were noted as normal. After that, molecular cytogenetic analysis were performed by fluoresence in situ hybridisation using DGS region-spesific probe (TUPLE1, Cytocell-LPU004). The results of this study will be showed and possible association between del22q11 and TOF will be discussed in this presentation.

P202. 22q11.2 deletion. An uncommon phenotypic presentation of the velo-cardio-facial syndrome : case report and review of the litterature.

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This is the report of a girl with incomplete VACTERL association consisting of multiple ventricular septal defects, a „butterfly“ vertebral body of D9, and anal malformation. Prenatal onset growth retardation was obsereved subsequent developmental delay with feeding difficulties and behavioural problems. She has some dysmorphic trades, microcephaly, atypical dental implantation and proximal implantation of the thumbs. This malformation complex includes features frequently occuring in VACTERL (cfr. above), in Wolff-Hirschhorn syndrome (prenatal onset growth deficiency, mental retardation, microcephaly, dysmorphic features and congenital heart defect) and in Velo-cardio-facial syndrome (heart defect, mental deficiency, feeding difficulties). The girl has normal karyotypes (G-banding). A deletion on the short arm of chromosome 4 at 4p16.3 was excluded with fluorescence in situ hybridisation (FISH) analysis and molecular analysis. FISH analysis with TUPL1 locus specific probe identified a microdeletion on chromosome 22q11.2 (del22q11.2). The association of Velo-cardio-facial syndrome and VACTERL was already described, although it remains an unfrequent observation. This observation further supports the clinical variability of del22q11.2,although the genetic defect is remarkebly homogeneous in ~90% of the patients. Phenotypically discordant monozygotic twins with del22q11.2 suggest that clinical variability may not have strong genetic basis. Though recent experiments in mice models indicate that phenotypic modifiers play a role in dtermining penetrance, the nature of these modifiers is still unclear. Additional molecular research is in process in order to evaluate the size of the deletion including or not new regulatory sequences.

P203. European DiGeorge syndrome registry

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As a part of a larger concerted action undertaken by the European Society for Immunodefficiencies (ESID) to map immunodefficiencies in European population, a collaborative database of diGeorge syndrome patients is being created in Prague. Following the standards of ESID databases, basic information about the individual cases is collected together with data describing the changes of the immune functions in these patients.
Sufficient number of of patients in different age groups will allow to reduce the otherwise problematic length of prospective studies aimed to map the age-related development of immune parameters in patients with this syndrome. A larger cohort will also allow to revise and diversify the immunological prognosis of these patients.
Concurrently, by collecting details about the molecular cytogenetic aspects of the individual cases, their clinical findings and their family history, the project may enhance knowledge about the genotype-fenotype correlation in this extremely variable condition. Last but not least, further studies may be facilitated by the information about DNA availability in clinically described patients.
The project is supported by the EURO-PID-NAS QLRT-2001-02742 project of the EU 5th Framework Programme.

P204. The first case of Kallmann syndrome in St.Petersburg population

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Kallmann syndrome is a rare heterogeneous hereditary disorder. It consists of congenital, idiopathic hypogonadotropic hypogonadism secondary to deficiency of hypothalamic gonadotrophin-releasing hormone (GnRH), and hyposmia or anosmia. We found the 14-year-old proband among 24 males with hypogonadotropic hypogonadism. He was born after the 2nd physiological pregnancy by the 1st spontaneous normal delivery. At birth his weight was 3350g (<75th centile), his length was 52cm (<75th centile). His parents are non-consanguineous. Proband has no eunuchoid habitus, gynecomastia and the mirror movements but his height is short. He has hyposmia, small dense testes (2 ml) in scrotum, small penis (8x1.5 cm) and decreased sexual hair (lanugo hair). Serum concentrations of testosterone (T), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were low. The thyroid gland function is not disturbed. Proband showed small enlargement of testes and increased content of T in blood plasma as a result of multiple hormonal agent supplements (Busereline acetate, chorionic gonadotrophin). His smell testing was stimulated by small doses of Busereline and he began to distinguish perfume and food odors. The usual doses of Busereline were a reason of an inadequate response of smell testing (worsened olfaction, hyposmia transformed into anosmia), but without hormonal supplement his sense of smell was impaired. His transmitting mother had partial anosmia especially after exacerbation of allergic rhinitis and acute respiratory tract diseases. His father is healthy man without phenotypic sings of KS.

P205. Hypomelanosis of Ito caused by mosaic balanced reciprocal translocation

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Mosaic balanced reciprocal translocation is rare. Most cases were ascertained via prenatal diagnosis or in the course of investigations for miscarriages. In the majority, the phenotypes were normal. Only 2 cases of confirmed mosaic balanced reciprocal translocation have congenital abnormalities.
A 6 year-old boy presented with hypotonia, learning difficulties and infantile onset epilepsy. He has macrocephaly and hypopigmentary streaks on his trunk which followed the Blaschko lines. The standard lymphocyte karyotyping was normal. His skin biopsy however showed an apparently balanced reciprocal translocation, 46,XY, t(1;9)(q21-23;q22) in 4 out of 50 cells. Using his buccal cells, we have painted chromosomes 1 and 9 and this has confirmed the mosaic reciprocal translocation (5%)reported in his skin biopsy.
Like many other cases of Hypomelanosis of Ito, the underlying cause in this boy is a chromosomal mosaicism. In this particular case, it was an apparently balanced reciprocal translocation. Submicroscopic deletion, gene disruption or gene position effect could be the underlying explanation for the phenotype.

P206. Diagnosis of Pallister-Killian syndrome - remember the mouth swab!

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Pallister-Killian syndrome is a rare chromosomal condition, often

with a normal karyotype in lymphocytes but mosaicism in other cell types. Because of the trauma involved in a skin biopsy, there is a certain threshold to perform this diagnostic procedure thus potentially delaying the diagnosis.
This case presentation re-emphasizes that the clinical diagnosis can be confirmed by interphase FISH analysis of cells from buccal scrapings. The need for a skin biopsy can be avoided, especially in children.
A simple protocol for the preparation of the buccal specimen is presented. An additional step involving protease K in the hypotonic treatment significantly reduced the background signal due to cytoplasm and improved the quality of the interphase FISH signals.

P207. Trisomy 12 mosaicism: about two reports

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Among the autosomal trisomy mosaicisms, those involving chromosome 12 are very uncommon. At the present time, only five cases have been reported post natally. The phenotype is extremely variable, ranging from severe malformations to a fortuitous finding in an adult studied for infertility. Several factors, specially the number and type of tissues involved, and the proportion of abnormal cells determine phenotypic variability. The abnormal clone is detected either in blood lymphocytes and/ or in skin fibroblasts, usually at low rate. Pigmentary dysplasia and or corporal hemihypertrophy are frequent but non-specific findings. According to clinical context and when these signs are present, chromosome analysis of skin fibroblasts must be performed, even if the blood karyotype was apparently normal.
We report two patients with a similar phenotype including dysmorphic features, lower limb hemihypertrophy, pigmentary anomalies that appeared during infancy and moderate mental retardation. Cytogenetic analysis showed a normal karyotype on peripheral blood. Skin biopsy revealed trisomy 12 in 38% and in 3% of cultured fibroblasts for the first and the second patient respectively. This latter with the lower percentage of aneuploid cells presented an ovarian tumour. Interestingly, this chromosome abnormality has been described associated with isolated ovarian tumour.
Our observation demonstrated that constitutional trisomy 12 mosaicism could predispose to cancer.

P208. Pregnancy outcome in carriers of translocation involving the Miller-Dieker critical region

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In a recent study of 14 families segregating a reciprocal translocation involving the Miller Dieker Syndrome (MDS) critical region (Pollin *et al*. Am J Med Genet 1999,85:369), the overall risk of MDS in the offspring was found to be 18.9±3.5% (microdeletion, abnormal phenotype, or both). Here we report a family segregating a 11p;17p translocation as balanced through two males in one generation, and as unbalanced at a high rate in the following generation. In two girls (first cousins on paternal side) with retarded development, epilepsy, and craniofacial dysmorphology suggesting MDS, FISH analysis with specific probes (Vysis) revealed microdeletion in the 13.3 region of chromosome 17p in both cases. FISH on metaphases of the patients' fathers showed cryptic balanced translocation between 11p and 17p in both cases. Cytogenetic examinations of the patients' phenotypically normal sibs (one and two sibs, resp.) identified two further carriers and one brother who has no translocation. Microdeletion was identified by prenatal FISH analysis in two further offspring of one of the fathers (fetal pathological examination confirmed MDS in both) which means that 3 out of the 4 offspring of this carrier father had deletion of the MDS critical region. Comparing this family to those analysed by Pollin et al., it is noteworthy that the transmission of the balanced rearrangement involving the MDS critical region which was silent in the the fathers' generation turned into unbalanced in the next generation resulting 4 microdeletions (two live-born patients and 2 fetuses) out of the 7 offspring.

P209. Three new microsatellite markers for a PCR-based diagnostics of the Miller-Dieker syndrome and isolated lissencephaly sequence

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Miller-Dieker syndrome (MDS, MIM*247200) is a rare autosomal dominant disorder characterized by lissencephaly and facial abnormalities. About 90% of MDS patients have variable visible or submicroscopic deletions on chromosome 17p13 involving *LIS1* and other genes distal to *LIS1*. Point mutations in or deletion of the *LIS1* gene only result in isolated lissencephaly sequence (ILS, MIM*601545). Two markers, *D17S5* (YNZ22) and *D17S379* are usually used for a rapid PCR-based diagnostics of the MDS deletions. However, both of these markers are telomeric to the *LIS1* gene. Additionally, the *D17S5* marker is situated too far from the *LIS1* gene to be efficient for detection of some MDS and ILS microdeletions.
We propose here three new highly polymorphic microsatellite markers suitable for a PCR-based diagnostics of MDS. One intragenic tetranucleotide repeat has observed heterozygosity of 41%. Two CA-repeats with observed heterozygosities of 65% and 73% were selected from the *LIS1* containing clone and are centromeric to the gene. All these markers were used for DNA-diagnostics in one MDS and two ILS families where cytogenetically visible 17p13 chromosomal deletions were not found.

P210. Lissencephaly phenotypes caused by missense mutations in the LIS1 gene.

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Classical lissencephaly is a cortical malformation secondary to impaired neuronal migration resulting in mental retardation, epilepsy and motor impairment. The clinical severity generally correlates with the degree of agyria and cortical thickening. Approximately 70 % of patients show abnormalities of the LIS1 gene on 17p13.3 or of the DCX gene on Xq22.3. A more severe lissencephaly in posterior brain regions is generally observed in patients with LIS1 mutations. Previous work suggest that patients with a LIS1 missense mutation have a wider and milder spectrum of cortical malformations and clinical sequelae compared to patients with other mutation types. Currently, among the 33 mutations reported in the LIS1 gene only 5 are missense mutations. Here, we describe 5 novel missense mutations (K64N, D129V, G314V, V363I, D401H), identified by direct sequencing of the LIS1 gene. Of these, one (V363I) was found in three relatives of the affected child: his mother who is mildly mental retarded but with a normal MRI scan and his grandfather and aunt who are normal. DCX was sequenced in this family but no mutation was found. To date, this is the first non-synonymous polymorphism found in the LIS1 transcript, which encodes a highly conserved protein. In addition, we describe the clinical and imaging features of the 10 patients with a LIS1 missense mutation. Our data support the hypothesis that, in most cases, the lissencephaly phenotype associated with LIS1 missense mutations is milder, but also questions the pathogenicity of certain LIS1 missense mutations identified in sporadic cases.

P211. 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 is so small that it may escape detection by standard karyotyping. The syndrome is characterised by severe growth and psychomotor retardation, microcephaly, ‚Greek helmet' facies and closure defects.
We report here a 4 month old girl (Patient 1 or Case 1) and a 9 month old boy (Patient 2 or Case 2) whose clinical signs strongly suggested WHS. Patients 1 and 2 both are products of the third pregnancy of healthy young non-consanguineous parents. The parents of Patient 1 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. The family history of Patient 2 was eventful. Chromosomal analysis by G-banding at 450 bands resolution indicated a normal karyotype of 46,XX and XY, respectively. Clinical signs were very suggestive of WHS and FISH studies were performed by one of us (OB) using DNA probes for chromosome 4p16.
In Case 1, FISH tests performed in the parents showed a balanced translocation between chromosome 4p and 20q in the mother/father. Consequently, Patient 1 had a distal trisomy of 20q in addition to the distal monosomy of 4p. In Case 2, the parents showed normal karyotype by FISH. In genetic counselling, prenatal diagnosis was offered to both couples.

P212. Clinical and molecular genetics and epigenetics of Beckwith-Wiedemann syndrome (BWS).

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BWS is a model imprinting disorder resulting from mutations or epigenetic events involving imprinted genes at chromosome 11p15.5. Thus germline mutations in CDKN1C, uniparental disomy (UPD) and loss of imprinting (LOI) of IGF2 and other imprinted genes have been implicated. Many (~40%) familial BWS cases have germline CDKN1C mutations. However, most BWS cases are sporadic and UPD or putative imprinting errors predominate in this group. Sporadic cases with putative imprinting defects may be subdivided into (a) those with H19 hypermethylation and silencing, and LOI of IGF2 in whom it is postulated that there is a defect in a distal 11p15.5 imprinting control element (designated BWSIC1) and (b) those with loss of methylation at KvDMR1, LOI of LIT1 and variable LOI of IGF in whom it is postulated that there is a defect at a more proximal imprinting control element (BWSIC2). We investigated genotype/epigenotype-phenotype correlations in 179 cases referred for molecular investigation of BWS. Hemihypertrophy was strongly associated with UPD ($p<0.0001$), whereas exomphalos was associated with BWSIC2 defect or CDKN1C mutation and not UPD or BWSIC1 ($p<0.0001$). Combining our data with other large studies revealed that the risk of embryonal tumours was highest (>25%) for UPD and BWSIC1 cases. However while <5% of BWSIC2 and CDKN1C cases developed tumours, tumours occurred in each molecular subgroup. Investigations to determine if there is a correlation between extent of segmental disomy and tumour risk in UPD cases are in progress.

P213. Detailed deletion breakpoint mapping alongside in-depth cognitive profiling as a tool to aid genotype-phenotype correlations in Williams-Beuren Syndrome

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Williams-Beuren syndrome (WBS) is a developmental disorder caused by a hemizygous deletion (~1.5Mb) at chromosome 7q11.23. The phenotype is complex with striking physical, cognitive and behavioural aspects that include overall IQs in the range of 50-60 alongside an uneven cognitive profile (verbal tasks outstripping spatial tasks). Physically, WBS phenotypes include a dysmorphic face, congenital heart disease (typically SVAS), growth retardation, hyperacusis, premature ageing, and infantile hypercalcaemia. Up to 25 genes have been identified within the critical region, however, only hemizygosity for elastin is associated with a phenotype (SVAS). Therefore, alone or in combination, some of the remaining deleted genes appear to be responsible for the other features of WBS. Generally, relations between genotype and phenotype in WBS are studied at the group level with rather gross measures of behavioural outcome, yet, much is to be gained by more in-depth cognitive studies. We have performed detailed cognitive analyses on a number of WBS individuals to identify those which show higher or lower functioning. One higher functioning patient (WBS-HF) has been genotyped using somatic cell hybrids and compared at both molecular and cognitive levels with patients exhibiting classic phenotypes or SVAS, to correlate genotype with phenotype. The results show that despite above-average scores for the syndrome, WBS-HF had the typical uneven profile of WBS and a characteristic WBS chromosomal deletion. In contrast, the SVAS patients, with a smaller deletion, displayed a normal profile suggesting that genes at the telomeric end of the WBS deletion are important in causing the WBS cognitive profile.

P214. An autosomal dominant hidradenitis suppurativa in a large Indian family.

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Hidradenitis suppurativa (HS) (OMIM 142690) is considered as a chronic disease of apocrine gland. It is usually develop in the groin and some times under the arms and under the breasts. The risk of developing any cancer with patients with HS is high as compared with others (Arch Dermatol 137:730-73, 2001. Several small and moderate families with autosomal dominant mode of inheritance have been reported (Br J Dermatol 142(5): 947-53, 2000), however the genes responsible for HS is yet to be identified. We have studied a large three generation Indian pedigree with an autosomal dominant HS. The pedigree consists of 65 individuals including twenty affecteds (12 males/8 females). The age onset was during puberty. The phenotype appeared to be 100% penetrant in this family. The expression of the phenotype was variable and ranged from very severe to moderate with typical features of HS. Detailed pathologic examinations were performed including histopathologiccal studies in selected affecteds. Majority of the examined individuals were severely affected and their findings included cutaneous scars, folliculitis, GI polyps, familial gall stones, sinuses axillae, polymorph function defects, pilosebaceous abscess and folliculitis. Hirsuitism was observed in affected females. Skin grafting was performed in some of these individuals and two affecteds died due to squamous cell carcinoma. Blood DNA samples were made from selected individuals for future plan of research. u_c_rao@hotmail.com.

P215. Effect Of Deferiprone On Skeletal Radiography Of Thalassemia Major Patients

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Thalassemia major patients require life long blood transfusion along with iron chelation therapy. Chelation therapy can cause a number of side effects. *Objective*: The study was undertaken to examine the effect of deferiprone on large joints of thalassemia major patients. *Material and Methods*: Sixty-two thalassemia major on hypertransfusion treatment regime aged between 4 and 19 years were assigned to three groups. Group I included 42 patients on deferiprone, Group II included 10 patients on desferioxamine, and

Group III included 10 patients who were not taking chelation therapy. Radiographs of wrist, elbow, knee and ankle were taken to examine bone age, bone density,bone expansion and articular changes. *Results*: Radiographs of 3 patients (4.9%), one from Group I and 2 from Group II were normal. Rest (96%) of the patients had mild to moderate bone marrow expansion and reduction in bone density. 19/42 (43%) patients from Group I showed articular changes in the knee joint which clinically correlated with complaints of joint pain, stiffness, limping, swelling, inability to squat and/or climb stairs. Articular changes were alsopresent in the wrist 9/42 (21%), elbow 9/42(21%) and ankle 4/42(9.5%); reduced joint space in elbow in one and soft tissue swelling in another. No such changes were observed in Group II and III patients. *Conclusion*: Our study reports highest incidence of arthritic changes in the knee joints of thalassemic patients. Since these changes were present in patients who were on oral iron chelator, this suggests that these symptoms are related to deferiprone therapy.

P216. No evidence of HESX1 mutation in five cases of septo-optic dysplasia with variable phenotype, including metabolic cardiomyoapthy and further brain anomalies

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Septo-optic dysplasia (SOD), also called de Morsier syndrome, is a highly heterogeneous condition defined by any combination of optic nerve hypoplasia, midline neurological abnormalities such as agenesis of the corpus callosum and absence of the septum pellucidum, and pituitary hypoplasia. The most frequent endocrine defect is growth hormone deficiency. Our patients fulfill these defining criteria, and somw have additional brain anomalies and metabolic cardiomyopathy. Previous data showed an important role for Hesx 1 / HESX 1 in forebrain, midline and pituitary development in mouse and human. Recently a homozygous missense mutation in the homeobox gene HESX 1 was detected in one familial case. We screened 5 Taiwanese patients with sporadic SOD for HESX 1 mutations by direct sequencing. There were no mutations or polymorphisms in the coding and boundary regions of the HESX 1 gene. Therefore, mutations of this candidate gene seem not to be frequently involved with sporadic SOD cases. Further research for mutations in regulatory regions of the HESX 1 gene or other developmental gene disruptions (such as cytochrome b, PIT) which might be involved in the pathogenesis of SOD.

P217. ATM Gene Mutations Detection in Iranian Patients with Ataxia – Telangiectasia

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Ataxia-Telangiectasia is an inherited autosomal recessive disorder characterized by defect in a number of distinct organ systems. Symptoms include a progressive cerebellar ataxia, telangiectasia, immunodeficiency, chromosomal instability radiation sensivity and increased incidence of malignancies. The ATM gene of human chromosome 11q22.3 has recently been identified as the gene responsible for human recessive disease ataxia – telangiectasia (A-T).
ATM is encoded in 66 exons and spans 150kb of genomic DNA.
In this study 20 families with at least one affected child with clinically suspect for ataxia – telangiectasia were examined and DNA was extracted and amplified by using standard methods.11 exons which were hot spot for point mutations in ATM gene were detected by PCR-RFLP. Sequencing methods was used to detect the new point mutation. We suggest that the sequencing is the best method for investigation of the genes with high rate of mutations.

P218. A study of the clinical and genetic features of thiamine-responsive megaloblastic anaemia in UK patients

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Thiamine-responsive megaloblastic anaemia (TRMA) is an autosomal recessive disorder resulting in diabetes mellitus, anaemia, optic atrophy and deafness. The SLC19A2 gene is mutated in TRMA patients and codes for a thiamine transporter protein (ThTr1). The diabetes is insulin dependant and non auto-immune. The disorder has a variety of symptoms and varying response to addition of thiamine, which leads to diagnostic confusion. We aimed to characterize the syndrome in the UK population, define the mutation spectrum, and assess the feasibility of a mutation screening strategy for affected families.
We identified 12 patients with TRMA: a prevalence of 1 per 5 million. Sensorineural deafness presented at a median age of 7 months. Non-autoimmune, insulin deficient diabetes mellitus presented with a median age of onset of 2 years, with a variable anaemia. Other abnormalities included optic atrophy in 4 patients. Treatment with thiamine reduced insulin requirements in 7 patients, but the effect reduced over time.
We identified mutations in the SLC19A2 gene in all patients: 3 nonsense, and one insertion mutation in the UK cohort and a novel missense mutation in a non-UK TRMA patient. No phenotype/ genotype relationships were observed, but the same mutation appeared in 3 of the 6 UK families suggesting a founder effect and a common ancestor. Childhood onset diabetes mellitus, deafness and a variable anaemia are the best available diagnostic criteria for TRMA, the differential diagnosis of which includes other inherited diabetes syndromes. Screening of the SLC19A2 gene identified mutations in all patients confirming the symptomatic diagnosis.

P219. Genetic variation of methylen tetrahydrofolate reductase and methionine synthetase reductase genes in Iranian patients with coronary artery disease

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Several studies showed that elevated plasma homocysteine is a risk factor for coronary artery disease. Two common mutations in the methylenetetrahydrofolate reductase (MTHFR) gene C677T and A1298C are reported to be associated with decreased enzyme activity in which homozygotes mutated genotypes accumulate significant levels of non-methylated folate derivatives. This study was to analyze the frequency of these mutations in 100 patients with CAD compared to the 100 normal control. The preliminary study shows the higher prevalence of the mutation in the CAD patients compare to the normal control in Iranian patients. Final statistical analysis in the time of conference will show the prevalence and significance of these mutations in studied cases compared to the normal control in Iranian cases.

P220. Cytogenetic analyses in mentally retarded school studens

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Mental retardation is defined as an IQ of less than 70, and it accounted for approximately 2 to 3 percent of population. Chromosomal abnormalities are the important cause of mental retardation. In order to study the frequency of chromosomal abnormalities and to determine the types of chromosomal abnormalities that play a major role in the causation of mental retardation, we recruited 427 moderate to severe mentally retarded students (253 males and 174 females) from school for the mentally retarded under parental consent during the period from November 1999 to December 2002. In our study, all metaphase chromosome preparations were obtained from peripheral blood cultures and GTG banded chromosomes were examined at a 500 to 550 band level.

The results revealed chromosomal abnormalities accounted for 23.89 % in the recruited students (102 cases, 44 males and 58 females). Obviously, the number of 75 cases of Trisomy 21 (17.56 %, 33 males and 42 females) is a major contributor of mental retardation. Three cases associated with sex chromosome abnormalities are numerical (0.70 %, two 47, XXY and one 47, XXX), which may not directly cause mental retardation. Among 24 cases of autosomal abnormalities (5.62 %, 9 males and 15 females), involving deletion, markers and translocations, 2 cases with cryptic rearrangements are inherited from maternal balanced reciprocal translocations. In conclusion, the total frequency of cytogenetic anomalies in afflicted students is high. Still, conventional cytogenetic analyses remain the simple, inexpensive and effective way of investigation in mentally retarded patients.

P221. Unusual chromosomal abnormalities associated with mental retardation in children

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Objective: The authors present two unusual chromosomal abnormalities identified in two children with mental retardation (MR).
Material and methods: The children were admitted to the Department of Pediatric Neurology of the Clinical Hospital Al. Obregia, Bucharest for evaluation of a delayed psychomotor development. They were included in a large study, part of a national research program, which investigate the cytogenetic causes of MR in children. First case, a six months old boy, showed: dysmorphic features, hypotonia, sever psychomotor retardation. Cerebral MRI revealed complex brain malformation, including agenesis of corpus callosum, large ventricles. The second case, an eight-year-old girl, showed: dysmorphic features, sever MR, hyperkinesias with self-injurious behaviors. The children were investigated cytogenetically by karyotype with GTG-banding.
Results: In the first case, the cytogenetic investigation revealed a deletion of the long arm of chromosome 1 at band q42. By our knowledge this is the fifth case of del(1)(q42-qter) reported by now. In the second case, the karyotype showed a partial trisomy 18pter-18q21.
Conclusions: The chromosomal abnormalities are one of the most important causes of MR. For a better description of the sequences involved in the breakpoints in these two cases, we intend to extend the study by applying molecular genetic methods.
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P222. Approach to rare chromosomal disorders under the prism of minor dysmorphic features

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Minor dysmorphic features are defined as unusual morphogenetic patterns that do not have medical importance for the individual, but may be of great importance for syndrome recognition.
Chromosomal abnormalities (numerical or structural) cause abnormal morphogenesis of many organs and systems, as well as distinct patterns of dysmorphic features. Such minor defects are present to a variable degree in all chromosomal abnormalities. Some of them, such as Down syndrome, are easily recognized, but in rare chromosomal rearrangements it is difficult establish the diagnosis only by dysmorphic signs.
Children with different chromosomal rearrangements (except Down and Turner syndrome) have been evaluated for the presence of minor anomalies. Anomalies were divided into groups, according to the location (face, ears, hair, neck, limbs, body, and skin). Most of them were on the face (epicanthic folds, abnormal slanting paplpebral fissures, micrognathia, facial asymetry), and hands (brachydactily, clinodactily, abnormal dermatoglyphs, etc.). The incidence of each single minor malformation will be given. The number of these anomalies per individual case will be correlated with the severity of chromosomal change (deletion, duplication, translocation) and particular chromosomes that are involved. Also, a correlation of the minor anomalies with the major anomalies (cardiopathies, brain anomalies) will be made.
Minor dysmorphic features, although not important for the individual's health, have to be recognized because of the possibility of an underlying chromosomal or genetic disorder. Presence of three or more of those signs isolated or together with major birth defects, can raise the question of necessity of chromosomal analysis.

P223. Analysis of One Year of Referrals for Undiagnosed Developmental Delay.

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Undiagnosed developmental delay is a common reason for referral to a genetics clinic. One year of referrals for undiagnosed developmental delay to South West Thames Department of Clinical Genetics was analysed to establish the overall diagnosis rate and observe the types of diagnoses that are being made. In addition the data has been examined to ascertain any variables in the history or examination that may predict the department findind an underlying aetiology. The investigations undertaken have also been scrutinised to see which are giving a high diagnostic yield.
The overall rate for establishibg a firm diagnosis was 29% with an additional 12% of patients having tentative or partial diagnoses being made. It is not suprising that a genetics department is not diagnosing Downs syndrome, but perhaps unexpected that there is only one case of a microdeletion syndrome in the list of patient diagnoses. Analysis of features evident from initial history of examination that predict an aetiology being established show the presence of dysmorphic features and a milder level of delay to be the only factors with positive predictive value. The presence of autistic features, positive neurology (inc. microcephaly, macrocephaly, epilepsy) other system anomalies (eg cardiac etc)were all found not to have any predictive value in whether or not a firm diagnosis would be made. Analysis of the investigations that had been useful in establishing a diagnosis showed X-rays, skin chromosomes, opthalmological opinion and haematology to have been the most diagnostically helpful, and telomeres the least.

P224. Recent advances in diagnosis of genetic disorders

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The aim of the present study was to bridge the gap between conventional cytogenetics and molecular genetics using molecular cytogenetics approach and to apply new technologies for patient care- in diagnosis, prevention and management.
Prenatal diagnosis was done to screen for aneuploidies of chromosomes 13, 18, 21, X and Y using FISH on both dividing and non-dividing (interphase) cells obtained from amniotic fluid and chorionic villi sampling. Postnatal diagnosis of congenital anomalies was carried out to screen for minor cell lines in interphase cells obtained from peripheral blood in cases of primary amenorrhea, male hypogonadism and mental retardation. The cases with ambiguous genitalia were further analyzed using probe specific for the sex-determining region (SRY) on the Y chromosome. In leukemias, FISH was used for diagnosis and evaluation of minimal residual disease. Low-level mosaicism was detected using FISH in cases of primary amenorrhea, hypogonadism and submicroscopic gene rearrangements were detected in cases of ambiguous genitalia and cancers. Some patients of leukemias on therapy revealed presence of malignant clone in spite of complete cytogenetic remission. To conclude, results were obtained within 24 hours using interphase FISH that was of immense importance especially in prenatal screening and leukemias.

P225. A Genetic Study of 100 Patients with Autism Spectrum Disorders

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A known genetic condition was identified in several patients: five males were diagnosed as the fragile X syndrome (with FMR1 gene expansion), three females as suspected Rett syndrome, two patients as the Aarskog syndrome, and one patient each as the Smith-Magenis syndrome (confirmed by FISH), ring chromosome 17 syndrome, Borjeson-Forssman-Lehmann syndrome, suspected Cornelia de Lange syndrome, an inborn error of metabolisms, and preclinical familial hypothyreosis. The phenotype of most of the remaining patients was not remarkable with the exception of several patients with a non-specific facial stigmatisation, macrocephaly, or unusually shaped ears. In 14 individuals the pregnancy or the pre- or perinatal history were complicated. Three patients might have suffered from foetal hydantoinate syndrome, and one patient from herpetic encephalopathy. In five patients autism might be secondary to deafness. Several patients had a family history of psychiatric disorders. Behaviour in relatives typical of the broader autism phenotype was reported for several patients, mainly those affected by the Asperger syndrome or by highly functioning autism, possibly reflecting a higher genetic load in these pedigrees. Cytogenetic findings were normal in most of the patients. One patient had a t(21;22) translocation, one a mosaic of ring chromosome 17, two brothers a translocation t(9,17)(p13,q22), and one patient a mosaic of chromosome 8 trisomy [1/50]. A possible increase of the frequency of the ADA 8Asn allele was also analysed by molecular genetic methods. It was very low and did not even reach the frequency observed in control samples of the published studies.

P226. Chromosomal abnormalities in children with mental retardation assosieted with dysmorphisms

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About 15% of persons with psychomotor retardation (PMR) have chromosomal abnormalities associated with dysmorphic features. The aim of study was to determine the frequency of chromosomal abnormalities in persons with PMR and dysmorphic features. The karyotype has been done in persons with PMR and dysmorphic score by Waldrop method ³ 7. The investigations comprised 217 persons with PMR.

Chromosome abnormalities were found in 59,5% of children with dysmorphic signs and MR, predominantly in those from the group with severe and profound MR. Only 4.3% of group with milder forms of MR had chromosome abnormalities (autosomal and gonosomal abnormalities were found in equal number). In the group with severe and profound MR, all pathological changes were found on autosomal chromosomes (16,5% children from this group). Leading chromosome abnormality was trisomy 21. This was present in 6,9% children with MR, and in 15,2% of children with severe and profound MR.

In children with dysmorphic features an MR, the most frequent cause is a chromosomal abnormality, especially in the group with severe and profound MR (72,2%). Assessment of dysmorphic score, by Waldrop protocol is a simple, quick and applicable method, which can be used as a criterion for cytogenetic analyses and revealing the MR etiology.

P227. Our experience in Meconium Plug Syndrome in the Last Decade

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Objectives: *to establish the rate of occurence of the meconium plug syndrome in newborn as a cause of intestinal obstruction; *to assess the role of different clinical presentations of the syndrome in a proper diagnosis; *to verify the main methods used in early differentiation from Hirschprung' s disease.

Methods: We studied all newborns hospitalised in our departments presenting signs and symptoms of intestinal obstruction between 1993-2002 using the prenatal history, clinical features before and after admission and also the radiological images of the abdomen. Histological aspects were obtained after rectal biopsies. Also sweat test, glucose and magnesium dosage were performed to demonstrate colonic hypomotility.

Results: The authors hospitalised 58 cases of newborns with intestinal obstructions including intestinal atresia and stenosis (19 cases), meconium ileus (6 cases), necrotizing enterocolitis (3 cases), Hirschprung' s disease (10 cases), malrotation (4cases), meconium peritonitis (3 cases) and meconium plug syndrome (13 cases). We correlated meconium plug syndrome with prematurity (84,61%), sweat test significant for cystic fibrosis (15,38%) and hypoglycemia and increased glucagon production (7,69%).

Conclusions :1). The rate of occurence of the meconium plug syndrome in our study was 22,41% of all newborns hospitalised for intestinal obstruction.

2). Prematurity was present in 84,61%.

3). In two cases (15,38%) the sweat test was significantly abnormal indicating cystic fibrosis.

4). One of our patients (7,69%) had a diabetic mother and hypoglycemia at the newborn was corelated with increased glucagon production.

5). Hypermagnesemia was not shown to be a cause of hypomotility

P228. The prevalence of the Arnold Chiari malformation in premature babies

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Purpose: Diagnosis integration of the echographically tracked down disease; establishing a relation between the echographic signs and the clinic ones; establishing a relation between evolutive stadium and the moment of surgical intervention.

The study was performed at Neonatology and Puericulture Clinic between 1992-2000 and it contains 27 cases of preterm newborns diagnosed with CNS Malformations through both a transfontanel ultrasound examination (TF) and CT. TF was dynamically performed, according to a standardized protocol, using a convex 5 MHz transducer. The sonographic approach was through the anterior fontanel and all specific anatomic structures were studied.

The malformation types were as follows: cranio-vertebral dysraphia - 15 cases (55.55%), Arnold Chiari II syndrome - 5 cases (18.51%), Arnold Chiari III syndrome - 3 cases (11.11%), Dandy Walker syndrome – 3 cases (11.11%), Corpus callosum agenesis – 5 cases (18.51%), Malformation of Galen's vein – 2 cases (7.40%), Arachnoid cyst – 2 cases (7.40%). In most of the cases multiple malformations were associated. The association of meningomyelocele, with other CNS malformations was present in 44.66%.

Enlarged ventricles were found to be present with all types of CNS malformation in this study. It had an evolutive character being accompanied by severe clinical signs (recurrent convulsive syndrome, lower limb paralysis accompanied by urinary incontinence and absence of both sucking and swallowing reflex with cases of Galen's vein malformation, Arachnoid cyst, and Arnold Chiari III malformation. Only in 2 cases (7.40%) of hydrocephalus was a ventriculo-peritoneal drain inserted, although the diagnosis was established early.

P229. Chromosomal pathology revealed in children before the age of 1.

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2594 children were ascertained by the monitoring system in newborns which is conducted at state level for the whole population of the Republic of Moldova. These children received medico-genetic counselling at the Scientific Research Institute of Motherhood and Childhood Health Care before the age of 1. 714 children, 88.0% of whom had multiple congenital malformations, were investigated cytogenetically. Chromosomal pathologies were revealed in 41.0%. Aneuploidies comprised 87.9% of abnormalities, of which 84.6% were trisomy 21, 03% trisomy 13 and 0,3% trisomy 18. Structural chromosomal aberrations were noted in 8.1%. Anomalies of sex chromosomes constitued 2.7% of chromosome abnormalities. 1.4% were cases of chromosomal polymorphisms. The clinical diagnosis

of Down syndrome was confirmed in 99.9%. Among anomalies of chromosome 21, 85.5% of the cases were simple trisomic type, 13.2% - mosaic and in 1.3% structural aberrations. In 66 parents of the children with Down syndrome that we investigated, 12.1% showed chromosomal polymorphisms: 46,XX,22st in one mother; 46, XY, yq+ in 6 fathers; 46, XY,yq+21st+ in one father. In a father we found 46, XY,dup4(p11). We investigated the frequency of Down syndrome according to parental age. The highest frequency of parents were over 35 years old (30.8%), but also 24.7% of mothers and 30.2% of fathers were aged 21-24.

P230. Analysis of deletions of the MECP2 gene in Rett's syndrome (RTT) and Rett's syndrome "plus" (RTT+) patients

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Rett's syndrome (RTT) is a significant cause of mental retardation affecting 1 in 10-15000 girls. Mutations in the MECP2 gene which encodes the methyl CpG binding protein, account for >70% of cases of classical RTT.
Over 500 UK samples have been analysed by the Cardiff diagnostic service using a PCR, sequencing based strategy. 90 MECP2 mutations have been identified the majority of which are nonsense and missense mutations. Patients with a high clinical suspicion of RTT but no mutation were further investigated using a quantitative fluorescent PCR assay. This was designed to detect multi- and single exonic MECP2 gene deletions. This approach has allowed the identification of five MECP2 gene deletions in 29 mutation-negative classical RTT patients tested. Genotype-phenotype correlation showed classical RTT in 3 patients with intragenic deletions while the 2 patients with deletions extending beyond the 3' end of the gene have a "RTT-plus" phenotype, including cleft palate and congenital heart malformation.
In conclusion our data show that MECP2 deletions are an important cause of classical RTT syndrome, and that additional features suggest deletions extending beyond this gene.

P231. Transcriptional profiling in Rett syndrome using spotted cDNA microarrays.

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Rett Syndrome (RTT) is a severe neurological disorder affecting exclusively females. Its prevalence is about 1 in 15,000 live borns and it accounts for approximately 10% of severe mental retardation in women. The clinical course of the disease is typical and consists of a normal neonatal period followed by an arrest of the development between 6 and 18 months of age. The patients show a number of clinical signs indicative of a neurodevelopmental defect : arrest of brain development, regression of acquisitions, and behavioural troubles (stereotypic hand movement, autism).
Mutations in the MeCP2 gene were identified in 70-80% of Rett syndrome cases. The MeCP2 gene encodes a methyl-CpG-binding protein that is expressed ubiquitously and acts as a global transcriptional repressor through its interaction with mSin3A. Defects in MECP2 function are thus expected to lead to the abnormal transcription of a number of currently unidentified genes. To test this hypothesis, we have performed global expression analysis using cDNA microarrays. We have compared the expression level of 12.000 non redondant cDNAs and EST in five RTT lymphoblastoid cell lines (mutations R168X, R255X, R270X, R294X and 608insA) and two clonal cell lines expressing only the MeCP2 transcripts with the R106W and R194X mutations. Several genes show a significantly different expression level in RTT samples when compared to wild type lymphoblastoid cell lines. These results are being confirmed by Northern Blot analysis and we expect to identify a subset of genes that are (directly or indirectly) regulated by the MeCP2 protein.

P232. Rett syndrome: a study of the face

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Rett syndrome is a neurodevelopmental disorder caused by mutations in *MECP2*. The face is said to resemble that of Angelman syndrome, which is characterized by a prominent mandible, wide mouth with spaced teeth, and microcephaly by age 2. In this study of the face in Rett syndrome, we have evaluated 37 females, from 2 to 60 years of age, performing a dysmorphology examination and detailed set of measurements.
Most individuals with Rett syndrome were not unusual looking and resembled their family. Many persons examined had a wide face with broad lower jaw and full cheeks. This was particularly noticeable in the young girls. In addition, eye fissures had a mild upslant and appeared slightly close-spaced. A low hanging columella was frequently found. The philtrum often was short with a shallow groove, an everted upper lip and full lower lip.
Objective study revealed microcephaly, a relatively short round head, broad forehead and face, and slightly wide nasal base. Most dimensions fell within the normal range. In the three children under 3, there was a striking and consistent broadness of the head and upper face. The pattern of craniofacial dimensions was fairly consistent over time, suggesting that the face in Rett syndrome follows the expected sequence of age-related changes.
In conclusion, although there are some subtle subjective and objective facial differences in Rett syndrome, the diagnosis cannot be made solely by inspection of the face. While Rett and Angelman syndromes have similar clinical, neurological and behavioural phenotypes, the faces are different.

P233. Molecular Characterization And Genotype-phenotype Correlation Italian Patients With Rett Syndrome

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Rett sindrome is a X-linked dominant neurodevelopmental disorder, presenting almost exclusively in females. Clinical diagnosis of classical form rely on a battery of obligatory criteria (normal pre/perinatal period and normal head circumference at birth, followed by loss of acquired skills as communication and purposeful hand use and deceleration of head growth, gait posture dyspraxia) and a sequence of characteristic stages. Diagnosis is usually confirmed by supportive manifestations. More severe variants forms (congenital), as well as milder clinical presentations have been described.
About 80% of classical forms shows mutation in coding region of MECP2 gene, which acts as global repressor of transcription, while the molecular cause is unknown in the remaining 20%. Mutation analysis on 93 Italian patients presenting with classic (24), variant (8) and Rett-like forms (61), with only a few obligatory criteria, showed sequence changes in 75% of classical and variant forms and 13.1% in the Rett-like subset, with three yet undescribed mutations and a somatic mosaicism case. Rearrangement analysis by Southern blot of MECP2 gene on 27/61 patients negative on mutational analysis, no found any alteration, suggesting involvement of others genes not yet identified. The pattern of X-inactivation was random in 27/29 MECP2 mutated patients, but the limited size of the sample does not allow to drawn conclusions about its role in clinical presentation.
Our study shows statistically significant correlation between mutation type and phenotype: N-terminal mutation are more severe than C terminal deletions (p=0,0017) and C terminal deletions lead to milder presentation than proximal truncanting mutations (p=0,0101)

P234. Characteristic X-ray sign of Rett syndrome: extreme thin diaphysis with narrow medulla of tubular bones

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As part of a long term follow-up study including clinical and genetic examination of Hungarian Rett syndrome patients X-ray evaluation of tubular bones was performed. Previously published reports of the X-ray signs of Rett syndrome described thin metacarpals and metatarsals, shortened ulna, curved radius and osteoporosis.
21 patients were examined with the clinical diagnosis of Rett syndrome of whom 19 cases had the typical form and 2 cases the atypical form of the disease. Clinical diagnosis could be confirmed by mutation analysis of the methyl-CpG-binding protein 2 gene (MECP2) in 17/21 patients.
In 14 patients with various mutations of MECP2 X-ray examination has found the signs of hand already known from previous reports. In 9 patients X-ray picture was made also from the long tubular bones of the upper and lower extremities, originally not included in the protocol of the study. Out of these 9 patients, a surprising feature was detected in 7 cases not mentioned in the case reports yet, namely, an extreme thin diaphysis with narrow medulla in particular of the fibula. Based on these preliminary results X-ray examination of long tubular bones is recommended to look for this characteristic X-ray sign in patients with Rett syndrome, however, further investigations are needed to prove the specificity of this change.

P235. Sporadic Rett patients: prevalence of inherited versus de novo MECP2 mutations.

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A causative MECP2 mutation was found in 111 apparently sporadic cases of Rett syndrome (RTT). 75/111 (67%) mutations were represented by hot spots, 13/111 (12%) were C terminal deletions, while 23/111 (21%) were private mutations. To confirm the de novo origin of the mutation, both parents of the 111 cases were tested. Surprisingly, in one case (1/111, 0.9%) the mutation was present in the apparently asymptomatic mother. The proband is a 18 years-old girl in the stage four of the disease (late motor deterioration). She developed normally until 15 months when rapid developmental regression and stereotypic movements appeared. She is now in a wheel-chair. The analysis of MECP2 revealed the presence of the hot spot mutation R306C in both the proband and her mother. Analysis of X inactivation in blood was not informative in the mother and balanced in the proband. Extension of the MECP2 analysis to the mother's relatives (parents and sister) revealed that it occurred "de novo" in one of the gametes of the mother's parents. This result allowed us to define a recurrence risk of 50% for the mother and 0% for her sister. In conclusion, these data underline the importance to perform molecular analysis in parents of apparently sporadic RTT cases, in order to assess the exact recurrence risk and to suggest the appropriate prenatal diagnosis.

P236. Deletion of exons 3 and 4 of MECP2 gene, revealed by MLPA technique, in a RTT italian girl with "classical" phenotype

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Mutations in X-linked methyl-CpG-Binding Protein Gene 2 (MECP2) have been found to be a cause of Rett Syndrome (RTT). Mutation detection was achieved in 79% of classical and 25% of non-classical RTT patients, by conventional techniques.
A novel mutation detection approach, called Multiplex Ligation-Dependent Probe Amplification (MLPA), was able to reveal a genomic deletion not identified by direct sequencing of MECP2 coding, and flanking intronic regions, in a 15 years old Italian girl with classical RTT.
In the patient and both parents, long-distance PCR coupled with long-read direct sequencing of the entire MECP2 coding region, did not reveal any mutation of the MECP2 gene, but identified in the mother an heterozygous C->A transversion at 375 (1125), in exon 3. An homozigous wild sequence is present in the father. RTT patient shows only maternal allele with C->A substitution: we hypothesised lack of entire exons in the RTT girl.
MLPA technique (MRC-Holland) is useful to reveal alterations of genomic DNA escaping detection by conventional diagnostic tools. This quantitative multiplex PCR approach was useful to determine the relative copy number of each MECP2 gene exons. Deletion of exons 3 and 4 was confirmed in patient.
This is the first demonstration of a deletion of entire exons of MECP2 gene; such large deletion escaped direct sequencing.
We are now extending the same study to our RTT patients not showing mutations of MECP2 gene by classic molecular methods.
The help from Jan Schouten (Department of Clinical Genetics, Free University of Amsterdam) is gratefully acknowledged.

P237. Rett patients with both MECP2 mutation and 15q11-13 rearrangments.

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Rett syndrome (RTT) and autism (A) are classified as separate disorders. Recently, the identification of the Preserved Speech Variant (PSV), where autistic behavior is usual, stressed the phenotypic overlap between these two conditions. In 1999 it was shown that about 80% of RTT cases have a *de novo* mutation in the transcriptional silencer *MECP2* gene, classifying RTT as a monogenic X-L dominant disorder. Subsequently, we and others provided evidence that this model was too simple and not sufficient to explain why germline *MECP2* mutations may result in X-L mental retardation in males while they may be silent in carrier females. On the other hand, we and others have provided evidence that 15q11-q13 maternally inherited duplications are found in autistic children, suggesting that an abnormal dosage of gene(s) within this region might be responsible for A. Now we show that a proportion of RTT patients are carriers of both a *MECP2* mutation and a 15q11-q13 duplication and that other RTT patients are carriers of a *MECP2* mutation and a 15q11-q13 duplication. Therefore, we hypothesize a complex model in which a *MECP2* mutation is necessary but not sufficient to cause the RTT phenotype in females, based on the status of a second gene which, at least in a proportion of cases, may be abnormal dosage of one or more 15q11-q13 gene. In summary, we provide preliminary evidence that A and RTT (clinically related for a long time) may have a common overlapping molecular basis.

P238. Mutation analysis of MECP2 gene in patients with Rett syndrome from Czech and Slovak Republics

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Rett syndrome (RTT), an X-linked disorder, is caused by mutations in the methyl-CpG-binding protein 2 gene (*MECP2*). MeCP2 protein contains two main functional domains, an N-terminal methyl-CpG-binding domain (MBD), followed by a transcriptional repression domain (TRD). It binds specifically to CpG-methylated DNA and is thought to act as a global transcriptional repressor. There are 5 prevalent mutations known in *MECP2* that cause RTT. Four of them are detectable by restriction analysis. We report mutation analysis of 51 patients, all girls, with clinical diagnoses of RTT from the Czech and Slovak Republics. Genomic DNA was used to amplify coding

sequence and exon/intron borders of the *MECP2* gene. Products were examined by restriction analysis and direct sequencing. The analysis revealed 15 different disease-causing mutations in 38 sporadic patients (75%). Three have not been previously published: a missense mutation, P302S, a small deletion of 3 bp - 1069delAGC and deletion of 172 bp along with insertion of 41 bp, 1063del172bp+ins41bp. Nineteen patients had missense mutations (R133C, K135E, T158M, R306C), fourteen patients carried nonsense mutations (Y141X, R168X, S204X, R255X, R270X, R294X) and two had frameshift mutations (806delG and 1157del141bp). Two novel polymorphisms, 587C>G (T196S) and 815C>T (P272L) were detected in patients carrying mutations R133C and R255X, respectively. Our results facilitate diagnosis of RTT at the molecular level in the Slavonic population and provide insight into the molecular pathology of Rett syndrome. *Supported by grants from GACR 301/01/P068 (D.Z., R.R., J.Z) and MSMT-LN00A079 (P.M.)*

P239. Abnormal methylation of a *MECP2* mutant allele in a boy with "male Rett syndrome" and his unaffected heterozygous mother.

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Rett syndrome is a severe neurodevelopmental disorder affecting principally females and characterized by a normal postnatal development followed by stagnation and regression of acquired skills. We report a mentally-handicapped 4-year-old boy and his unaffected carrier mother. The propositus was born at term after a pregnancy marked by diminished fetal movements and growth retardation. Birth weight and head circumference were below, and length was at the 10th centile. The baby was placid and showed axial hypotonia. Milestones were delayed: sitting at 10 months, standing with help at 15 months, first words at 20 months. Independent walking has never been achieved. Facial phenotype showed low-set anteverted ears and hair „cowlick".

At 46 months, the child could no longer crawl nor stand. Seizures with tonic limb movements, eye revulsion and loss of contact were observed. Neurological examination revealed microcephaly, stereotypic hand and hand-to-mouth movements, intention tremor, head nodding, intermittent convergent strabismus, multidirectional nystagmus, bruxism, absent speech, spasticity of inferior limbs with hyperreflexia and bilateral Babinski sign.

The patient and his unaffected heterozygous mother have a 44 bp truncating deletion in *MECP2* (c1158del44 or 386fs388X). The mutation arose on the grandpaternal allele. Maternal leukocytes expressed mutant mRNA, at a lower level than the normal allele. X-inactivation studies were uninformative but we showed that the mutation is associated in the patient and his mother with complete methylation of a normally unmethylated intragenic CpG dinucleotide. The occurrence of this typical "Rett" mutation in an unaffected female greatly complicates genetic counselling for this disorder.

P240. The spreading of X inactivation in X;Y translocations

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We have studied 25 individuals with segments of either Yp or Yq translocated onto the X. In each case we mapped X and Y breakpoints, determined X inactivation ratios, performed expression studies of translocated Y genes by allele-specific RT-PCR, and attempted phenotype-genotype correlations. Of 11 Y-genes studied only *SMCY* was silenced by the spread of X inactivation. Consistent with previous evidence suggesting that LINE elements promote the spread of X inactivation, sequence analysis showed a marked accumulation of LINEs within *SMCY* relative to *SMCX*, in contrast to other X-Y homologues in which LINE content was relatively low. We also observed that *TMSB4Y* was expressed at approximately 10% of the level of *TMSB4X*. This suggests decay of the Y homologue, providing evidence that *TMSB4Y*/*TMSB4X* is in the process of evolving from an X-Y gene pair escaping X inactivation to an X-linked gene subject to X inactivation.

There was no apparent relationship between X inactivation ratios and phenotype in the five Yp translocations studied with hermaphroditism or hypospadias. However, Y breakpoints in three were located only 30-70kb proximal to *SRY*. Although the translocated portion of Yp was much larger in the two other cases, RT-PCR showed that expression of *ZFY* (located 170kb proximal to *SRY*) was disrupted in both individuals. We hypothesise the presence of cryptic rearrangements of Yp11.31 in these two cases, and suggest that incomplete masculinisation in cases of X;Y translocation might be a result of disruption of normal *SRY* expression by position effect, rather than X inactivation.

P241. Neuroanatomical distribution of ARX in brain and its localization in GABAergic neurons

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Recent human genetics approaches identified the Aristaless-related homeobox (ARX) gene as the causative gene in X-linked infantile spasms, Partington syndrome, and non-syndromic mental retardation as well as in forms of lissencephaly with abnormal genitalia. The ARX predicted protein belongs to the large family of homeoproteins and is characterized by a C-terminal aristaless domain and by an octapeptide domain near the N-terminus. In order to learn more about ARX function, we have studied in detail ARX expression in the central nervous system during embryonal development as well as in the adult. We show that ARX is likely to not only play an important role in the developing telencephalon, diencephalon and spinal cord, but also in the olfactory bulb, neocortex, hippocampus, hypothalamus and amygdala in adult. Using anti-GABA antibodies, we show that ARX is predominantly expressed in GABAergic neurons in the cortex, suggesting that mutations in ARX alter their differentiation and/or migration and thus potentially causing the seizure disorders observed in humans. We also performed ARX wild-type and mutant over-expression experiments in COS7 and PC12 cells, and found that the different ARX mutations tested, including a poly-alanine expansion, do not modify the morphology of the cells even after nerve growth factor treatment. Moreover, no abnormal cell death or protein aggregation was observed, hence suggesting that more subtle pathogenic mechanisms are involved.

P242. Development of a full-coverage X-chromosomal BAC array for high throughput screening of genomic alterations in patients with X-linked mental retardation.

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Approximately half of the mentally retarded patients have a genetic origin and a significant proportion thereof is due to defects on the X chromosome. The European XLMR consortium (Leuven, Paris, Tours, Berlin, Nijmegen) has established a cohort of 200 well-characterized families with nonspecific X-linked mental retardation (MRX) which are being studied by various approaches. This unique cohort has been instrumental for the isolation of 6 of the 9 MRX genes that are known to date. Since most of the cytogenetically visible X-chromosomal deletions have already been studied, novel methods are needed to efficiently identify small submicroscopic deletions and duplications in MRX patients. Identification of such aberrations will immediately result in novel candidate genes for MRX. The technology that we have focussed on is array-based comparative genomic hybridisation (arrayCGH). We have constructed a full-coverage X-chromosomal BAC array consisting of approximately 1500 clones. The sensitivity and specificity of the technology were tested in a series of normal versus normal control experiments and a series of patients with known chromosome X copy number changes (including an Xq26 duplication, several Xq21 deletions, an Xp and an Xpter deletion, two PLP duplications and an XXXY). The results show that our array allows the detection of copy number changes >50 kb on the human X chromosome.

This study clearly demonstrates the power of the arrayCGH technology. Deletion and amplification mapping can now be

performed at the submicroscopic level and will allow high throughput identification of novel X-chromosomal regions harboring genes involved in X-linked mental retardation.

P243. Characterization of two novel genes, *PRKWNK3* and *CXorf17*, at the Xp11.2 chromosome breakpoint in a patient with intellectual disability and a balanced X; autosome translocation

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Nonsyndromic X-Linked Mental Retardation (XLMR) is a common, heterogeneous disorder which is currently estimated to affect approximately 1 in 1000 males. At least 17 families with XLMR have been described which have linkage intervals that colocalize to Xp11.2. In order to identify candidate genes for XLMR within this region we have used large insert genomic clones (BACS and PACS) to map the Xp11.2 breakpoint in several patients with learning disabilities and cytogenetically balanced X;autosome translocations by conventional fluorescence in situ hybridization (FISH).
The X chromosome breakpoint in one patient with a 46, X, t(X;16)(p11.21;q12.1) translocation has been characterized. The patient has mental retardation but no other distinguishing clinical features and has skewed X inactivation. PAC 390O13 was shown to span the X chromosome breakpoint by FISH. Southern blot analysis was used to further define the X breakpoint which was located to a 2.7 kb NdeI restriction fragment. This was found to lie less than 9 kb from two novel genes for which full length coding cDNAs were isolated from human brain cDNA. *PRKWNK3* is a member of a novel family of protein kinases, two of which have been recently shown to cause pseudohyperaldosteronism type II. *CXorf17* is a member of a previously undescribed family of putative transmembrane proteins without homology to any previously described proteins. By RT-PCR we have shown that transcripts from both of these genes can be detected in lymphoblastoid cell lines from the patient. They are therefore unlikely to underly this patient's intellectual disability.

P244. Identification of a submicroscopic duplication at the X chromosome breakpoint in a patient with an apparently balanced translocation 46,X,t(X;8)(q28;q12)mat and significant developmental delay

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Reports of patients with apparently balanced translocations who have duplications at their chromosome breakpoints are rare. We have investigated a female patient with developmental delay who was shown by high resolution GTL banding to have inherited an apparently balanced X;autosome translocation, 46,X,t(X;8)(q28;q12)mat. Her mother also had significant learning disability from childhood. Replication studies in the mother and daughter showed a skewed X-inactivation pattern in lymphocytes, with the normal X chromosome preferentially inactivated. To investigate the possibility that a novel candidate gene for X linked mental retardation was disrupted at the X chromosome translocation breakpoint, we mapped the breakpoint using fluorescence in situ hybridisation (FISH). This showed that the four known genes involved in non-syndromic mental retardation in Xq28, FMR2, SLC6A8, MECP2 and GDI1, were not involved in the translocation. We found that the X chromosome breakpoint in the daughter could not be defined by a single breakpoint-spanning genomic clone and further analysis revealed a 650kb submicroscopic duplication between DXS7067 and DXS7060 on either side of the X chromosome translocation breakpoint. This duplicated region contains eleven characterized genes, of which 9 are expressed in brain. Furthermore, for 5 of the genes in the duplicated region (NEMO, DKC1, MPP1, F8 and C6.1A) there are ESTs showing expression in the hippocampus, the region of the brain considered important in the processes of learning and memory. Duplication of one or several of the genes within the 650kb interval is likely to be responsible for the mental retardation phenotype seen in our patient.

P245. X-linked mental retardation: A novel candidate gene encoding a DHHC zinc finger domain identified from a balanced reciprocal t(X;15)

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X-linked forms of mental retardation (MR) is highly heterogeneous. We have characterised a 27-year-old woman with severe non-specific MR and physical features resembling Prader-Willi syndrome. Karyotype showed a balanced reciprocal translocation between chromosomes X and 15 [46,XX, t(X;15)(q13,cen)]. Physical mapping of the breakpoints using FISH, mini-FISH and Southern analysis localised the Xq breakpoint to the immediate vicinity of the first exon of a gene encoding a protein with a DHHC zinc finger domain of yet unknown function. Chromosome replication analysis using BrdU incorporation shows a late replication of the normal X chromosome in 81% of the cells analysed. We are currently investigating the expression pattern of the candidate gene in the patient and in normal controls.

P246. X-linked mental retardation, autism and epilepsy with disruption of a specific TM4SF2 isoform

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X-linked forms of mental retardation (MR) are highly heterogeneous and affect approximately 1 in 600 males. We have examined a girl with a balanced translocation t(X:10)(p11.4:q11.1) associated with severe MR, autism and epilepsy. To fine map the X-chromosome breakpoint, we performed fluorescent in situ hybridisation (FISH) analyses on metaphase chromosomes from the patient. The translocation breakpoint was localised with the BAC clone RP11-709P23. This clone is localised close to the *TM4SF2* gene which has previously been shown to be involved in X-linked MR. Further investigation showed that the translocation disrupts only one specific isoform of *TM4SF2*; the gene structure encoding other isoforms is intact.
This specific isoform has not previously been associated with MR. The findings may further clarify the role of different *TM4SF2* isoforms in severe MR, autism and epilepsy.

P247. A familial form of mental retardation, suspected for Coffin-Lowry syndrome

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Background:
Coffin-Lowry syndrome (CLS) is an X-linked disorder characterized by severe psychomotor retardation, facial and digital dysmorphisms, and progressive skeletal deformations. CLS had previously been mapped to Xp22.2. Recently, mutations in the ribosomal S6 kinase (Rsk-2) gene were shown to be associated with CLS.
Case report:
A 12-year old girl with dysmorphic facial features, congenital heart malformation (mitral regurgitation and mild aortic stenosis), skeletal deformations and moderate mental retardation was referred to the Genetic Clinic mainly because of her progressive kyphoscoliosis. Her mother was of short stature, with broad facial features, peculiar appearance of the hands and mild mental retardation. Her father died at the age of 32 years from no diagnosed congenital heart malformation. At the detailed clinical examination characteristic features of Coffin-Lowry syndrome were observed in the mother and daughter. However, the daughter was more affected than the mother. The molecular analysis in IGMBC, INSERM, CNRS, ULP, Strasbourg, is in process.
Conclusion:
In case of confirmation of the diagnosis of Coffin-Lowry syndrome in these patients, they will be demonstrative as a clinical manifestation

and variable expression of this X-linked recessive mental retardation syndrome in two females in a family. The presented clinical data emphasize again the necessity of detailed clinical examination of family members in the diagnosis of children with MCA/MR syndromes.

P248. X chromosome inactivation status in the X-autosomal translocations with breakpoint positions on X chromosome short arm.

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It has been suggested that in X-autosome translocations with breakpoints in Xp22 (critical segment), incompletely skewed X inactivation could influence the phenotype. We studied four different X-autosome translocations with breakpoints at Xp: t(X;5)(p22.2;q32), t(X;6)(p11.2;q21) t(X;7)(p22.2;p11.1) and t(X;22)(p22.1;p11.1). These were ascertained in normal women with repeated miscarriages and/or malformed progeny. The breakpoint positions were interpreted using GTG, RBG and FISH–wcp methods. X inactivation status was evaluated by analysis of replication banding patterns using the RBG technique after incorporation of BrdU. The der(X) chromosome was late replicating in 5/100 cells of t(X;5)(p22.2;q32) and 10/180 of t(X;7)(p22.2;p11.1). In both these cases break points were clustered at the critical segment Xp22.2. Fully skewed inactivation was seen in two other cases with breakpoint positions either within the critical region [t(X;22)(p22.1;p11.1)] or outside the critical region [t(X;6)(p11.2;q21)]. Therefore we suggest that neither the distribution of the breakpoints at Xp22.2 nor abnormally skewed inactivation can influence the phenotype of the women we observed.

P249. Cytogenetical and Clinical Delineation of Familial (X;3) Translocation. Case Report and Literature Review

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At least three cases of t(3p;Xp) have been reported resulting in 3p trisomy and Xp monosomy involving regions 3p21 to 3pter and Xp22.2 to Xpter. Two of the cases were maternal derivatives and the third a de novo deletion. The first case reported a 3p trisomy phenotype without an effect of Xp monosomy. The second case did not show the classical 3p trisomy phenotype but Aicardi syndrome attributed to Xp22.2 region. The third case reported a milder form of 3p trisomy phenotype and variable derivative X inactivation pattern. We report an eight years old female child with a balanced translocation 46,X,+der mat (X;3)(Xqter->pter::3p14->pter) exhibiting a trIsomic segment bigger than those previously reported, being enough to explain the 3p trisomy phenotype observed. Although the phenotypic effect of 3p trisomy has been well delineated, if Xp monosomy is involved variable severity and clinical variations are observed as suggested by this and previous reports. X inactivation patterns could be involved. In our case, X inactivation studies by BrdU incorporation revealed that the abnormal X;autosome chromosome in the mother remained active but in the child the derivative inactivation pattern was observed only in the X segment, the autosomal region remaining active. The proposita showed seizures and mild expression of 3p trisomy. Probably the non random X inactivation pattern and the minimal Xp deletion are involved.

P250. Mutations in ARX is apparently not a frequent cause of mental retardation in Denmark

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Mutation of ARX has recently been reported to be a major contributor to X-linked mental retardation. ARX belongs to the aristaless-related paired-class homeodomain proteins. Two recurrent mutations both leading to a polyalanine tract expansion have been reported (431-454dup(24) and (GCG)10+7).
We have screened 705 Danish patients for the two recurrent mutations in the ARX gene. The patients were males who had previously been referred to us for fragile X testing. We found three patients with small expansions (not large enough to be one of the recurrent mutations), and one patient with a deletion. Further studies will be reported.
In addition we investigated 10 families with confirmed X-linked mental retardation. We found the 431-454dup(24) mutation in one proband. However, further investigations showed that this was a de novo mutation, and thus not the cause of mental retardation in the rest of the affected family members.
In conclusion, in a cohort of Danish males referred on the indication of mental retardation, we did not find any of the two recurrent mutations. In comparison we had found 15 probands with full mutations of FMR1. In 10 X-linked mental retardation families, we found one patient with the 431-454dup(24) mutation.

P251. Localisation of anonsyndromic X-linked mental retardation gene (MRX80)to Xq22-q24

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Mental retardation is a common and genetically heterogenous disorder. We report here a linkage analysis in a large family including 15 members, 6 of whom presenting X-linked nonsyndromic mental retardation (XLMR). Two-point linkage analysis using 23 polymorphic markers covering the entire X chromosome demonstrated significant linkage between the causative gene and DXS8055 with a maximum LOD score of 2.98 at theta = 0.00. Haplotype analysis indicated location for the disease gene in a 23.1 cM interval between DXS1106 and DXS8067. This interval overlaps with 7 XLMR loci (MRX23, MRX27, MRX30, MRX35, MRX47, MRX53 and MRX63) and contains 2 genes associated with nonsyndromic mental retardation, namely the PAK3 gene, encoding a p21 activated kinase (MRX30 and MRX47) and the FACL4 gene encoding a fatty acyl-CoA ligase (MRX63). Therefore, the PAK3 and FACL4 genes should be considered as candidates for this novel MRX locus.

P252. Complex subtelomeric rearrangement of gonosome X associated with familial mental retardation.

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Recent studies showed subtelomeric aberrations contributing to a significant proportion of idiopathic syndromic mental retardation. We report about a boy with mental retardation, microcephaly, growth retardation, hypotonia and dysmorphic trades.
A maternal uncle died at the age of 9 from important encephalopathy associated with microcephaly. A maternal nephew is treated for epilepsy. No particularity is observed in the mother except a short stature. Metabolic screening, ophtalmologic investigations and EEG were normal. Fragile-X syndrome was excluded by DNA mutation analysis (FMR1).Karyotypes (G-band) of the boy and his parents appeared normal.
However, multisubtelomeric analysis identified a microdeletion of Xp22.3 (SHOX-, DXYS129-) associated with a translocation of the duplicated-Xq28 (DXYS61++) replacing the Xp22.3 deleted region. The same rearrangement was found in the mother.
Firstly, we investigated phenotypical expression of the Xp22.3 deletion syndrome. Among the six gene-regions mapped to Xp22.3, the X-linked non specific mental retardation (MRX) region seems

interesting. In particular, haploinsufficiency of VCX-A , PRKX and FGS3 genes is important to investigate.
Secondly, mutations of genes in Xq28 are responsible for at least 10 mental retardation syndromes and 5 non-syndromal forms. However, we focused on the XYxq syndrome that shares clinical manifestations with our case : mental retardation, microcephaly, seizures, hypotonia and short stature.
Lahn et al.(1994) suggest that some of the clinical findings in the XYxq syndrome may result from increased gene dosage.
We conclude that our familial observations constitute a unique model for studying molecularly gene haploinsufficiency of Xp22.3 region and double dose effect of Xq28.

P253. Clinical study of a new case of Metatropic dysplasia

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We present a case of Metatropic dysplasia to illustrate a rare genetic disorder and to present some particularities.
Metatropic dysplasia is a short-limbed dwarfism with changing pattern, defined by: prominent joints, narrow chest and sometimes a caudal appendage. Radiographs reveal metaphyseal flaring with flattened deformed epiphyses, short ribs and flattened vertebrae. AR inheritance seems probable, but a dominant form has been described.
Our proband is a 15 years old female, third child of a normal couple. There are no similar cases in the family. Pregnancy and birth: uneventful. Postnatal development: delayed, with delayed and abnormal tooth eruption. Measurements: short- limbed dwarfism (Ht = -11.26 SD, Wt = -5.71 SD, OFC = -4.58 SD). Physical examination: normal face, marked scoliosis, short limbs with prominent joints, hyperextensible fingers and short nails. Radiographs: marked scoliosis, short ribs, metaphyseal flaring, flattened irregular epiphyses, short femoral neck with flat femoral head. Psychologic examination: moderate mental retardation. Karyotype: normal.
Positive diagnosis of Metatropic dysplasia was based on the typical clinical and radiological features. Differential diagnosis with other bone dysplasias will be presented. The case presents with the following particularities: abnormal and delayed tooth eruption, abnormal proximal femoral extremity, short nails, moderate mental retardation and delayed diagnosis.
In conclusion, we present this case to illustrate a rare bone dysplasia, to present some particularities and to discuss the importance of early diagnosis.

P254. Long term follow-up in a patient with metatropic dysplasia

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Metatropic dysplasia (MD) is a rare chondrodysplasia characterised clinically by short limbs with limitation and enlargement of joints and usually severe kyphoscoliosis. X-rays manifestations include severe platyspondyly, severe metaphyseal enlargement and shortening of long bones. The term "metatropos" means changing patterns in greek and emphasises the evolutive changes in the body proportions of patients with MD. The classification proposed by Beck et al, based on the radiological anomalies, distinguish three different types: a lethal autosomal recessive form, an autosomal recessive non lethal form and a non lethal autosomal dominant form with less severe X-rays manifestations and better clinical outcome.
Here, we report the clinical and radiological follow-up of one of the sporadic original cases described by Maroteaux et al, from birth to 30 years of age. At birth, X-rays anomalies were consistent with the non lethal autosomal recessive severe form but later on, X-rays changes were more consistent with the less severe autosomal dominant form. A review of the literature showed the same overlap between the different forms in a girl having a severe form of MD whereas her father had a mild form of MD. We suggest therefore that the distinction in three different types, especially in isolated cases, may be questionable. Additional reports of long-term follow-up will help to better delineate the natural history of MD.

P255. Diagnostic criteria for Marfan syndrome : application to two large families.

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In 1991, mutations in the FBN1 gene, coding for the microfibril protein fibrillin 1, were discovered in patients with Marfan syndrome. Examination of FBN1 genotypes in families showed that some individuals who were not mutation carriers had been assigned a diagnosis of Marfan syndrome on the basis of minor clinical findings. An international consortium revised the diagnostic criteria in 1996 to minimise such misdiagnosis (De Paepe et al, Am J Med Genet, 62: 417-426). Since 1995 the Prince Charles Hospital in Brisbane has run a multidisciplinary clinic for Marfan syndrome and all patients have been assessed using these 1996 criteria. A diagnosis of Marfan syndrome has been extremely difficult to assign, with only 50% of over 100 index cases fulfilling the criteria, although many had involvement of a number of systems. We evaluated two large pedigrees using the 1996 criteria. In each family DNA analysis (haplotyping plus mutation analysis in family 1 and haplotyping in family 2) was used to identify mutation carriers. The clinical status of family members was assessed without knowledge of mutation status. Several mutation carriers failed to meet the criteria. Our examination of these families highlighted anomalies and difficulties in applying the diagnostic criteria. Application of the strict criteria for Marfan syndrome in the absence of DNA analysis may fail to identify some individuals who are mutation carriers and who may be at risk of developing serious complications of Marfan syndrome and of having affected children.

P256. Use of Synteny Analysis to Identify Candidate Genes for Congenital Scoliosis

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Congenital scoliosis (CS) is defined as a lateral curvature of the spine due to a developmental abnormality. Because most cases of CS are sporadic, conventional linkage studies to identify human genes are not feasible. Using synteny conservation, a systematic review of mouse mutations with skeletal, tail, or neuromuscular phenotypes with the goal of identifying potential human candidate genes for CS was performed.
A search of the Mouse Genome Database (MGD) was performed for 'genes, markers, and phenotypes' in the categories 'neurological and neuromuscular,' 'skeleton,' and 'tail and other appendages.' An OMIM search was performed to determine whether each mouse locus has a known human homologue, and if so was accorded candidate gene status. Linkage maps of the chromosomes carrying loci with possibly relevant phenotypes but without known human homologues were examined in order to identify documented synteny conservation between the mouse and human genomes in the region surrounding such loci. Mouse loci mapping to regions in which synteny is not well-conserved or mapping close to syntenic region endpoints were excluded. We then used the Genome Database (GDB) world-wide-web sites to identify human genes mapping within ~5% of the map position specified by the synteny map.
Searching MGD by phenotypic category yielded 100 mutants, of which 66 had been mapped, including phenotypes of scoliosis, kinky or bent tails, other vertebral abnormalities, or disturbances of axial skeletal development. Twenty-seven loci of interest corresponded to human syntenic regions to which plausible candidate genes involved in different stages of vertebral development have been mapped.

P257. DHPLC analysis of Marfan Syndrome: a rapid sensitive exon screen of the *FBN1* gene.

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Mutations in the gene for fibrillin-1 (*FBN1*) have been shown to cause

Marfan syndrome, an autosomal dominant disorder of the connective tissue that primarily affects the skeletal, ocular and cardiovascular system. The complex 65-exon, genomic structure of *FBN1* combined with a diverse mutational spectrum and few common mutations have previously inhibited the setup and provision of a diagnostic service for this disease.
We describe a rapid highly sensitive screen of all 65 coding exons and splice junctions using a 96-well microtitre plate format with multichannel pipette transfer. Mutations are detected using denaturing high performance liquid chromatography at 2-4 melt temperatures (derived using Transgenomic Wavemaker 4.1.4 utility software). Since establishing the service in 2002 we have to date screened 37 patients and detected a range of mutations including deletions, splice-site and
missense to give a pick up rate of 24%. This relates to the quality of referrals rather than the sensitivity of the test; when applying stricter diagnostic criteria, the pickup rate increases to 53%.

P258. Detection of germline mutations in OI patients with abnormal levels of COL1A1 RNA determined by quantitative real-time PCR.

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Real-time PCR quantification (Taqman) showed abnormal levels of COL1A1 RNA relative to COL1A2 RNA in 15 out of 40 OI patients with mild to moderately severe presentations. Single Stranded Conformational Variant (SSCP) analysis of individual COL1A1 exons from genomic DNA detected at least one SSCP shift in every patient. All PCR products with unique shifts and a single representative product for those seen in 2 or more patients were sequenced (ABI 377). To date, in addition to one previously published mutation and 10 previously published polymorphisms, six novel mutations (one in two patients) and four novel intronic polymorphisms were detected. The novel mutations include 2 small deletions and 1 small insertion resulting in frame-shifts and premature stops, an amino acid substitution, an intron/exon splice site mutation and an insertion/deletion generating a premature stop. The novel intronic polymorphisms were found in introns 2, 12, 19 and 44, consisting of single nucleotide substitutions in introns 12,19 and 44 and a 14 base tandem repeat insertion in intron 2.
The relationship between polymorphisms and causative mutations requires further study. Novel intronic polymorphisms may prove useful as markers in OI or in osteoporosis and may contribute to our understanding of phenotypic variation in OI.

P259. Mutation analysis of the *FBN1* gene among patients with Marfan Syndrome from Bashkortostan, Russia

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Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder with an incidence of 1 in 5000. MFS is clinically highly variable and characterized mainly by the involvement of cardiovascular, skeletal and ocular systems. Mutations in the gene coding for fibrillin-1 (*FBN1*) are known to cause Marfan syndrome. The aim of our investigation is to study the spectrum and frequency of mutations in *FBN1* in patients with Marfan syndrome from Bashkortostan.
We analyzed exons 24, 25, 27, 32 and 34 of the *FBN1* gene in 22 patients with MFS from 20 families of Russian and Tatar origin by SSCP analysis. .
An abnormal band was detected in exon 24 in 3 patients of Russian origin. Sequence analysis revealed an A to T transition at position 2909 in exon 24.
SSCP analysis of exon 25 showed aberrant bands in 2 patients of Tatar origin. No mutations were found in exons 27, 32 or 34 in patients from Bashkortostan.
Further studies of other exons and an increase in the number of patients is necessary for definition of ethnic-specific mutations in patients with Marfan syndrome from Bashkortostan.

P260. Identification of Novel FBN1 Mutations in Patients with Marfan Syndrome using DHPLC Analysis.

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Marfan Syndrome (MFS, MIM#154700) is an autosomal dominant inherited connective tissue disorder (prevalence:1/5000) caused by mutations in the fibrillin-1 gene (FBN1, 15q21). The disorder is characterised by highly variable phenotypic manifestations, mainly in cardiovascular, ocular and skeletal systems. The FBN1 (230 Kb, 65 exons, 2871 amino acids) has revealed more than 500 mutations. We describe 11 novel mutations that were identified in 12 probands (one with sporadic and ten with familial disease). The MFS diagnosis was evaluated following the revised diagnostic criteria of the Ghent nosology. The FBN1 gene was analysed using DHPLC technology (Transgenomic) and automated sequencing (ABI 3100).

Proband Age	Sex	FH	Exon	Mutation	CS/OS/SS
24	M	+	10	Arg439Gly	+
53	M	+	12	Arg516tt	+
7*	F	-	27	Cys1153Ser	+
35	F	+	29	Tyr1219Cys	+
27	F	+	29	Tyr1219Cys	+
8	F	+	30	Ins 5bp	+
29	F	+	41	IVS40 –2A-G	+
7	F	+	43	Arg1790tt	+
45	F	+	46	Cys1900Tyr	+
16	M	+	52	Ala2160Pro	+
25	M	+	57	Ala2383Thr	+
11	F	+	61	Cys2535Trp	+

FH: Family History; CS: Cardiovascular System; OS: Ocular System; SS: Skeletal System.
*neonatal: de novo mutation
All family members were tested for the mutations found. These mutations were absent in 50 controls.
Our results suggest that DHPLC is a reliable and cost-effective technique for the screening of such a large gene and that FBN1 screening could be a helpful tool to confirm and possibly anticipate the clinical diagnosis in familial cases.
Supported by Grants RC and RF IRCCS Policlinico San Matteo, Pavia.

P261. Analysis of the Procollagen type 1 genes in the Israeli Osteogenesis Imperfecta patients

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Over 200 mutations in one of the 2 genes encoding for type I procollagen (*COL1A1* and *COL1A2*) have been reported in Osteogenesis Imperfecta (OI). To define the spectrum of mutations in these genes in Israeli patients, we employed exon-specific PCR amplification, Denaturing Gradient Gel Electrophoresis (DGGE), and DNA sequencing. Of 63 patients, 35 were mildly affected (type I), 2 fetuses had lethal OI (type II), 12 had severe OI (type III), and 14 had OI type IV. All 51 *COL1A1* exons and 10 *COL1A2* exons were screened in all the individuals. Mutations were detected in 13 OI type I patients: 2 missense, 6 nonsense, 1 out of frame insertion, 2 out of frame deletions and 2 splicing mutations, with 10/13 being novel mutations. One missense mutation (known) and one in frame deletion (novel) were detected in 2 OI type III patients.
Cultured skin fibroblasts from patients with OI type I synthesized about half the normal amount of type I procollagen, typical of OI type I. Cells from two OI type III patients, for whom mutations are not yet characterized, synthesized overmodified chains of type I procollagen, consistent with mutations that result in subtitutions for glycine residues of the triple helix of either chain. There are no founder

mutations in Jewish OI patients and DGGE is an effective technique to screen for mutations in these genes.

P262. New collagen alpha-1 chain gene mutation in a Czech girl suffering from severe brittle bone disease

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The authors report a new 4 base pairs insertion polymorphism in the 3´- end of the gene in a female patient with atypical severe skeletal picture of osteogenesis imperfecta (OI). This insertion is different from described small insertions in "Cardiff Mutation Database". The proband is a Czech girl born after a normal pregnancy and delivery at 40 weeks gestation. Birth weight was 2.45 kg, length 49.0 cm. After delivery the fracture of left clavicle was observed. She comes from healthy mother and father of average height; nobody in family suffers from OI. The skeletal disorder was previously classified as a fresh mutation of severe type of OI type IA according to Sillence et al. (1979). The case is presented from a particular radioclinical point of view and special course of severe disease is described and documented. The small insertion (CCGT) in exon 30 of COL1A1 gene was determined in blood sample of the proband by classical molecular genetic techniques. (polymerase chain reaction /PCR/, horizontal and vertical electrophoresis and sequencing of PCR ampificates). There are still many OI entities in which the basic defect is unrecognized and on the other hand, there are hypothetically some OI cases (especially those with severe course) in which some other gene mutations should be supposed.
Acknowledgement: The research has been supported by grant of Ministry of Education EuroMISE – Cardio No. LNOOB107.

P263. Cryptic terminal deletion of chromosome 9q34: a novel cause of syndromic obesity in childhood?

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Obesity is a symptom of diagnostic value in multiple congenital anomaly-mental retardation syndromes. While acquired non-specific weight gain related to drug intake or associated behavioural disorders, occasionally occurs in the course of mental retardation, obesity is known to be a specific feature of several well-defined conditions, including Bardet-Biedl syndrome, Prader-Willi syndrome, Cohen syndrome, Fragile X syndrome and several chromosomal anomalies. Yet, a number of mentally retarded children with apparently early-onset overweight remain undiagnosed. Here, we report on a de novo deletion of chromosome 9q34 in two unrelated mentally retarded children with early-onset overweight, distinctive facial features (brachycephaly, synophris, anteverted nostrils, prognathism) sleep disturbances and behavioural problems. FISH and microsatellite DNA analyses revealed that the two children carried a similar small deletion (3 Mb) of the terminal long arm of chromosome 9 (del9q34). We suggest therefore that the del9q34 is a novel cause of syndromic obesity and mental retardation. Its association with distinctive facial features and behavioural problems should help recognizing this novel phenotype. Based on this observation, we suggest giving consideration to cryptic deletions of chromosome 9q34 in the diagnosis of unexplained obesity/mental retardation syndromes

P264. Clinical features of a child with a submicroscopic deletion of 1q43->qter detected by comparative genomic hybridization

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Using conventional cytogenetic methodology a number of 1q deletions have been delineated. The pattern of phenotypic features resulting from these deletions is relatively constant and constitute a recognisable syndrome. Smaller submicroscopic deletions of 1q43 are not as well characterised, but a recent report of two cases cases suggests that these children are less severely affected. Here we report the clinical features of a child with a submicroscopic deletion of 1q43 defined by comparative genomic hybridisation. This girl was small for gestational age and showed early failure to thrive and microcephaly. When evaluated by us at 10 months of age, she had gross psycho-motor delay, microcephaly, hypotonia and growth retardation (weight 7 kg, length 66 cm and head circumference 39 cm). She had a small ASD; brain CT was normal. Dysmorphic features included prominent metopic ridge, hypertelorism, ud-ward slanting of the palpebral fissures, anteverted nostrils, down-turned corners of the mouth, a high palate and a small maxilliary bone. Metabolic investigations were all normal as was conventional cytogenetic analysis. Comparative genomic hybridisation showed a del(1q43>qter), which was absent in both parents. The result was confirmed using a 1q subtelomeric probe. We are presently defining the deletion breakpoints by quantitative PCR.

P265. Further delineation of the 22q13 deletion syndrome. Clinical, cytogenetic and molecular data.

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A chromosomal deletion syndrome associated with a 22q13 microdeletion has been reported in approximately 60 children. We describe 5 patients from Denmark with a deletion of 22q13. One was cytogenetically visible by conventional karyotyping, one was diagnosed by high resolution karyotyping after the demonstration of low arylsulfatase A activity. Two were diagnosed by CGH analysis and one was diagnosed serendipitously as lack of the control signal in a FISH analysis for 22q11 deletion.
The phenotype of the children included: Generalized developmental delay, compromised language development, hypotonia, normal or accelerated growth and minor facial dysmorphism. Other features were partial agenesis of corpus callosum, bilateral ureteropelvic stricture, gastroesophageal reflux and hearing loss.
The extent of the deletion was studied by quantitative PCR analysis of a number of DNA markers in the 22q13 region. Results will be presented correlating clinical phenotype with molecular data.

P266. Variable clinical expression of familial interstitial microdeletions detected by comparative genomic hybridization

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In an ongoing high resolution comparative genomic hybridisation (HR-CGH) study of dysmorphic and mentally retarded individuals having an apparently normal G-band karyotype we have found three familial interstitial microdeletions. The first is a girl with a deletion in1p, dim(1p31p32)mat. The mentally retarded girl shows hypotelorism, epichantus, broad nose and lowset and backward rotated ears. The mother is not retarded. She has no epichantus but a peculiar inner eye ankle and a broad nose and backward rotated ears like her daughter. The second is a boy with a deletion in 2p, dim(2p10p12)mat. The mentally retarded boy has macrocephaly, frontal bossing and low set abnormal ears. His mother is boarder line mentally retarded, but dysmorphism has so far not been reported. The third is a boy with a deletion in 13q, dim(13q31.1q31.1)pat. The mentally retarded boy is hypotonic, has an asymmetric scull, epichantus, deep set eyes and a preauricular fistula. His father is apparently normal. All microdeletions have been confirmed by quantitative PCR. We are currently mapping the deletion breakpoints.

We will discuss the possible mechanisms that might explain the clinical differences between the children and their parents carrying the same deletions.

P267. Idiopathic mental retardation – results of clinical and FISH studies for subtelomeric rearrangements in 75 families

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Subtelomeric chromosomal abnormalities have been reported to occur in 4-7% of patients with moderate/severe idiopathic mental retardation and dysmorphic features or congenital malformations. Results of clinical, cytogenetic and FISH studies carried out in selected group of patients from 75 families with unexplained developmental delay are reported. Subtelomeric rearrangements were identified using fluorescence in situ hybridisation with Chromoprobe Multiprobe T system (Cytocell). The following inclusion criteria for subtelomeric studies were used:1. idiopathic MR with dysmorphic features, 2. family history of MR (non-obligatory), 3. normal G banded karyotype at the 400-650-band resolution level, 4. exclusion of FMR1 gene mutation. Detailed clinical analysis including family history, dysmorphic features, congenital malformations, pre- and postnatal development was performed. The overall prevalence of subtelomeric rearrangements was 7/75 (9,3%). Dysmorphic features and congenital malformations were found in all identified subtelomeric cases. All but one identified aberrations were familial and majority of them paternal in the origin. In families with subtelomeric aberration 24 cases of MR, 5 cases of miscarriages and 8 of early unexplained deaths were noted. Our results confirm other observations that subtelomeric aberrations play significant role in the etiology of mental retardation especially in cases of familial moderate / severe MR. We suggest that detailed analysis of the phenotype should precede qualification for subtelomeric testing.

P268. 1p Deletion Syndrome: Further Characterization Of A Common, Important And Often Missed Cause Of Developmental Delay/mental Retardation.

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We report on 11 patients aged, at the first observation, 1 month to 7 1/2 years, whose karyotype showed monosomy 1p36.3, associated in two of them with del 2qter, and dup 17pter respectively. Subtelomere FISH analysis was necessary for diagnosis in eight patients, whereas HRB was sufficient in the remaining three. Two of the patients were karyotyped at ages 1 and 4 months, respectively, due to multiple congenital anomalies, whereas the other nine were studied (aged 3 1/2 to 18 years) as part of a work-up for developmental delay/mental retardation (DD/MR). On examination, the OFC was at/below the 2nd percentile, and height and weight ranged between the <3rd and 50th percentile. Tower skull, prominent forehead, deep-set eyes, straight eyebrows, epicanthus, midface hypoplasia, broad nasal root/ bridge, long philtrum, high arched palate, thick alveolar ridges, small wide-spaced teeth, pointed chin, brachydactyly/ camptodactyly, and short feet were observed in all patients. Additional findings include, ear/eye/brain anomalies, heart defects, pyloric stenosis, cecum malposition. Moderate-severe DD/MR with very poor/absent speech, and hypotonia were present in all; seizures were reported in five. It seems that del 1p36 has a distinct clinical phenotype. From the recent literature it appears as if 1p36.3 deletions account for 0.5-0.7% of idiopathic DD/MR. In view of the commonness of this condition and of the difficulty and delay in making the diagnosis in most patients, we wish to alert clinicians and make them cognizant of the need to pursue subtelomere FISH analysis for 1p36 in all patients with similar clinical features.

P269. Clinical and genetic evaluation of two unrelated children with an overlapping deletion of 7p15.2-p14.3 containing 32 annotated genes including the entire *HOXA* gene cluster.

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High-resolution comparative genomic hybridisation, HR-CGH, is a powerful technique for the detection of both subtelomeric and interstitial microdeletions. The technique is estimated to have a detection limit as low as 3 Mb. At our clinical cytogenetics laboratory HR-CGH is used as a diagnostic tool for screening of dysmorphic and idiopathic mentally retarded children with normal or apparently balanced G-banded karyotypes. A surprisingly high proportion of these children (~12%) carry structural aberrations initially not detected by conventional karyotyping, although some of these aberrations could be confirmed by traditional banding techniques retrospectively. In our screening program we identified two unrelated children both having an interstitial deletion of the short arm of chromosome 7. Based on the HR-CGH data the two deletions appeared to be confined to the same locus. Both children had distinct similar phenotypic characteristics opening up the possibility of a new deletion syndrome. The two children were therefore selected for a more detailed clinical and genetic evaluation. A real time quantitative PCR approach was developed and used to determine the exact size and location of the two deletions. Interestingly, although the two deletions had different breakpoints they both encompassed the same 6.12 Mb region containing 32 annotated genes including the entire *HOXA* gene cluster. The thorough characterization of the two children might therefore elucidate central developmental aspects of the *HOXA* cluster.

P270. Rapid high resolution dosage analysis and refinement of 2q37 deletion breakpoints using multiplex amplifiable probe hybridization.

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Deletion of 2q37.3, the telomeric region of chromosome 2q, is associated with an Albright hereditary osteodystrophy-like syndrome. It is characterized by developmental delay, hypotonia, facial dysmorphism and shortening of the metacarpals and metatarsals. Additional features may include obesity, short stature, strabismus, eczema, sparse or coarse hair and autism or other behavioral disturbances. The deletions have traditionally been studied by conventional cytogenetics, FISH and microsatellite analysis, but these methods offer limited resolution. To better define the commonly deleted region, we have developed new assays for systematic assessment of gene dosage across the 2q37.3 region by multiplex amplifiable probe hybridization (MAPH) and quantitative real-time PCR (Q-PCR). The MAPH assay covers 20 known genes and 5 microsatellite loci from 2q37, together with 8 control loci, in two hybridizations. Selected genes were also analyzed by Q-PCR. Known deletion and deletion/duplication patients were used as controls for verification. Previous microsatellite results were confirmed in all cases. Furthermore, significant refinement of the proximal breakpoint was achieved in one deletion patient. We have subsequently used MAPH to exclude major deletion in another patient with an AHO-like phenotype in whom multiple microsatellites were uninformative. MAPH therefore offers a rapid means of high-resolution dosage analysis across the 2q37.3 region and represents a significant improvement over current diagnostic methods. Analysis of a wider panel of patients is ongoing to further narrow the commonly deleted region and to search for microdeletions that might pinpoint the candidate gene(s) responsible for this phenotype.

P271. Monosomy 1p36: Report of first two patients from Saudi Arabia and review of the literature

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Monosomy 1p36 is a common relatively newly delineated contiguous gene deletion syndrome. The syndrome is characterized by hypotonia, severe developmental delay, growth abnormalities, and craniofacial dysmorphism. We describe two patients with monosomy 1p36 who were referred to our institute and compare their features with cases of pure monosomy 1p36 previousy described in the literature. The first patient was referred to our institute at the age of 11 months with global developmental delay primarily to investigate for neurometabolic eitiology. The second patient was referred at 4 months of age for patent ductus arteriosus (PDA) ligation. The clinical features of both patients included postnatal growth delay, motor delay, hypotonia, mental retardation, microcephaly, large anterior fontanelle, low set ears, flat nasal bridge, long philtrum, seizures, and congenital heart defect and the chromosomal breakpoint was at 1p36.2. The above features were fairly consistent with the discription in the literature, however both patients were seen by multiple specialites and many unneccessary investigations were done before reaching the final diagnosis. Furthermore, chromosomal analysis done for the second patient at an oustside institute was reported to be normal. In conclusion we hope that our observation of this specific 1p36 phenotype in Saudi Arabia should assist in clinical diagnosis of this chromosomal abberation since the condition is likely to be underrecognized and commonly missed on routine chromosomal analysis especially in less experienced laboratories.

P272. Subtelomeric Chromosome Rearrangements In Mentally Retarded And/or Dysmorphic Patients From North-eastern Slovenia.

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A significant diagnostic challenge in medical genetics is to identify new causes of mental retardation (MR). MR is present in about 3% of the general population and is explained in only about half of all cases. Previous work has shown that subtelomeric chromosome rearrangements can cause idiopathic MR in 7.4% of patients with severe or moderate MR and about 0.5% of mild MR. The screening method generally used is multiprobe telomere fluorescent *in situ* hybridisation.
We report results of our study done on 61 MR and/or dysmorphic patients (children and young adults, 0-21 years old) from north-eastern Slovenia. All patients had a normal G-banded karyotype at resolution 400-550 bands. FISH using the Chromoprobe Multiprobe T-System (Cytocell) was performed. For confirmation of the results and determination of breakpoints, FISH was performed using locus specific probes (YACs, BACs, PACs). In our screen four patients with subtelomeric rearrangements were detected (6.5%). One of them was apparently normal variant : 2qtel deletion (1.6%). Three cases had unbalanced cryptic rearrangements with phenotypic consequences (4.9%). One patient has two terminal deletions; del 2qtel and del Xptel. Del 2qtel is a polymorphism, the del Xptel was significantly larger than the normal variant. The second case was a *de novo* unbalanced translocation, and the third case was a meiotic rearrangement after a maternally inherited pericentric inversion. Our results confirm previous findings, indicating the important role of subtelomeric rearrangements in the aetiology of MR and the usefulness of subtelomeric FISH as a screening method.

P273. Subtelomeric chromosome rearrangements in mentally retarded and/or dimorphic patients from north-eastern Slovenia

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A significant diagnostic challenge in medical genetics is to identify the new causes of mental retardation (MR). MR is present in about 3% of individuals in general population and is explained only in about the half of all cases. It has been shown that subtelomeric chromosome rearrangements may be a common cause of idiopathic MR in 7,4% of patients with severe or moderate MR and about 0,5% of mild MR. Screening method generally used for detection of subtelomeric rearrangements is multiprobe telomere fluorescent in situ hybridisation (FISH).
We report results of our study done on 61 MR and/or dysmorphic patients (children and young adults, 0-21 years old) from north-eastern Slovenia. All patients had a normal G-banded karyotype at resolution 400-550 bands. FISH technique using Chromoprobe Multiprobe T-System (Cytocell) was performed. For confirmation the results and determination the breakpoint regions, FISH using locus specific probes (YACs, BACs, PACs) was performed. In our screening four patients with subtelomeric rearrangements were detected (6,6%). One of them was apparently normal variant: 2qtel deletion (1,6%). Three cases had unbalanced cryptic rearrangements with phenotypic consequences (4,9%). One patient has two terminal deletions; del 2qtel and del Xptel. Del 2qtel was polymorphism, del Xptel was significantly larger than normal variant. The second case was de novo unbalanced translocation, and the third case was a meiotic rearrangement after maternally inherited pericentric inversion. Our results confirm previous findings, indicating the important role of subtelomeric rearrangements in aetiology of MR and usefulness of subtelomeric FISH as screening method.

P274. Detection of cryptic chromosome aberrations by high-resolution CGH.

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During the last decades, the possibility to detect small chromosome aberrations has increased significantly by the use of banding techniques and FISH. However, the resolution of genome wide screening methods such as chromosome analysis and CGH remains comparatively low.
In order to increase resolution, we developed a modified CGH protocol using fresh high-quality metaphases slides and chemical labeling and tested the method on 9 previously identified cases with cryptic chromosome aberrations. Seven of the cases consisted of subtelomeric rearrangements; 5 unbalanced translocations, one derivative chromosome and one terminal deletion, and two cases had interstitial deletions. Thirteen of the fifteen aberrations could be detected by this modified CGH, including a terminal deletion, size-mapped using BAC and PAC clones to 2.1 Mb. Two subtelomeric aberrations size-mapped to 1 and 1.3 Mb respectively could not be detected.
The subtelomeric regions have been the most difficult to evaluate using CGH. This modification of CGH thus improved the resolution, also in the subtelomeric regions and can be used as a screening method for genome-wide chromosome imbalances in individuals with idiopathic mental retardation

P275. A prospective study of mentally retarded and dysmorphic individuals using HR-CGH and subtelomeric FISH for revealing cryptic chromosome aberrations

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We have previously shown that by HR-CGH analysis cryptic chromosome aberrations were found in 11% of mentally retarded and dysmorphic individuals with normal conventional karyotype. Studies using subtelomeric assays have reported 0-13% cryptic abnormalities in such patients. A direct comparison of results from different studies remain problematic, as detection rates are expected to be highly dependent on the strategy used for selection of patients. Subtelomeric screening offers high sensitivity but is confined to chromosome ends, while HR-CGH enables whole-genome screening albeit at lower sensitivity. In order to suggest a scheme for the most beneficial use of these techniques for investigation of mentally retarded and dysmorphic individuals, we performed a prospective study, which included 100 patients investigated by both subtelomeric FISH and HR-CGH.
We present the results of this study, which showed that HR-CGH was superior to subtelomeric FISH in detecting submicroscopic chromosome aberrations. More aberrations were detected by

HR-CGH and the majority of the aberrations were interstitial. We concluded that, except for familiar cases, HR-CGH should be offered as a first choice for investigation of mentally retarded and dysmorphic patients with normal karyotypes and for whom known syndromes are excluded.
We will also review another approximately 300 HR-CGH analyses performed in our lab on this indication. Cryptic chromosome aberrations were found in 12% of all investigated patients. In addition the types of aberrations will be discussed, as well as the strategy used for confirmation and further characterization of the findings.

P276. Subtelomere FISH analysis in over 2000 specimens tested at Genzyme Genetics

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Small rearrangements at the ends of chromosomes can often be overlooked in standard cytogenetic analysis. These rearrangements have been shown to be responsible for a small but significant percentage of otherwise unexplained mental retardation.
Fluorescence in situ hybridization (FISH) analysis using subtelomere probes detects the presence and location of gene sequences near the ends of the chromosomes. This has proven to be a useful technique for detecting rearrangements that are not visible on traditional cytogenetic analysis. Genzyme Genetics has performed over 2000 subtelomere FISH analyses using the ToTelVysion multi-color FISH probe panel from Vysis, Inc. (Downers Grove, IL). In our laboratory, approximately 4.4% of specimens have an abnormal subtelomere FISH result. The clinical implications of these findings are not always clear; approximately 1.1% of probands have subtelomere rearrangements that have been inherited from a parent reported to be phenotypically normal. These rearrangements are therefore possibly normal variants with no clinical significance. The remaining 3.3% of specimens have an abnormal subtelomere FISH result that is assumed to be clinically significant. We will present a summary of the abnormal results and a discussion of the inheritance patterns in cases where follow-up studies were performed.

P277. FISH analysis of cytogenetic detectable deletions of 21q22-qter in two patients with unusual mild clinical symptoms

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The phenotype of „21q- syndrome“ is highly variable and critical regions involved in the major and minor aspects of this syndrome were deduced from partial monosomies of 21q resulting from translocations, ring chromosomes or pure partial monosomies.
Here we report on clinical, cytogenetic and molecular cytogenetic analyses of pure partial monosomy 21 in two unrelated patients, both with apparently overlapping cytogenetically detectable deletions of 21q22.2-qter and with unusually mild clinical phenotypes. FISH mapping revealed that the 21q material missing in the chromosome 21 of both patients greatly overlaps, but the deletion breakpoints do not coincide. FISH analysis with region-specific large insert clones and small single copy probes demonstrated that the proximal deletion breakpoint of one patient is located between exon 5 and exon 8 of *ETS2* and the deletion extends to a region less than 300 kb from the telomere. Thus the deleted region encompasses about 8 Mb. To our knowledge, this is the first pure partial monosomy 21q22.2-qter characterised with a deletion breakpoint within *ETS2*. Particularly FISH analysis for the second unrelated patient with a cytogenetically overlapping deletion demonstrated that the proximal deletion breakpoint is located in a region 50 kb distal to *ETS2*. Our finding elucidate that the region distal to *ETS2* deleted in our patients may not lead to the classical anomalies of 21q- syndrome and may present a mild subtype, even when larger deletions (Mb) are present.

P278. Monosomy 18p due to whole arm translocation

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Monosomy 18p is one of the most frequent autosomal deletion syndrome, with variable clinical presentation. Most cases are due to terminal deletion, and only few cases from de novo whole arm translocation. Unbalanced whole arm translocations of chromosome 18 usually involve chromosomes 21 and 22. The mechanism of whole arm translocation is unknown yet.
We present the results of clinical and cytogenetic study of a case with partial monosomy 18p due to unbalanced translocation. Our patient is 8 years old girl with mild mental retardation and facial dysmorphism. Cytogenetic analysis was performed on slides obtained by peripheral blood culture. We used high resolutin GTG-banding method and FISH analysis with chromosome 15 and 18 painting probe, ToTelVysion Multi-color DNA Probe Mixtures for subtelomere detection and Prader Willi/Angelman probe for precise chromosome 15 characterization.Analysis revealed aberrant karyotype with 45 chromosomes, missing chromosomes 15 and 18, and the presence of the rearranged chromosome. Banding pattern and FISH analysis excluded complex chromosomal rearrangement and showed that the aberrant chromosome was composed of the long arms of chromosomes 15 and 18, with the presence of the centromeric region of both chromosomes involved and the absence of 18p subtelomeric sequences.
This study presents evidence that unbalanced whole arm translocation between chromosomes 15 and 18 retain centromeric regions of both chromosomes involved in the rearrangement.
This work was supported by a grant from the Ministry of Science and Technology of the Republic of Croatia (project No. TP-01/072-01).

P279. Case Report: A girl with unstable ring chromosome 18

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A 10 year old girl admitted to our department for chromosomal analysis because of multiple malformations, mental and developmental delay. She is the first child of non-consanguineous, healthy parents. At birth the mother was 19 and the father was 23 years old. The girl was born after normal term pregnancy by spontaneous vaginal delivery. At birth she was noted to have ptosis of the right eye. On physical examination at the age of 10 years; her weight was 24 kg (<3rd centile), height was 119 cm (<3rd centile). She was noted to have developmental delay, microcephaly, brachycephaly, temporal narrowing, asymmetric and flat face, hypertelorism, mongoloid slant, ptosis of the right eye, short nose, small alae nasi, carp-shaped mouth, long philtrum, high arched palate, geographic tongue, low-set ears, asymmetric chest, low hair-line on the neck, bilateral clinodactyly and pes planus. Her GTG banded metaphases prepared from peripheral blood culture revealed a ring chromosome. The ring was too small to discriminate its nature but one homolog of chromosome 18 was missing in all metaphase spreads with the ring chromosome. The ring was not stable in all metaphases. Some had a large ring, some had isochromosomes and some had lost the abnormal 18 at all. We tried to deduce the break points by clinical findings because of the difficulty in determining by classical cytogenetic methods.

P280. Do all 8p rearrangements result from the same mechanism ?

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Structural rearrangements of the short arm of chromosome 8 such as the invdup(8p), the 8p23 interstitial deletion and the 8p23pter analphoid marker are frequently observed in the population having chromosomal structural abnormality. To explain their recurrence in the population, Florida et al. (1996) postulated that an unequal crossing over involving repeat sequence was involved. More recently, Giglio et al. identified these inverted repeat sequences as belonging to the olfactory receptor (OR) genes family. They demonstrated that the

OR genes clusters were the substrate for a non allelic homologous recombination leading to recurrent rearrangement of the short arm of chromosome 8. To further explore this mechanism of origin and using FISH we characterised at the molecular level 5 cases of invdup(8p) and 2 cases of 8p23 deletion. In six out seven cases investigated, the mechanism of origin of the rearrangement was in agreement with Giglio's hypothesis. In one case however, the duplicated and deleted segments were different. The implication of this observation to define a common strategy to detect structural rearrangement of the short arm of chromosome 8 is discussed.

P281. Anal atresia in a patient with the 18q21 deletion

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The 18q deletion syndrome is one of the commonest deletion syndromes with a wide clinical spectrum (mental retardation, growth deficiency, microcephaly, minor facial anomalies, hearing impairment and limb anomalies). Most patients with this syndrome have deletions from 18q21 to qter.
We present a patient with the deletion of 18q21, with typical clinical features and a new phenotypic finding: anal atresia.
Case report: The girl was born as the first child to a 20-year old mother and a 25-year old father. The pregnancy had been uncomplicated. The girl was born at 39 weeks of gestation by emergency Cesarean section due to bradycardia. Apgar scores were 2-7-7. She had a low birth weight (2020 g), length: 46 cm, head circumference: 30.5 cm. Physical examination revealed hypotony, microcephaly, facial anomalies, tapering fingers, proximal thumbs and feet in adductus varus position. Echocardiography showed a complex heart defect. Cerebral ultrasound revealed agenesis of corpus callosum and dilated lateral cerebral ventricle. Abdominal ultrasound was normal.
On day 8 abdominal distension and rectal bleeding were observed. Abdominal radiological examination revealed anal atresia with fistula and colon perforation. The girl was operated. Ileocecal resection was done.
Classic cytogenetic and FISH analyses showed a terminal deletion of the 18q (46,XX,del(18)(q21.3)).
The patient shows many clinical features described in 18q deletion syndrome, yet anal atresia has not been previously reported. It might be a coincidental co-occurrence, but due to possible severe complications, it is recommended to add it to the list of abnormalities observed in patients with 18q deletion syndrome.

P282. The subtelomeric 18q deletion syndrome including SALL3 is the cause of Rasmussen Syndrome

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Since the development of effective assay for screening all subtelomeric regions in mentally retarded patients, we have developed an alternative strategy. Our hypothesis is that each pure subtelomeric deletion is associated with one specific clinical phenotype. Previous data demonstrated that deletion of the distal bands of some chromosomes has been recognised as the cause of characteristic clinical syndrome. Examples included Wolf Hirshhorn syndrome and 4p16.3 deletion, Miller dieker syndrome and 17p13.3 deletion. High resolution analysis have also improved the identification of subtelomeric chromosomal syndromes and allowed to clearer definition of clinical phenotype (1p36.3 deletion, 2q37.3 deletion). We have undertaken an extensive review of all distal chromosomal deletion reported in the literature and in our personal experience to delineate the associated phenotype spectrum. This study permit to create a "catalogue" of specific phenotype associated with deletion of virtually all subtelomeric regions.
This approach led us to identify on clinical orientation the first cryptic deletion of the 18q23.3-qter sub-band only detectable using a specific 18q subtelomeric probe by FISH. The male patient exhibited three main manifestations of the 18q- syndrome including the characteristic facial dysmorphism, hearing loss with external auditory canal atresia and vertical talus. Interestingly, this clinical association was also described by Rasmussen in 1979 as a distinct syndromic entity with a probable autosomal dominant inheritance.
The localisation of SALL3 in this region, its expression in limb buds, otic vesicle during human embryonnic development suggested that the haploinsufficiency for this gene might be responsible of both 18qter deletion and Rasmussen syndrome.

P283. Turner Syndrome variant 46 X,del(X) (q23)in three generations

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Turner syndrome (TS) is the most common sex-chromosome abnormality in females. The great majority of women with 45,X present with short stature, do neither develop any sign of secondary sexual characteristics nor go spontaneously through puberty and remain infertile. TS-variants resulting only from an effective partial X-monosomy are described occasionally associated with fertility.
Here, we describe a family history over three generations with transmission of a sexual chromosome aneuploidy 46,X del (X)(q23) where index patient, mother and grandmother presented with short stature. Mother's and grandmother's adult height without growth promoting treatment (GHT) is 152cm (-2.3SDS) and 148cm (-2.54SDS), respectively. Both women entered puberty at an early age (menarche at the age of 11 and 11 1/2 years) and later presented with menopause at the age of 28 and 32 years. Beforehand the grandmother gave birth to one girl at the age of 24 years. The patients' mother experienced 4 pregnancies. Two of them were abortions and two female offspring. One presented the 46,X del(X)(q23). At the age of 9 3/12 the height was 123.4cm (-1,55SDS), the height velocity was slightly decreasing (4.8cm/yr; -1SDS) and, therefore, GHT was started. During the first year of treatment the height velocity increased up to 9.8cm/yr (+5.28SDS). In the follow-up, the girl started early pubertal development with 11 years (P4).
We suspect, therefore, that TS variants with partial deletion of Xq23 leads to an early menarche and premature ovarian failure and that this part of the X-chromosome might be most important for gonadal maturation.

P284. Familial transmission of a derivative marker chromosome 19 trough at least three generations with variable phenotype.

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Here we report on two sisters with a high percentage of a constitutional mosaicism for a partial trisomy 19, which was also found in additional affected relatives. Due to psychomotoric retardation, delay of speech development and dysmorphic facial features the two sisters were subjected for cytogenetic analysis at an age of 6 and 4 years respectively. The elder girl developed seizures at an age of 5 years, whereas her younger sister does not show any seizures yet.
Anamnesis revealed that their father suffered from epilepsy during his early infancy following a traumatic attack leading to open head injury. Their mother shows a considerable reduction of her mental capacity without obvious dysmorphy. Cytogenetic analysis of lymphocytes of both sisters revealed a mosaicism for a small marker chromosome in about 50% of more than 50 mitoses analysed.
An identical marker chromosome was identified in the mother and maternal grandmother, the very aggressive father was not available for further analysis unfortunately. FISH-analysis demonstrated that the marker chromosome is derived from chromosome 19 in all four family members. Further investigations using region specific BAC clones allowed to characterise the derivative chromosome 19 in more details, in particular nearly complete absence of the short arm and distal long arm, leading to the following karyotype: mos 47,XX,+mar.ish der(19)(wcp19+,D19Z1+,2017_d_11-,452_L_11+)[55]/46,XX[45]mat. Percentage of partial trisomic cells was similar in all affected family members in lymphocytes and fibroblasts

as well.
Mosaicism of partial trisomy 19 is rare especially with stable transmission through several generations therefore further investigations will be performed.

P285. Cytogenetic, molecular, and clinical study in two males with short stature, hypogenitalism and rearrangements of chromosome Y

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We present a long follow-up study of two unrelated males, originally referred for severe statural developmental deficiency, and hypogenitalism. The first patient shows, since the birth a generalized hyperpigmentation, not obvious in his parents and other consanguineous.
In the first child, preliminary cytogenetic analysis, performed by using several banding and DAPI techniques, revealed a 46,XY/46,X,dic(Y)(qter—p11::p11—qter) mosaicism. Molecular studies, by using PCR screening of genomic DNA to detect SRY and the sequence-tagged sites (STS) with several, different, sequences of Y chromosome, didn't reveal any deletion.
In the second child, the karyotype showed an inv dup (Y). The breakpoints were mapped using FISH and PCR for a deletion analysis of chromosome Y: several loci resulted deleted also in the AZFb and AZFc regions.
Further molecular studies were later performed in both subjects by PCR, and sequencing analysis of SRY gene (HMG and non-HMG box regions), and SHOX gene.
From the clinical point of view, the two subjects, now 21 and 20 year-old, show normal intelligence. Their final stature is respectively 145 and 146 cm.

P286. Interstitial de novo deletion of the long arm of chromosome 5q?15 to 5q?22: molecular characterisation of the deleted region

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Interstitial deletions involving the central segment of the long arm of chromosome 5 are rare, and fewer than 30 cases have been reported. Because of the repetitive banding pattern of this region, the extent and localisation of the deleted segment has not been well characterised in most reported cases. This has complicated attempts at establishing a definite karyotype-phenotype correlation. The APC gene is located within the 5q21-q22 region, and patients with deletions encompassing this gene tend to develop FAP. We report a further patient with a de novo interstitial deletion of the region 5q?15 to 5q?22 identified by standard G banded karyotype. He presented with learning disability, dysmorphic features (down-slanted narrow palpebral fissures, micrognathia), sensorineural hearing loss and associated structural anomalies (amongst them cleft palate, iris colobomata and horseshoe kidney, which have previously been reported in 5q deletion cases). In addition, this child had an Arnold-Chiari type I malformation that required surgical decompression. FISH studies using BACs spanning the 5q15 to 5q22 region revealed that these were all present in both homologues, confirming that the deletion must be actually distal to BAC RP11-124B6, located at 5q22.1. This finding is consistent with the more severe phenotype observed in distal (5q22 to 5q31) deletions. Further analyses are currently under way to define the deleted segment, and the results will be presented.

P287. Computer assisted diagnosis of chromosomal aberrations using the Human Cytogenetics Database

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The possibility to diagnose chromosomal aberrations using a computerized database was tested with the clinical desriptions of 101 patients with an established chromosomal aberration using the „The Human Cytogenetics Database" (HCDB) which is a commercially available computerized catalogue of postnatally ascertained, cytologically detectable human chromosomal aberrations. For this database positive associations between chromosomal aberrations (deletions and duplications) and congenital malformations have been shown by its author.
For every case, two kinds of searches within the database were performed:
First all the features described were used for the diagnostic process; second only those symptomes, which were present in the description of the respective aberration within the database. Three levels of precision were applied assessing the diagnoses: suggestion of the correct (1) chromosome number, (2) chromosome arm, (3) aberration type and rough location.
We yielded less than a third true positive results on the lowest level of precision. These results were further processed taking into account the number of (inevitably wrong) differential diagnoses, of similar cases within the database, numbers of symptomes used for the description of the respective clinical case and all the other cases within the database.
Our results show, that the suggested diagnoses are unprofitable in a clinical context. In an epistemiological connection they indicate that no real standard phenotypes exist for the respective chromosomal aberrations, clinical findings are nonspecific and a dramatic overlap of anomalies exists among the different genotypes thus raising questions about genotype-phenotype correlations and putting an emphasis on the disruption of homeostasis.

P288. Monocentric, inverted duplication of 8p due to a maternal paracentric inversion: phenotype and proposal of a novel mechanism

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Classically, individuals heterozygous for paracentric inversions have little risk of producing chromosomally unbalanced offspring since meiotic recombination usually produces acentric and dicentric chromosomes. We report a 2 year old boy with a monocentric chromosome 8 with an inverted, duplication in the short arm [46,XY,inv dup (8)(p23.1p12) ish dup (8)(wcp+, D8S504+, D8S574+)] whose mother is heterozygous for a paracentric inversion [46,XX,inv(8)(p12p23)]. Features of the child concordant with other reports of duplication of 8p include normal birth parameters, normal visceral anatomy, and severe encephalopathy characterized by developmental delay, brain malformations, hypotonia, and strabismus. He has similar dysmorphic features including frontal bossing, hypertelorism, a thin upper lip with prominent lower lip, high arched palate, widely spaced teeth, and low set ears. This case is unique since it is only the second reported of an inverted, duplicated 8p arising from a paracentric inversion. Additionally, it lacks the concomitant terminal deletion that is typical of inverted, duplicated 8p. We propose a molecular mechanism involving misalignment of inverted repeat segments followed by anomalous recombination in maternal meiosis leading to this pure trisomy 8p.

P289. De novo complex chromosomal rearrangement with six breakpoints in a rhizomelic mentally retarded child: molecular cytogenetic study

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By definition complex chromosomal rearrangements (CCRs) involve three or more chromosomes or/and breakpoints. They are often found in tumors but very rarely as constitutional anomalies.

Apparently balanced constitutional CCRs in dysmorphic/mentally retarded patients are a powerful resource for gene mapping and identification.
We describe cytogenetic, molecular cytogenetic and clinical data of a constitutional de novo derived apparently balanced CCR which involves multiple translocations of five chromosomes, 1, 2, 11, 13 and 18 with six breakpoints altogether. It was identified in a newborn male followed up until the age of two years who presented with mental retardation, short statute due to limbs rhizomelia, dysmorphic features, congenital heart disease and cryptorhid testes. Routine cytogenetics, FISH analysis with whole chromosome paints probes of chromosomes 1, 2, 11, 13 and 18, multicolor FISH and multicolor banding studies specified the following derivative chromosomes:
der(1) t(1;13)(pter>q31::q?31>qter),
der(2)t(2;11)(qter>p16::q24>qter),
der(11)t(11;18)(pter>q13::q12.1>qter),
der(13)t(2;13)(pter>p16::q?31>pter),
der(18)t(1;11;18)(qter>q31::q13>q24::q12.1>pter). No cytogenetically apparent deletion or duplication that could account for the clinical findings in the patient was established. We suggest that causative gene(s) for this condition and especially for rhizomelic shortening is most likely located at one or more of the above given breakpoints. Further refinement and characterization of these breakpoints is expected to identify such gene(s).

P290. De novo der(13)t(10;13)(q23.3;q34) with features of distal trisomy 10q : a case report

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Partial trisomy of the long arm of chromosome 10 is a well-defined but rare syndrome. Clinical features of this chromosomopathy include a distinctive dysmorphic appearence, developmental delay, growth retardation, and in some cases, abnormalities of extremities, renal, cardiac and ocular anomalies.
Here we report on the boy with intrauterine growth retardation, psycho-motor retardation, dysmorphic facial features, toe anomalies, right choanal hypoplasia, coloboma, micropenis and other genital anomalies, bilateral renal hypoplasia and initial chronic renal failure. The chromosome analysis of the proband showed a male karyotype with the derivative chromosome 13 : 46,XY,der(13). Both parents have normal karyotypes.
Fluorescent in situ hybridisation (FISH) using whole chromosome painting probes (Chromoprobe Multiprobe OctoChrome System, Cytocell) and subtelomeric DNA probes (Chromoprobe-T, Cytocell) identified the additional material on the long arm of derivative chromosome 13 as to be derived from the distal part of the long arm of chromosome 10. To determine the breaking regions on chromosome 10 and 13 locus specific BAC‘s from 13q33.3-13q34 region and 10q23.32-10q24.1 region were used (M. Rocchi, University of Bari, Italy). FISH results demonstrate that the patient was trisomic for 10(q23.3-qter) and monosomic for subtelomeric region of chromosome 13. The resulting karyotype after FISH analysis was : 46,XY,der(13)t(10;13)(q23.3;q34)de novo.

P291. A new case of partial trisomy 16q

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Trisomy 16q is a rare condition in livborne infants. Some 28 cases have been reported and most cases are due to unbalanced translocation. Clinical presentation is not well defined because it is usually associated with loss or gain of an other chromosome involved in the rearrangement, while duplications are quite rare.
In this report we present the results of clinical and cytogenetic investigation in 10 - years old girl. Clinical examination showed dysmorphic traits including hypoplastic supraorbital ridges, epicantus, dysplastic, low set ears, full cheeks, thin lips, micrognatia. Her somatic and intelectual development were within normal range, although she experianced learning difficulties, mostly due to behavioural problems, aggressiveness and disobedience. Cytogenetic analysis was performed on peripheral blood culture of the patient and her parents. Both parents presented with normal karyotype, while cytogenetic analysis in the proband identified an extra GTG-positive band in the long arm of chromosome 16. FISH with chromosome 16 painting probe stained the aberrant chromosome completely. Analysis thus suggested the duplication of the long arm of chromosome 16 most probably involving 16q13-->q22 region.
It has been proposed that the duplication of the distal long arm segment may cause typical features of trisomy 16q, including short survival. On the other hand, the comparison of our patient to other reported cases revealed that trisomy for the proximal segment of the long arm of chromosome 16 may be associated with mild clinical presentation and behavioural problems as a major manifestation.
This work was supported by a grant TP-01/072-01.

P292. Identification and molecular characterization of a de novo balanced translocation associated with polymicrogyria.

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Polymicrogyria is a malformation of the human cerebral cortex which is characterized by an abnormal organization of neurons within cortical cell layers leading to an excessive number of small gyri. This anomaly belongs to the group of cortical dysplasias which are responsible for a significant number of developmental disabilies often accompanied by epilepsy.
We present clinical, cytogenetic and molecular data for a 33-weeks-old female fetus. This fetus is the product of a pregnancy terminated following identification of severe brain malformations and the presence of a chromosomal rearrangement. The fetus presented with a polymicrogyric cortex (predominantly in the posterior areas), heterotopic nodules throughout the white matter and hydrocephaly. Bilateral ventricular dilatation and syndactyly were also present. The brain malformations, strongly suggestive of a neuronal migration defect, were found to be associated with a de novo balanced translocation t(2;7) (q36;p22).
We hypothesize that one of the translocation breakpoints disrupts a gene causing the observed neuronal migration defect. In order to clone the translocation breakpoints, we performed fluorescent in situ hybridation (FISH) experiments using bacterial artificial chromosomes (BAC) clones as a probe. Once cloned, the breakpoint region will be analyzed to determine if a gene is interrupted by one of the translocation breakpoints. We believe that the identification of such a gene will improve our understanding of the mechanisms responsible for the organization of the normal human cerebral cortex.

P293. Further delineation of the 4q- syndrome about a new case and review of literature.

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The proband was a male infant born at 37 weeks gestation. Prenatal US examinations were considered normal, except a doubt about a hypoplastic corpus callosum. Prenatal growth was normal. Three hours after birth he had laryngeal dyspnea which required intubation and ventilation. The following distinctive features were noted : wide fontanelle and dissociated sutures, severe retrognathia, low set ears with a triangular aspect of the upper part, downward slanting palpebral fissures, camptodactyly of the fifth fingers, high implanted thumbs, abnormal deep plantar crease between first and second toes, over-riding third toes, hypoplastic scrotum, cryptorchidism, neonatal hypotonia, hypertrophic left cardiac auricle and left to right shunt, hypoplastic corpus callosum, bilateral sensorineural deafness. Evolution was marked by several episodes of supraventricular tachycardy. He developed a pyramidal syndrome and died at 6 weeks of age of general failure. Chromosome analysis showed 46,XY, del(4)(q31-q34). Karyotypes of parents were normal.
Out of 37 reported cases of del(4q), 22 had breakpoint at q31: 30% died before 2 years of age; 83% of survivors had a severe developmental delay; predominant abnormal features were heart defect (74%), cleft palate (74%), retrognathia (70%), finger malformations (70%), post-natal growth failure (68%), abnormal ears (61%). The phenotype was similar in cases with terminal deletions (20 cases) or interstitial deletions as in our case (2 cases). 15

cases were reported with a breakpoint at q32 (3 cases) or q33 (12 cases): the phenotype was less severe with normal growth, mild developmental delay and slight dysmorphic signs.

P294. Deletion of chromosome region 13q21.2-13q22 in a female with Hirschsprung-like complaints

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Chromosome analysis was requested in a 37-year-old male and his 36-year-old wife because of one spontaneous abortion and one intra-uterine death at 17 weeks of pregnancy. Analysis of GTG-banded metaphase chromosomes showed that the male patient had a normal 46,XY karyotype. Analysis of GTG-banded metaphases of his wife showed an aberrant chromosome 13 in all cells examined, with deletion of a Giemsa dark band in the q-arm. Subsequent chromosome painting showed that no other chromosome was involved in this chromosome aberration. As it was not clear after the analysis of the GTG-banded chromosomes which chromosome band was actually deleted, chromosome microdissection was performed. After the dissection of the aberrant chromosome 13, degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) and reverse painting on a normal control the deleted region was clearly visible and we concluded that the patient had a 46,XX,del(13)(q21.2q22) karyotype. The length and weight of the patient were within normal limits. Her mental development was normal, although she had learning problems at regular school. She showed minor dysmorphic features: her nose tip was broad, her upper lip was small and she had simple ear lobes. Furthermore, she appeared to have two open vertebrae causing back pain for already 23 years, and periods of constipation alternated with diarrhoea. In the literature, the EDNRB gene (located at chromosome band 13q22) is reported in connection with the genetics of Hirschsprung disease. Chromosome analysis of the parents of the patient was not possible.

P295. Terminal deletion of the long arm of chromosome 10 in a girl with dysmorphism and mental retardation, revealed by subtelomeric FISH analyses.

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Partial pure monosomy of distal 10q is an uncommon chromosomal disorder. Clinical findings in these patients include mental retardation with hypotonia, growth retardation, triangular face, broad nasal bridge with a beaked or prominent nose, hypertelorism, strabismus, clinodactyly of little fingers, genital/urinary tract anomalies, and behavioural problems.

We report on a 11-year old girl with moderate mental retardation, behavioural problems with as a mean feature hyperactivity, short stature, severe myopia, bilateral hypoplasia of the optic nerve, high palate, prominent nasal bridge, retrognathia and low posterior hairline. There was no family history of mental retardation. We first saw her at the age of 3 years for delayed development, convergent strabismus, growth retardation and facial dysmorphism. Complementary investigations were all normal, including brain magnetic resonance, abdominal and renal ultrasounds, auditory and visual evoked potentials, metabolic screen, skeletal X-rays and bone age. Standard karyotype was normal 46,XX. FISH analyses excluded CATCH 22 and Smith-Magenis syndrome (17p). Fragile X syndrome was excluded by DNA analysis. No specific diagnosis could be given. Recently subtelomeric FISH analyses were performed and revealed the presence of a subtelomeric deletion of the long arm of chromosome 10. This deletion occurred de novo, since parental karyotyping and FISH studies were normal. Subsequent FISH analyses with specific probes enabled us to define the breakpoint in 10q26.2.

So far, only a few other cases with a similar small terminal deletion were described. The clinical findings in these patients are reviewed and compared with the present patient.

P296. Familial reciprocal translocation between chromosome 3p and 8q: A severe mentally and motor retarded 5 years old female patient with der(3) t(3;8)(p25q24)(wcp8+sp) mat due to maternal reciprocal 3;8 translocation and a prenatally diagnosed balanced translocation 46XYt(3;8)(p25q24)

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A severe mentally and motor retarded 17 months old female patient was admitted to our genetic department. She had never talked and walked. Family history revealed two spontaneous abortions and there was no consanguinity. In physical examination she was microcephalic and spastic, had bilateral clinodactyly, high arched palate, thick philtrum, micrognathia and a small hemangioma on the upper lip. The mother was found to have a reciprocal translocation t(3;8) break point 3p25 and 8q24. The patient had inherited the unbalanced translocation with the derivative chromosome 3p resulting in ish der(3) t(3;8)(p25q24)(wcp8+sp)mat (abnormal findings, maternal unbalanced 3;8 translocation). The mother was pregnant again and we performed amniocentesis. The fetal karyotype was found as 46,XY t(3;8)(p25q24). The baby was born by a cesarean section and was healthy. Maternal grandmother also was found to have a similar balanced translocation fish t(3;8)(p25q24)(wcp8+sp;wcp8+sp;D8S547/TRPS+st, D3S1270/D3S1297+mv) (balanced familial 3;8 translocation). Maternal aunt was pregnant and we performed peripheral blood chromosome and FISH analysis. Both were normal. The baby was also phenotypically normal. This is a quite interesting and new finding because reciprocal familial translocation between chromosomes 3p and 8q and maternal unbalanced 3;8 translocation (ish der(3)t(3;8)(p25q24) and abnormal findings with 8q breakpoint which does not disrupting the region of TRPS1 has not been reported in the literature previously.

P297. Enlarged chromosome 13 p-arm hiding a cryptic partial trisomy 6p22.2-pter

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Here we report on a 2 years old male child with developmental delay, short stature, blepharophimosis, clinodactyly, hypolastic philtrum, abnormal ears and microcephaly. Banding-analysis revealed a normal karyotype apart from an enlarged short arm of one chromosome 13. Rather than NOR- and CBG-staining, we applied the so-called acro-cenM-FISH probeset containing a probe specific for the acrocentric human p-arms, a NOR-specific probe, a probe for Yq12, and all available centromere specific probes for the human acrocentrics. The results allowed to exclude that the enlargement on the suspect chromosome was due to a centromere or p-arm- polymorphism, an enhanced NOR-region or Yq12-derived additional material. As M-FISH was unable to resolve the nature of the rearrangement, the case was studied by glass needle based microdissection and reverse painting. As a result, the region p22.2-pter on both homologous chromosomes 6 was painted with the microdissection-derived probe. The presence of a partial trisomy 6p was proven by multicolor banding, too. In the present case a parental origin of the rearrangement could not be excluded, as the child was adopted and parents remained unknown. The literature revealed that the symptoms of the present patient are in high concordance with those reported for three similar cases with trisomy 6p22or23-pter. In summary, every abnormally large acrocentric short arm marker should be studied in detail by different molecular cytogenetics methods. It must be kept in mind, that M-FISH may not always be reliable methods to detect translocations at the end of acrocentric p-arms. Supported in parts by the DFG-436RUS17/40/00-RUS17/49/02

P298. Distal partial trisomy of 10q with additional clinical features: A new case identified by FISH analysis.

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Partial trisomy of chromosome 10q is a very rare and well defined condition characterized by mental retardation and distinct facial features. Only few cases with 10q duplications have been reported, most of them involving the distal portion of the chromosome. They frequently are the result of unequal segregation of balanced translocations. In some cases the extra chromosome material may be associated with deficiency of other autosome. In the present study, we describe a 17 year-old female with mental retardation and clinical features associated with the distal 10q duplication. There was a familial history of miscarriage and two apparently affected sibs that was not possible to contact due to geographic conditions. FISH analysis using the whole probe of the chromosome 10 and cytogenetic studies were performed according to conventional methods and supplier‘s conditions. The kariotype was 46, XX, - 4, + der (4), t(4;10) (q 34.3; 24.3). ish (wcp 10+). Clinical findings were similar to those reported in the literature except for the Sprengel anomaly and the long life expectative (17 year-old). In conclusion we report a new case of partial trisomy of 10q with clinical features not previously observed.

P299. A Maternally Inherited Balanced Reciprocal Translocation in a Moderately Mentally Retarded Dysmorphic Girl.

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A 23 years old girl with various dysmorphic features including short stature and webbed neck with mild to moderate mental retardation was referred to our center for chromosome investigation. The mother has had two previous miscarriages, one deceasd son(12 years old) with apparently similar abnormal features to the proband and one normal son.Both the patient and her mother were found to have an apparently balanced reciprocal translocation between the long arms of chromosomes 4 and 10. The breakpoint on chromosome 4 is at the distal end(4q35), while for chromosome 10, it is proximal to the centromere(10q11.2). The Multi-FISH studies using sub-telomeric probes are being carried out in search of a genetic imbalance , and so far there appears to be no telomeric rearrangements in the patient. To date this is the first reported case of t(4;10)(q35;q11.2). Complete results and their implications on mental retardation and dysmorphism in the patient will be presented.

P300. A t(10;13)(q24;q22)de novo in a patient with mental retardation, epilepsy and ataxia.

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We report a, at the cytogenetic level, balanced translocation t(10;13)(q24;q22)de novo in a patient with mental retardation, microcephaly, bilateral neurosensorial deafness, strabismus, epilepsy, ataxia, autistic behaviour, advanced bone age and precocious puberty. Part of this phenotype is similar to infantile onset spinocerebellar ataxia (IOSCA) (OMIM 271245) described in 19 finish families by Nikali et al. (1997) who found linkage between D10S192 and D10S1265, a narrow interval within 10q24 containing PAX2 and CYP17. Since our patient also has a breakpoint in 10q24, we have started to map this breakpoint by Fluorescence In Situ Hybridisation (FISH) using bacterial artificial chromosomes (BACs) from the critical region of IOSCA. Linkage to 10q24 has also been reported for familial temporal lobe epilepsy with aphasic seizures (OMIM 600512) (Brodtkorp et al. 2002), where mutations have been demonstrated in affected members in LGI1. Physical mapping of the 10q24-breakpoint will shed light on the potential association with these disorders.
In addition, the CLN5 gene mutated in the Finnish variant of late infantile neuronal lipofuscinosis (LINCL) (OMIM 256731) with mental retardation, ataxia and myoclonic epilepsy, maps to the region of the other breakpoint, at 13q22. The objectives of this study are: (1) to contribute to the understanding of the etiology of mental retardation associated with ataxia and epilepsy, (2) to counsel the family at risk and (3) search for a candidate gene for ataxia.

P301. Duplication of chromosome 8P23.1P11.2 in two unrelated families

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

P302. A Case Of Chromosome 22 Monosomy Syndrome

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The proband, a boy, was born from the third normally developing pregnancy, the third timely confinement, from aged parents, the mother at the age of 47. The parents had no kinship ties between them, it is their first marriage.
The boy was born in asphyxia of the 1-st degree, with the weight of 3150 grams, the body length 53 cm, the head circumference 33 cm. Since the 20-th day of life the baby developed flaccidity, weakness, adynamism, lethargy, poor appetite, belching, nutrition problems. The baby put on his weight poorly, developed signs of nutritive allergy, absence of auricular and visual concentration
By the two months‘ age the baby had acutely manifested signs of postnatal hypotrophy, retardation of the psycho-motor development, infectious toxemia.
The clinical examination showed the following:
- (NSG) neurosonography - intraventricular hemorrage of the first degree on the stage of cystic degeneration;
- (EEG) electroencephalography - moderate general cerebral changes, signs of cortex immaturity. The threshold of auricular and visual potentials is acutely decreased.
- (Echo-CS) echocardioscopy - congenital heart disease, atrial septum defect
- (ECG) electrocardiography - sinus arrythmia, AB block of the first degree
The proband‘s phenotype: acrocephalia, low hairline on the forehead, narrow forehead, large and wide auricles, flat face, expressed hypertelorism, wide straight nose bridge, short nose with front-opened nostrils, short filter, thin upper and lower lips, small hidden penis, anomalous palm folds, singular palm fold, calcaneal-valgus foot, microophthalmia, short neck, short upper and lower limbs,

hypotony, retardation of psycho-motor development.
Karyotype: 45,xy,-22,monosomy 22.

P303. Translocation t(10;15) in a man with pigment dispersion syndrome and infertility

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Pigment dispersion syndrome (PDS) affects up to 4% of the white population. It is an autosomal dominantly inherited condition, in which granules of iris pigment flakes off in the intraocular fluid. Patients have findings like the presence of transillumination defects, Kruckenberg's spindle and dense trabecular meshwork pigmentation. In out half of time, PDS progresses to pigmentary glaucoma. The gene responsible for the PDS has been mapped to 7q35-36. We report on the first case of PDS in a male with translocation between chromosomes 10p and 15q. Examination at the age of 34 years showed a man suffering from the PDS. He had normal intellect but was infertile with oligo-astheno-teratozoospermia (sperm density 5M/ml, motility 17% and morphology 0% normal forms). Studies of his pedigree involving 24 members showed PDS to be maternally inherited as an autosomal dominant trait. Chromosome analysis from peripheral blood lymphocytes culture using GTG banding method revealed translocation t(10;15)(p14;q15) in all cells examined. These results show that the existence of PDS and t(10p;15q) in our patient may be a coincidence. But this finding might also be a causal connection and may be helpful in the localization of the new additional candidate loci for the PDS in the regions of 10p14 and/or 15q15. It is known that male infertility may be caused by autosomal translocations. Probably, regions 10p14 and/or 15q15 might be responsible both for the eye development and spermatogenesis.

P304. Deletion 14q11.2q13: a new clinical case and bibliographic review

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Few cases of chromosome 14 interstitial deletions have been reported, including only four with involvement of q11.2 q13 bands. Different phenotypes were observed with the same karyotype abnormality. Several theories were considered, such as: 1) tissue specific mosaicism; 2) molecular differences; 3) parental origin of chromosome deletion. Here we present a new case for comparison with those already described, in an attempt to outline the phenotype of this syndrome.
The patient is a single female child, aged 1 year, born to a young non-consanguineous marriage. She was born at 36 weeks of gestation, weighing 2380g. She had hypoglycaemia, hypotonia, congenital hip dislocation and recurrent respiratory infections. Physical examination: weight, height and head circumference were within normal percentiles. Synophyris, epicanthus, upward slanting palpebral fissures, flattened nasal bridge, anteverted nostrils, protruding upper jaw, down-turned corners of the mouth, high arched palate and abnormal palmar creases were noted. Cerebral CT-scan showed bifrontal cortical atrophy. There was psychomotor retardation and hypothyroidism. Karyotype of peripheral lymphocytes: 1) patient: 46,XX,del(14)(q11.2q13); 2) maternal karyotype: 46,XX; 3) paternal karyotype: 46,XY.
Comparison of the chromosome 14 polymorphisms between the patient and her parents showed that the deleted chromosome was maternal. The most frequent clinical features described in the literature, but in a mild degree, were observed in this patient. Although the comparative evaluation points out coincident anomalies, a greater number of patients are necessary to clinically outline this syndrome in order to explain the phenotypic variability described.

P305. Cytogenetic and molecular detection of a de-novo translocation t(2p;7p) in a patient with kidney agenesia.

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Chromosomal translocations appear rarely (1:1000) and 50% of the subjects with an apparently balanced "de novo" translocation are phenotypically abnormal.
We describe a premature newborn child with left renal agenesis, right low functional kidney, neutropenia, recurrent pulmonary infections and altered chemical-clinical parameters.
Post-natal cytogenetic analysis, performed on peripheral blood and fibroblast cultures, using the QFQ and high resolution GTG banding, revealed a 46,XY,t(2;7)(p1?;p1?) de-novo karyotype.
The chromosome breakpoints were defined by using FISH. FISH was performed using BAC probes from the RP11 library. For the short arm of chr.2, BAC46H3, mapping in 2p13, gave hybridisation signals on der(7), BAC91O12 on der(2) while BAC110B12 shows signals both on der(2) and der(7). The region of the breakpoint is limited to about 123.000 bp from the end of 46H3 and the end of 110B12. A similar strategy was applied for the short arm of chr.7: BAC16L13, mapping in 7p12, gave signals on der(2) and BAC194H16 on der(7), delimiting a 84.600 bp region (from the end of 16L13 to the beginning of 194H16) that contains the breakpoint.
Bioinformatic analysis showed two candidate genes that are likely involved in the rearrangement: *SEC15B* for chromosome 2 breakpoint and *TEM6* for chromosome 7 breakpoint. *TEM6* shows significant sequence identities to tensin 1, a focal adhesion phosphoprotein, expressed in many different tissues during embryogenesis. Interestingly, tensin 1 knock-out mice show a progressive kidney degeneration. Experiments at molecular level are in progress to better elucidate the role of *SEC15B* and *TEM6* in the pathogenesis of phenotype in the patient.

P306. Partial interstitial duplication 3q de novo with breakpoint characterization by YAC-probes

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Chromosome investigations were carried out in a male newborn with severe asphyxia at birth, feeding difficulties, deep seated eyes, prominent front hip dysplasia. A de novo structural aberration of the long arm of a chromosome 3 which proximally contained additional material of unknown origin was diagnosed.
Fluorescence in situ hybridizations (FISH) were performed with a whole chromosome painting probe (wcp 3, commercial probe) and with arm specific probes (pcp 3p, pcp 3q; NIH, Bethesda). A heterologous insertion was excluded and the additional material demonstrated to originate from 3q. In order to further characterize the duplicated interstitial region, hybridizations with a number of yeast artificial chromosome (YAC) probes (CEPH library; MPI, Berlin) were performed. The breakpoints of the duplication were narrowed down to the chromosomal regions 3q12~q13 and 3q21~q22, respectively. The karyotype of the patient could thus be delineated: 46,XY,der(3).ish dup(3)(q12~13q21~q22) (wcp3+,pcp3q+,744e02+,939d05+,949c10+ +,967f11++,967h01+).
Technical particularities of the FISH investigation with the non-commercial probes are given.
As far as we know, this is the first description of a de novo dup(3)(q12~13q21~q22). The clinical findings in the patient will be demonstrated and compared to observations from the literature.

P307. Structural Abnormalities of Chromosome 18 in 2500 Clinical Cytogenetic Analyses

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Human Chromosome 18 is more susceptible for breakage and commonly involved in structural aberrations. Partial trisomy or monosomy of different regions of chromosome 18 due to structural rearrangements like translocations, isochromosomes, inversion-duplication are reported to be 1 in 4500 live births. We report structural aberrations involving chromosome 18 found in our 2500 clinical cytogenetic analyses. Chromosomal analysis was done in

peripheral blood cultures using differential chromosome banding and fluorescence in situ hybridization (FISH) was carried out whenever needed for the molecular cytogenetic characterization. Of the 2500 cases investigated, chromosome 18 abnormalities were found in 5 cases: deletion of 18p (monosomy 18p); satellited 18 due to maternal reciprocal translocation involving chromosomes 18 and 21 resulting in monosomy 18p; pericentric inversion of 18(p11.2q21); proximal monosomy 18p(p10--11.2) and proximal trisomy 18q(q10--q21) due to maternal pericentric inversion; de novo reciprocal translocation involving chromosome 18 and ? resulting in monosomy 18p and trisomy? The prevalence of structural rearrangements of chromosome 18 in this referred population is 0.2%. The genotype-phenotype correlations of these abnormalities will be presented, which was useful in genetic counseling and in prenatal diagnosis.

P308. Large de novo duplication of chromosome 15 in two unrelated children. Clinical, cytogenetic, and molecular studies

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Partial, proximal trisomy of chromosome 15 was very rarely reported in live subjects.
In this study, we present the cytogenetic, molecular - cytogenetic, and molecular findings, and the detailed clinical phenotype, of two unrelated children with “de novo”, large duplication of chromosome 15.
The first child is an Albanian newborn girl, referred for severe congenital cardiac, renal defects, associated with minor facial dysmorphisms. The follow up shows global developmental delay. The karyotype revealed, in all metaphases, partial trisomy 15(pter--q22), with a “de novo” 8;15 translocation detected by conventional cytogenetic and FISH techniques.
Further application of FISH using whole chromosome specific library probes, locus specific, repetitive probes allowed us to well characterize the translocation between chromosomes 8p and 15q
The satellites, the centromere region and alpha-satellite DNA were normally detected at the site of the primary constriction of the two normal chromosomes, and also of the extra derivative chromosome 8;15
The second child is an Italian newborn boy, referred for microretrognatia and complete palatoschisis. The karypotype was 47,XY,+mar. The extra chromosome was identified as a deleted 15, del(15)(q22) with C-band positive heterochromatin, and satellite to the distal end of the short arm. FISH with whole chromosome 15 painting confirmed that the marker was a fragment of chromosome 15 and excluded the involvement of other chromosomes. The karyotype of both parents was normal.
Looking at the karyotype/phenotype correlation, we can confirm that the duplication of the same subtotal chromosome 15 is associated with an apparently different clinical spectrum in the two patients.

P309. Currarino syndrome and holoproencephaly associated with an inv(7) recombinant chromosome

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Pericentric inversion involving chromosome 7 have been reported infrequently. We report here a rare case of a boy with a recombinant 7, rec(7) dup p del q, resulting from a paternal pericentric inversion. This child presented with prenatal and postnatal growth retardation, facial dysmorphism (plagiocephaly, bilateral coloboma, posterior rotated ears, median incisor, glossoptosis and retrognathism), metatarsus varus, mental retardation. Radiological assessment showed agenesis of the corpus callosum, cerebellar atrophy, interventricular defect, hemisacrum and tattered cord. Karyotype of the proband showed an add(7q) interpreted as a rec(7) after the study of paternal karyotype and producing a 7p15.1-pter segment duplication and a 7q34-qter deletion. The patient had signs of the Currarino syndrome (tattered cord and hemisacrum) with a single maxillary central incisor (holoproencephaly spectrum) and abnormal extremities. Molecular cytogenetic investigations showed a deletion of HLXB9 and SHH locus. Further molecular characterisation of the trisomy 7p size is in progress.

P310. Homologies of human chromosome 9p subtelomere

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Chromosome rearrangements are caused by mechanisms such as inversions, translocations, duplifications, fusions, fissions and insertions. These changes are involved not only in human diseases but also in karyotype evolution. Some of these rearrangements have occurred at telomeric regions and led to new telomeres or loose telomeres through evolution. As a result, the genome structures at telomeres have been changed due to the rearrangements. Telomeres are at the end of chromosomes and are essential to prevent fusions between chromosomes and keep chromosome structure stable. Subtelomeres, adjacent to telomeres, are known to have some relation with diseases such as a mental retardation. Normally subtelomeric probes consisting of single copy DNAs are used to identify specific telomeres. Some of subtelomeric markers, however, are not specific and show homologies between subtelomeres of different chromosomes. One BAC clone studied here shows its FISH signals at human chromosome 9p subtelomere, 9 pericentromeres and 2q13. As human chromosome 9 pericentromeres and 2q13 derived from subtelomeric regions, these homologies might have resulted from chromosome rearrangements through evolution. In this study, a cDNA which has homologous sequence in the BAC clone has been identified. Homologies in the human genome can be analyzed based on the database, but the original locus is not certain. In order to investigate the origin of the homologies, the cDNA was examined in primates by FISH and the signal was identified. Comparative mapping at subtelomeres will help us to understand the process of chromosome rearrangements.

P311. The *INSL3-LGR8/GREAT* ligand-receptor pair in human cryptorchidism

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Introduction. Testicular descent is a complex multistep embryonic process requiring the interaction between anatomical and hormonal factors. Failure in any of these steps results in cryptorchidism, the most frequent congenital anomaly of the urogenital tract in human males. Evidence for a genetic cause for cryptorchidism is numerous and supported by animal models. In particular *INSL3* and *LGR8/GREAT* proteins seem to act as ligand and receptor, respectively, and to have a role in gubernaculum development involved in testicular descent. **Methods**. In a cohort of 87 ex-cryptorchid patients and 50 controls, we looked for mutations in *INSL3* and *LGR8/GREAT* genes by sequencing. Patients were classified on the basis of seminal, hormonal and testicular cytological analyses. **Results**. We found 3 mutations in the *INSL3* gene in four patients and one *LGR8/GREAT* mutation in four patients (8/87, 9.2%). The eight patients show different phenotypes, ranging from normozoospermia to complete azoospermia, and from bilateral cryptorchidism to retractile testes. Furthermore, the endocrine function of the testis appears normal in all subjects. Mutations were maternally inherited. **Discussion**. The findings of our study demonstrate that *INSL3-LGR8/GREAT* mutations are a frequent cause of human cryptorchidism, and are maternally inherited. The only clinical consequence of alterations of the *INSL3-LGR8/GREAT* system seems to be failure of the testis to normally descend in the scrotum during embryonic development, without affecting the spermatogenic and endocrine components of the testis itself. Further studies would clarify the molecular events involved in INSL3-LGR8/GREAT interaction as well as possible interaction with other factors implicated in testicular descent.

P312. Clinical and molecular spectrum in a large series of patients with complete androgen insensitivity syndrome

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Androgen insensitivity syndrome (AIS) is caused by defects in the androgen receptor (AR). The complete form (CAIS) is manifested by a normal female phenotype. The AR gene comprises exons 4-8 coding for ligand binding domain (LBD), exon 1 for transactivation and exons 2-3 for DNA binding.
We studied 120 patients with CAIS. The majority of these patients presented with inguinal hernia (60%), 11 presented with primary amenorrhea; 14 were related to positive family history, and 3 were diagnosed antenatally. 20 had an hCG stimulation test; median testosterone rise was 10.8 nmol/L.
Genetic screening was done in 91 patients; 80% had mutations in the AR gene. The spontaneous mutation rate was 10%. The majority were missense mutations; 9 had nonsense mutations; 8 had deletions (one complete gene deletion), one duplication and one insertion. Most of missense mutations were in the LBD, and 67% of nonsense mutations were seen in exon 1.
We conclude that inguinal hernia is the commonest presentation of AIS in children; a karyotype is not currently routine for this surgical problem. We recommend an hCG test in support of the diagnosis, and to exclude an androgen biosynthetic defect. Exons 4, 5, 7 are preferential sites for missense mutations. The minority of CAIS patients in whom no demonstrable gene mutation is found may have a defect in RNA processing or in AR expression.

P313. Phenotypic variability in XX males in relation to Y specific DNA sequences

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Background: In XX sex–reversed males, various degrees of testicular differentiation can be observed. The correlation between genotype (SRY +/-), respectively breakpoint in Yp, and phenotype is still unclear.
Aim: to analyse genotype – phenotype correlation in our patients.
Patients: 3 patients (46,XX-males): 2 phenotypically normal males [14 ys (A) and 15.2 ys (B)] with small intrascrotal testes; 1 boy [C],(2.5ys), with Prader IV external male genitalia.
Methods: clinical examination; endocrine testing (T, DHT, FSH, LH, 17OHP, DHEA, AD, ACTH, hCG stimulation in all patients, GnRH stimulation in the pubertal patients); pelvic and testicular ultrasound; genetic testing (karyotype analysis, X-Y FISH and Yq11 multiplex PCR) after informed consent in all patients; gonadal biopsy in patient C.
Results: Endocrine status was normal in all 3 patients except low GnRH response in patients A and B. In patient B puberty was delayed. Pelvic ultrasound revealed normal internal male genitalia in all patients; the testicular biopsy, performed only in patient C, showed germinal and Sertoli cell hypoplasia. Genetic studies confirmed the 46,XX karyotype. FISH analysis for Y fragments on Xp was positive in 2 patients and negative in patient C. The Y-positive patients were also positive for the SRY locus and negative for the complete Y long arm (Yq11).
Conclusions: Our observations in this small number of cases suggest that Y specific material on the X chromosome results in a more masculinized phenotype. In the intersex patient, incomplete masculinisation without SRY suggests a mutation of one or more downstream non-Y testis – determining genes.

P314. Analysis of the SRY gene in 40 Turner syndrome patients from the Macedonian population

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Turner syndrome is characterized by short stature, gonadal dysgenesis, variety of somatic features and malformations of internal organs. Half of the patients have a 45,X karyotype while others have structurally abnormal sex chromosomes or mosaicsm with other cell lines including 45,X/46,XY. Patients with Y chromosomal material are susceptible to developing gonadoblastoma or dysgerminoma later in life. The SRY gene on Yp has a major role in sexual differentiation, being the primary testicular determinant. Detection of SRY gene in Turner syndrome patients with or without sex chromosome mosaicism has important clinical and therapeutical implications.
We performed a genetic study in 40 Turner syndrome patients. The investigation consisted of two phases: cytogenetics and PCR for the SRY gene using two primer sets.
Cytogenetics identified a 45,X karyotype in 50%, isochromosomes in 25%, proximal long or short arm deletions and markers in 7.5% and 45,X/46,XY in 5% of patients.
SRY gene was detected in blood leucocytes of 2 patients with 45,X/46,XY karyotype. All other patients were SRY negative including one ovary extirpated due to endometrial malignancy.
We showed low incidence of SRY positive results in Turner syndrome patients-5%. Other studies using more sensitive techniques have detected higher percent of SRY positive patients.
Due to increased risk of gonadoblastoma and necessity of timely referral for gonadectomy, analysis of SRY should be offered to all Turner syndrome patients regardless of karyotype.

P315. Jumping translocation in an adult male with hypogonadism.

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Jumping translocations, the translocation of a same chromosomal fragment to different recipient chromosomes in different cell lines of the same individual, are rarely observed as a constitutional chromosome abnormality. We report a jumping translocation, observed in a university-educated 26 year old male, referred by his endocrinologist because of hypogonadism. Karyotype analysis on peripheral lymphocytes revealed the following karyotype:
46,XY,r(18)(p11.1q23), -8,+der(8)t(8;18)(q24.3;p11.2) [66%]/
46,XY,r(18)(p11.1p23) [21%]/
46,XY,r(18)(p11.1q23),-20,+der(20)t(18;20)(p11.2;q13.3) [13%].
Thus, an apparently complete ring 18q chromosome was observed in all cells, while the 18p was translocated to the end of 8q or 20q. However, in 21% of cells analysed, 18p was completely missing. Despite the observed ring instability and the 18p monosomy in an appreciable percentage of cells, the phenotype of the patient was normal except for hypogonadism. FISH analysis of the translocation breakpoints and karyotype analysis on skin fibroblasts are currently underway.

P316. Chromosome 9 hetrochromatin polymorphism: retrospective investigation in infertile couples

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Chromosome 9 heterocromatin polymorphisms (increase of the long arm heterocromatin and pericentromeric inversion)have been associated with several diseases such as: infertility, fetal malformation, schizophrenia and immunodeficiency. We have evaluated a sample of 141 couples who had complained about their reproductive failure, spontaneous abortions, fetal malformation and/or stillbirth. They were distributed into three groups (Group I - difficulty of getting pregnant and one miscarriage; Group II - two or more miscarriages; Group III - fetal malformations, stillbirth and/or newborn with chromosomal abnormalities) and were submitted to cytogenetic analysis. Thirty eight out of 242 patients, 21 men and 17 women, had some kind of chromosomal alteration (60,53 % had chromosome 9 polymorphism). Statistical analysis, such Chi-square and Z-test,was performed.Our statistical analysis has shown similar proportions of abnormal karyotypes among the three groups. Therefore, there was no apparent correlation between the groups and the chromosome 9 polymorphism. However, a strong association was found for the polymorphism and the fetal malformations, stillbirth and/or newborn with chromosomal abnormalities (Group III) (p> 0,05).

P317. Rearrangements involving Chromosome 1 and Male Infertility

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A survey of chromosome rearrangements identified in the Oxford region through male infertility showed a surprising bias towards those involving chromosome 1. Total referrals were 1086: 947 normal male karyotype, 108 Klinefelter and mosaic Klinefelter, 17 other sex chromosome abnormalities, and 14 autosomal rearrangements. Of these 14 rearrangements, eight involved chromosome 1. Six of the seven reciprocal translocations involved chromosome 1 (breakpoints p21.1, p13, p10, q12, q21.1, q24.1). There was also a pericentric inversion of chromosome 1 (p36.1q21.3) and a partial duplication of 1q (q12q21.3). There appeared to be no specific breakpoint region within the 1 and thus a single gene effect seems unlikely. These highly significant findings add to the previously reported association between pericentric inversions of chromosome 1 and male infertility. The previously reported pericentric inversions associated with meiotic arrest showed partial or complete asynapsis within the inversion. It is hypothesised that even in normal individuals the large size of the chromosome 1 bivalent creates physical stresses when pairing occurs and the presence of a large heterochromatic block within it further destabilises the pairing. This may be tolerated when there are no other factors involved, but a structural change involving chromosome 1 adds to the difficulties of pairing and thus there is a tendency for asynapsis to occur in or around the rearrangement. The asynaptic regions may either disrupt pairing of other autosomes or the formation of the sex vesicle and thus cause meiotic arrest.

P318. Linear increase of structural and numerical chromosome 9 abnormalities in human sperm with age

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Four-colour fluorescence in situ hybridisation (FISH) assay was used to investigate the effect of age on the incidence of numerical and structural chromosome abnormalities in human spermatozoa. The sperm samples were collected from eighteen healthy donors, aged 24-74 years (mean 48.8 years). Specific probes for the subtelomeric 9q region, centromeric regions of chromosomes 6 and 9, and the satellite III region of chromosome Y were used for FISH analysis. A total of 190,117 sperm were evaluated (a minimum of 10,000 sperm scored per donor). A significant linear increase in the overall level of duplications and deletions of 9cen and 9pter ($p=0.002$), chromosome 9 disomy ($p<0.0001$) and diploidy ($p<0.0001$) was detected in relation to age. The percentage of increase for each ten-year period was 29.0% for chromosome 9 disomy, 18.8% for diploidy, and ranged from 14.6 to 29.0% for chromosome 9 structural aberrations. Our results indicate a linear increase in structural aberrations and disomy for chromosome 9 as well as diploidy in sperm with age.
This work received financial support from Ministerio de Ciencia y Tecnología (project BFI2002-01193) and Generalitat de Catalunya (CIRIT, 2001 SGR-00201), Spain.

P319. Frequency of chromosomal abnormalities in azoospermia infertile men

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Introduction: Human male infertility is often related to chromosomal abnormalities. To determine association with azoospermia and chromosome anomaly, we assayed the chromosomal condition of azoospermic men and in normospermic men as controls.
Materials & Methods: We studied 45 azoospermic men who had no spermatozoa in their ejaculated semen samples and 40 men who had semen samples with normal semen parameters by WHO 1999 criteria. Peripheral blood lymphocytes were collected and cultured in Ham's F10 supplemented with 10% HSA with PHA for 60-72 hrs. Chromosomal spreads were obtained by standard methods. The karyotype was arranged and analysed using CytoVision Ultra ver.4.0 from Applied Imaging.
Results: Of the 45 patients, 22 were azoospermic and 23 had severe oligospermia $<5x10^6$/ml, mean + SD 4.3 + 1.2 x 10^6/ml. G banding did not reveal any structural abnormalities in either the case or control groups. In the case group, 10 samples analysed as Klienfelter syndrome (47,XXY, n=6, and mosaic 47,XXY/46,XY, n=4). There were no numerical anomalies in the control group, who had a mean + SD pserm count of 65 + 12 x 10^6/ml.
Conclusion: Since chromosomal aberrations are encountered more frequently in azoospermic and severely oligospermic infertile men, cytogenetic analysis of the male prior to ICSI is recommended.

P320. Sperm aneuploidy analysis in men with Klinefelter offspring. A preliminary study

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The purpose of this work was to investigate whether there was an increase of aneuploidy in sperm from fathers of patients with Klinefelter syndrome (KS) of paternal origin. We used the polymerase chain reaction (PCR) of DNA from peripheral blood to determine the parental origin of the syndrome in 11 families with Klinefelter offspring. Only three of these 11 cases were of paternal origin (27,3%). We analysed the frequency of numerical chromosome abnormalities for chromosomes 6, X and Y in sperm by three-colour FISH. We compared the percentages of numerical chromosome abnormalities in sperm in 8 fathers of maternally (33 to 58 years old) and 3 paternally (33 to 44 years old) inherited KS cases. Significant increase in the XX and XY disomy incidences were observed in paternal origin KS group. These differences were due to an increased frequency of sex chromosome disomies in one of the father (33 years old) who had a karyotype 46,X,r(Y)/45,X. The presence of the Y-ring could be the cause of the XY nondisjunction increment in this father. Further studies of additional cases will be needed to establish the relationship between paternal meiotic nondisjunction and sex chromosome abnormalities in sperm. This work received financial support from Ministerio de Ciencia y Tecnología (BFI2002-01193) and Generalitat de Catalunya (CIRIT, 2001SGR-00201; CIRIT, 2001SGR-0202), Spain.

P321. Cytogenetic study in 110 romanian couples with reproductive failure in Timis county

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Introduction: Ideally, chromosomal studies should be performed on parents in cases with sterility, recurrent miscarriage and stillborn infants.
Patients and methods: We performed chromosomal investigations on 110 couples with reproductive failures. This study included 34 couples with sterility (30.91%), 56 couples with repeated abortion (50.91%) and 20 couples with a stillborn infant (18.19%) who were referred for cytogenetic studies between July 2000 and December 2002 at two of the Genetics Centers of Timisoara.
Results: We paid special attention to prior reproductive history. The number of previous abortions varied from 2 to 13 abortions (mean 2 abortions per couple). Using standard cytogenetic techniques (GTG banding, C banding) 19 (17.27%) cases of chromosome anomalies were identified. These included: 4 cases with balanced reciprocal translocations [t(2;6), t(2;11), t(4;12), t(6;7)]; 5 cases with pericentric inversions [inv(9), inv(15)]; 4 cases with duplications [1(dup) and 16(dup)]; 1 case with insertion ins(3); 2 cases with deletions del(Yq); mosaic forms of gonosomal aneuploidies in 3 cases. Also, we detected increased heterochromatic regions of several chromosomes (chromosomal variants) 1qh+, 9qh+, 13ph+, 14ph+, 15ph+, 16qh+, 21ph+, 22ph+, Yqh+ in 15 cases (13.63 %).
Conclusions: The authors believe that every couple diagnosed with sterility and infertility should be offered genetic counseling. We also believe that cytogenetic studies will remain one of the major options for detecting the causes of reproductive failure.

P322. An autosomal recessive form of male infertility: mutation of mitochondrial DNA polymerase gamma (POLG)

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The gene encoding the mitochondrial DNA polymerase γ (POLG) is localized in 15q24-15q26. The N-terminal part of the polymerase includes a stretch of glutamines encoded by a CAG repeat of variable length. The most frequent allele consists of 10 repeats. Rovio *et al* studied the variation of this CAG repeat in infertile males (*Nat Genet*. 2001, **29**: 261-2). The presence of 2 alleles with a number of repeats different from 10 was found only in some infertile males with either asthenozoospermia or oligozoospermia.
We studied 177 infertile males and 28 normospermic controls. All control men have at least one allele with the 10 CAG repeat (10/10 or 10/x). Conversely, 2.8% of the infertile men (5 out of 177) have 2 alleles with a number of CAG repeats different from 10. Oligozoospermia and asthenozoospermia were present in these 5 patients. They represented 5.3% of our patients with oligozoospermia. In addition, the presence of only one allele with 10 repeats (10/x) was more frequent among our infertile male population: 25 % versus 14 % in our fertile population.
Our results are the first to confirm the data from Rovio *et al*. We conclude that, in the absence of any detectable cause, POLG mutation might contribute to 5% of oligospermic patients. It is noteworthy that this condition should be inherited as an autosomal recessive trait. Further studies are also in progress to understand the role of POLG polyglutamine variation in male infertility.

P323. A complex sex chromosome abnormality to a case of Turner syndrome

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Turner syndrome is characterised by sex chromosome abnormalities that lead to a highly variable phenotype which typically includes gonadal dysgenesis, pubertal arrest, amenorrhea, short stature. Intellectual performance often is normal. Approximately half of the affecteds show a 45,X karyotype, while the other half of the patients has been described to show a multiplicity of different chromosomal aberrations.
We report a 15 year old girl with proportionate short stature, pubertal arrest, primary amenorrhea. She is the first child of a young healthy non-consanguineous couple. Pregnancy and delivery were uneventful, and she was born at term. Fifteen years old she presented elevated FSH and LH levels, bilateral polycystic gonads and a normal intelligence. G-banded chromosome analysis (450 bands) showed a complex chromosome abnormality, karyotype 45,X[46%]/46,X,+mar[44%]/46,XX[6%]/47,XXX[4%]. Aiming to clarify the origin of marker, X- or Y-chromosomal, we performed FISH studies. Results demonstrated that the marker is a very small ring X chromosome (DXZ1+) lacking the gene for the X-inactivation specific transcript (XIST-) This may indicate that genes on the marker, if present, cannot be inactivated. The 47,XXX cells showed a small DXZ1 area on one X chromosome (normal polymorphism) and an unusually large DXZ1 area on the two other X chromosomes, suggesting the possibility of isodisomy.
We speculated the origins of the complex mosaicism. The 46,XX cells may possibly represent the zygote and the ring(X) may have arisen at an early postzygotic stage. Independent of that event, another 46,XX cell may have undergone a chromosomal nondisjunction leading to the 45,X and 47,XXX cells.

P324. Cytogenetic investigation of infertility (Report from Iran)

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Chromosomally derived sterility has long been recognized. We have investigated 355 azoospermic patients in our center and a constitutional chromosomal aberration were diagnosed in 92/355 (25.9%). Whereas the 47,XXY chromosome complement was the commonest (54/92 cases), the following abnormal karyotypes were also found: 46,XX; del(Y)(q11); inv(Y)(p11.2,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/48,XXY+mar/48,XXXY; 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 men. We have observed 29.5% chromosome abnormality in azoospermia and 20% in infertile men, which is compatible with the data from literature.
Cytogenetic analysis of 3497 samples showed thirty eight cases of inv(9)(p11;q13) (incidence of 1%), which confirms the data which is reported in literature. The main reason for referral in the male group was azoospermia (8/21), and in the female group spontaneous abortion (4/17) and primary amenorrhea (4/17). Taking into account that 50% of our cases with inv(9) were subfertile, maybe this abnormality is truly related to infertility, or perhaps this may only represent a selective bias.
We have also found 24 cases (24/498) with sex reversal which was more common in the male group as male pseudohermophrodism (17/24) in our other studies. The main reasons for referral were azoospermia and primary amenorrhea.

P325. Pericentric inversion of Y Chromosome : report of 5 new cases

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The prevalence of pericentrically inverted Y (inv(Y)) in the human population is approximately 1 to 1.15 per 1000, but less than 10 patients with inv(Y) have been described in details to our knowledge. Pericentric inv(Y) is commonly considered as a variant or an heteromorphism. We describe here 5 new cases of pericentrically inverted Y chromosome discovered in either a context of prenatal diagnosis (3 observations) or of infertility (2 cases). The breakpoints of the inverted Y chromosomes were established with conventional R, Q banding techniques and FISH procedure using specific probes for SRY and Y centromere loci. When possible molecular biology with markers of SRY and AZF loci was realised.
Inv(Y) is usually considered as a familial variant without effect on the phenotype but this has to be checked. New means like molecular biology or molecular cytogenetic are now available to confirm the breakpoints position on the inv(Y). We want to point out that is is necessary to precise those positions in order to better explain the different phenotypes associated with inv(Y).
We will discuss the genetic counselling which is difficult in both situation: spermatogenesis failure and inv(Y) or prenatal diagnosis of inherited inv(Y)

P326. Genetic Anomalies Detected in Patients with Non-Obstructive Azoospermia and Oligoasthenoteratozoospermia

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Introduction: Men with severely impaired spermatogenesis may have genetic anomalies as a cause of low sperm production. We present the results of genetic screening of men with non-obstructive azoospermia, and severe oligoasthenoteratozoospermia (OAT) patients.
Material and methods: We evaluated 436 oligozoospermic and 383 azoospermic men between years 1995-2003. All patients underwent evaluation, consisting of a complete history, physical examination, semen analysis, hormone testing, urinalysis and genetic testing. Each man had standard karyotype analysis of peripheral leukocytes. All men with chromosomal abnormality were referred for genetic counseling.
Results: Out of 436 men with oligozoospermia, only 4 (0,9%) patients had virtual azoospermia (spermatozoa detected in the pellet after centrifugation at 1800g for 10min) in their ejaculate; others had a mean sperm count of 3,22 million/mL ± 0,91 (0,1 – 10,8 million/mL). Of the 383 azoospermic patients, 47 patients (12,3%) and of the 436 oligozoospermic patients 20 patients (4,6%) had genetic abnormalities (Table 1).

Table 1.Chromosomal abnormalities detected in oligozoospermic and azoospermic patients

Cases	Total Number of Abnormalities	Sex Chromosomal Abnormalities	Autosomal Chromosomal Abnormalities
Azoospermic (n=383)	47 (12,3%)	36 (76,6%)	11 (23,4%)
Oligozoospermic (n=436)	20 (4,6%)	12 (60%)	8 (40%)

Conclusion: These results show that preoprative genetic screening is necessary in males who are azoospermic and severe OAT before referring to assisted reproductive techniques (ART).

P327. Rare chromosomal abnormalities in three mail patients with azoospermia

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This paper presents three cases of rare structural aberations of sex determing chromosomes.
A male patient with azoospermia and sterility, with de novo translocation ivolving the long arms of chromosomes Y and 9.
We performed cytogenetic analysis using conventional bending techniques, and found balanced reciprocal translocation. Karyotype is 46, X, t (Y ; 9) (q 11. 2 ; q 34).
Using PCR-method, we amplified 18 main fragments of AZF lokus, including DAZ, Kal-Y, SMCY genes and RBMI, DFFRY, DBY. Analysis of PCR products (on agarose gel with high resolution) showed that our patient has no delation in any of tested region. So, our hypothesis is that position effect has a main role in sterility of this case.
We also present two infertil male patients with azoospermia. The first case is with rare paracentric inversion of X chromosome, karyotype 46, XY, inv X (q 13 ; q 25). And the second case is with Y/Y translocation; karyotype 46, X, t (Y;Y) (p 11 ; q 11) [(Ypter - Yp11 :: Yq11 - Yqter)].Breaking points indicate that delation of DAZ gene, placed on Yq11, is the main reason of azoospermia. SRY gene is identified by FISH, which explains presenting of mail phenotype and genitalia.
Performed genetic counselling will be discussed.

P328. CFTR Gene: Frequent Haplotypes Involved in Azoospermic Patients and IRT Raised Samples

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Aims: To analyse the frequency of the IVS8-5T variant and the R668C-G576A haplotype in CBAVD (Congenital Bilateral Absence of Vas Deferens), CUAVD (Congenital Unilateral Absence of Vas Deferens) and CBASV (Congenital Bilateral Absence of Seminal Vesicles) patients and in neonatal screening samples with elevated IRT, and to extend the haplotypes in order to establish the differences and similarities between these phenotypes.
Methods: We analysed 23 exons from the CFTR gene in 19 azoospermic patients (16 with CBAVD, 2 with CUAVD and 1 with CBASV). 225 neonatal blood samples with raised IRT were also studied for 12 CFTR exons, which include 90% of the mutations causing cystic fibrosis in our population. The screening methods were PAGE, DGGE and direct sequencing.
Results: F508del was the most frequent mutation in both cases, but the IVS8-5T variant is the second in the azoospermic patients but not in IRT raised samples. There exist differences too when we extend the haplotype and analyse the $(TG)_m$ variant: $(TG)_{12}$ is the most frequent in CBAVD phenotypes and $(TG)_{11}$ in CUAVD and neonatal samples with elevated IRT. Another important difference is the linkage desequilibrium found between the 5T-11TG haplotype and the 875+40 A-G polymorphism in IRT elevated samples.
Conclusions: The penetrance of the IVS8-5T variant could be affected not only by the $(TG)_m$ but another polymorphisms such as 875+40 A-G, raising the IRT values at birth, or it is also possible that this haplotype is linked to another undetected mutation.

P329. Prognostic Value Of Y Chromosome Microdeletions For Sperm Retrieval

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Male infertility affects about 10% of the adult population. Considerable technical advances in the assisted reproductive techniques (ART), intracytoplasmic sperm injection (ICSI) and testicular extraction (TESE) in particular, revolutionized the treatment of male infertility. Recently, Y chromosome microdeletions proved to be an important etiologic factor in male infertility. Although molecular mechanisms have not been elucidated yet, several attempts have been made to find whether the Y microdeletion pattern may predict the success of TESE, and consequently, the infertility treatment. Therefore, we analyzed a database (YCMdb) of 512 infertile men with Y chromosome microdeletions. The aim was to find new associations between genetic markers, AZF regions and genes, and retrieval of testicular sperm at TESE. The YCMdb was constructed extracting data from papers, indexed by the Pubmed bibliographic database (1994-2002). Of the 512 patients included in the YCMdb, the data on testicular sperm retrieval was available for 331 patients: in 207 sperm was found present (176 OAT and 26 AZO patients) and in 124 no sperm was found. We identified three regions (AZFa, AZFb and the region AZFa-b), one gene (RBMY) and 11 STS markers (sY84, sY86, sY87, sY89, sY113, sY117, sY124, sY128, sY134, sY139, sY143), which were significantly (pŁ0.001) associated with sperm retrieval. In the identified loci sperm was retrieved in x-y%. We conclude that specific deletions may be associated with the success of sperm retrieval, however, larger datasets have to be analyzed to firmly estimate the prognostic value of the Y deletion analysis.

P330. Sexual ambiguity in a fetus due to a mosaic 45,X/46,idic(Yq) karyotype.

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A 35-years old woman presented in the 28th gestational week of her first pregnancy for a second opinion, since sonographic examination had shown ambiguity of the external genitalia of the fetus. An amniocentesis for karyotyping was offered and was performed in the 34th gestational week. This showed a 45,X[8]/46,X i(Yq)[2] karyotype. Fluorescence in situ hybridization with Y-specific probes showed an isodicentric Y chromosome with the breakpoint on the short arm of the Y chromosome.
An isodicentric Y chromosome is mostly found in combination with a 45,X cell line and causes intersex in approximately 30% of the cases (Hsu, 1994). In our case, this karyotype explained the sonographic findings but could not give more reassurance about the gender of the fetus (Tuck-Muller et al, 1995). The child was born in the 40th gestational week. No dysmorphism was found except for ambiguous external and internal genitalia. A penis was present with a testis on the right side. Sonographic investigation showed a uterus horn and fallopian tube on the left side. On this side no gonad could be identified. A mosaic isodicentric Y chromosome was the cause of this case of true hermaphroditism.
References.
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P331. Characterisation of HSFY, a novel AZFb candidate gene on the Y chromosome, with a possible role in human spermatogenesis

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Introduction. Microdeletions of Yq are the most common mutations in infertile males, involving one or more "azoospermia factors" (AZFa, b and c). We recently assembled a complete map of AZFb

determining its structure and gene content. Deletion analysis in infertile men suggested a possible role for a novel gene, HSFY. It contains an heat shock factor-type DNA-binding domain related to HSF2 on chromosome 6, and it is predicted to encode two transcripts. In this study we report preliminary results of genomic organization and expression analysis for HSFY. **Methods**. Genomic structure has been determined by Blast alignment with AZFb clones. Expression analyses were performed with primers specific for both transcripts in different tissues, spermatozoa, andSertoli cells by PCR, RT-PCR, and Northern blotting. **Results**. Transcript 1 (long, 1424 bp) was present in many tissues whereas transcript 2 (short, 1058 bp) amplified only in testis. A third transcript of 1500-1600 bp (transcript 3) was found only in the testis, confirmed also by sequencing. Northern analysis for this novel transcript confirmed a fragment of 1.5-1.6 kb expressed only in testis. Transcript 1 is expressed in spermatozoa but not in Sertoli cells, while transcript 2 and 3 are not expressed in these cells, suggesting that they are expressed in the first phases of spermatogenesis. **Discussion**. HSFY seems to be differently expressed during spermatogenesis and it may represent an AZFb candidate gene. Further studies are in progress to analyse its function during male germ cell development.

P332. Elucidation of murine *Theg* gene role in spermatogenesis

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Stage-specific interactions between Sertoli cells and germ cells are central in the regulation of spermatogenesis, but their molecular mechanisms are still poorly understood. In the present study, a differentially expressed gene, named *Theg*, expressed specifically in spermatids, is characterised. Its expression is up regulated by some unknown factor/s from Sertoli cells. The ORF of *Theg* encodes for a 42.7-kDa putative nuclear protein. To elucidate the function of Theg protein and its role in spermatogenesis, we decided to disrupt the *Theg* gene homologous recombination in mouse. Theg was identified as a novel protein therefore for functional analysis of Theg protein's domain structure, two different knock out approach were undertaken. In first knock out mice the C-terminal of Theg protein (which included exon 3-8) was deleted. Both male and female mice heterozygous for *Theg* deletion appeared normal and fertile. Homozygous male and female mice also exhibited normal phenotype and $Theg^{-/-}$ male mice were fertile. Thus suggesting that C-terminal of Theg does not play any important role for a successful progression of spermatogenesis. In the second knock out mice, we intended to delete the N-terminal domain of Theg protein by homologous recombination, which involved deletion of exons 1-4 of *Theg*. Both heterozygous and homozygous male mice were fertile. Further phenotypic and physiological analyses to identify any subtle abnormalities are awaited. However from our results from both knock out mouse model systems, we can conclude that Theg is a non-essential protein for spermatogenesis and perhaps its function is compensated by some other redundant protein/s

P333. Genetic findings among patients with Turner's syndrome referred to Uremia's cytogenesis center between 2000-2002

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Turner syndrome affects approximately 1 out of every 2,500 female live births worldwide. Almost all people with Turner syndrome have short stature and loss of ovarian function. In this program this syndrome situation were studied among 200 patients referred to university genetic center. The center is the only one in the whole province and the patients are from all ethnic groups since the city is near the border of Iraq and Turkey.When conventional karyotyping is done from lymphocyte cultures, about 50% of patients show a 45,XO chromosome constitution. Other karyotypes found with Turner syndrome are mosaicism of 45,XO with other cell lines such as 46,XX, 46,XY, or 47,XXX. Structural anomalies of an X chromosome, such as isochromosomes (46X iXq, 47X2iXq).But the major finding in about 90% of the cases is significant delay in diagnosing the patients. For example the average age of patients referred for karyotyping is about 16 year. It is mainly due to the cultural and social background. The majority of the patients are from rural area with no education. Therefore many of them have not received any treatment till adolescent age. Their short stature, and infertility make them isolated person among their tribe and relatives. Many of them have a lot of psychological and social problem. The only practical method for preventing and decreasing the side effects of the syndrome is: implementing screening programs for tracing genetic diseases in all hospitals from early stage of life as well as increasing people understanding about the disease.

P334. Genetic screening of 750 severely infertile men candidates for intracytoplasmic sperm injection

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Introduction. Children born after intracytoplasmic sperm injection (ICSI) performed for male factor infertility seem at increased risk of congenital malformations and chromosome aberrations. These anomalies seem to be related to the underlying spermatogenic damage of the father, that frequently is caused by genetic alterations. **Methods.** We studied 750 severely oligozoospermic men candidates for ICSI. Genetic tests included: 1. analysis of peripheral blood karyotype; 2. analysis of the Y chromosome long arm for detection of microdeletions in the azoospermia factors; 3. analysis of mutations in the cystic fibrosis gene; 4. analysis of mutations in the androgen receptor gene; 5. analysis of sperm chromosome aneuploidies. **Results.** A total of 104 genetic abnormalities were diagnosed, corresponding to a frequency of 13.9%. Chromosomal aberrations were present in 5.6% and they were in most cases alterations of the sex chromosomes. Y chromosome long arm microdeletions were detected in 6.0% and most frequently included AZFc. Mutations in the cystic fibrosis gene were diagnosed in 1.2%, and mutations in the androgen receptor gene in 1.1%. Sperm sex chromosome aneuploidies were increased in men with karyotype anomalies and Y chromosome microdeletions, and also in subjects without genetic abnormalities. **Discussion.** Genetic alterations represent a major cause of severe spermatogenic impairment leading to male infertility. The risk of transmission of chromosomal and genetic diseases by ICSI is therefore very high. Genetic tests and counselling are highly recommended in oligozoospermic men candidates for in vitro fertilisation techniques, to avoid transmission, persistence, or even an increase of genetic defects in future generations.

P335. High frequency of TTY2 gene family deletions in Caucasian patients with severe oligospermia and azoospermia

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Genes expressed in the human testis and involved in spermatogenesis are likely to be located on the Y chromosome.Micro deletions in the AZF locus (Yq11) are associated with spermatogenic failure and detected in approximately 10% of patients with idiopathic oligo-/azoospermia.Recently, TTY2, a Y-linked multicopy gene family of unknown function, has been identified by the screening of a Y-linked and testis-expressed cDNA selection library.Members of this family include the TTY2L2A gene (Yq11.2), and the TTY2L12A gene (Yp11).In order to examine the contribution of these two novel genes in male infertility, we studied 143 infertile men with normal karyotypes and 80 healthy fertile men of Caucasian origin.Patients were divided into group A (N=30) with idiopathic moderate oligospermia, group B (N=81) with azoospermia/idiopathic severe oligospermia, and group C (N=32) with azoospermia/oligospermia of known etiologies.DNA samples of all investigated men were analysed for deletions in TTY2L2A and TTY2L12A, as well as in the X-linked androgen receptor gene (control).No deletion was detected in group C patients and fertile controls.In group A, 2 patients had a deletion of TTY2L2A (6.7%) and 3 a deletion of TTY2L12A (10%).Twelve of the 81 group B patients (14.8%) had a deletion of TTY2L2A and 13 a deletion

of TTY2L12A (16.1%).Surprisingly, 8 of them had deletions in both studied genes, although they are located very far apart.Our data indicate that TTY2 gene family members may play a significant role in spermatogenesis, and suggest a possible mechanism of non-homologous recombinational events that may cause genomic instability and ultimately lead to male infertility.

P336. Semen cryoconservation in infertile men with AZFc microdeletion opting for ART

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Azoospermia Factor (AZF) loci on the long arm of Y chromosome is critical for germ cell development and differentiation. This region has three loci AZFa, AZFb and AZFc .One hundred and twentyfive infertile males with oligozoospermia and azoospermia were included in this study. Four cases showed AZFc microdeletion on PCR microdeletion analysis and a variation of testicular phenotype. Testicular phenotype varied from hypospermatogenesis to maturation arrest. In one case with hypospermatogenesis there was oligoasthenoteratozoospe rmia (OAT) the other 3 cases were azoospermic. This OAT case showed a decline in semen quality from 1.5 to 0.2 million over a one year period. Thus these cases are counseled to go in for sperm cryoconservation at the earliest age sothat the sperms can be used at a later date for ART. Thus the modifying effect of ones internal and external environment and various genetic factors augment the deleterious effect of AZFc microdeletion resulting in progressive decline in semen quality and hence such cases should be counseled to go in for sperm extraction and preservation at the earliest age.

P337. Cytogenetic analysis of patients with reproductive disorders

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We report here the results of cytogenetic analysis of 73 patients of primary amenorrehoea, spontaneous abortions, and male infertility referred to the Division of Genetics, AIIMS, and New Delhi during October 2001 to November 2002. Cytogenetic analysis was done using standard protocols and karyotyping done using the Image Analyzer. Out of the 55 cases of primary amenorrehoea referred for karyotyping, 45 (81.82%) had normal karyotype (46, XX) and 10 cases (18.18%) were mosaics (45,X/ 46,XX). The age of the patients ranged from 14years to 25years. Most of the patients had scanty pubic and axillary hair, underdeveloped breasts and hypoplastic uterus. Cytogenetic analysis was carried out in 10 cases (5 couples) with spontaneous abortions. All had a normal karyotype (46,XX for females and 46, XY for the males). During the same period 8 cases of male infertility were referred to us for karyotyping. Out of these, 3 cases had hypogonadism, 2 had undescended testes and 3 had suspected Klinefelter syndrome. These patients showed normal karyotype (46, XY) except one with suspected Klinefelter syndrome showed 46, XXY karyotype. Results show that cytogenetic analysis is imperative to establish the genetic etiology in cases with reproductive disorders.

P338. Structural autosomal rearrangements: intrachromosomal interactions affect spectrum of several defects

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The detailed analysis of already known phenotypic information about the patients with structural autosomal imbalance allows to demonstrate that interactions between several genes within the same autosome may significantly affect the phenotype.
Preaxial defects (absent or hypoplastic thumbs or preaxial polydactyly) are relatively common in patients with 4q trisomy, involving 4q31q32. These defects have been described in more than a dozen of patients, both with „pure“ trisomy 4q and in association with imbalance for some other chromosomes. In patients with more distal 4q duplications these defects have not been reported. Most likely, segment 4q31q32 contains one or several genes which, when triplicated, affect development of preaxial structures.
Among several hundreds patients with Wolf-Hirschhorn syndrome, caused by „simple“ 4p deletions, ectrodactyly was described only once (Bamshad et al., 1998).
Association of del 4p and dup 4q (usually due to parental inversion) has been described in at least 12 patients. No significant limb defects were found in 7 of them who had duplications of 4q33-qter (or more distal 4q). At the same time 3 out of 5 with duplication of more proximal segments had ectrodactyly or „bilateral underdevelopment“ of upper limbs (Kobori et al., 1993; Petek et al., 2000; Lemos et al., 2001). Hemizygous absence of distal 4p not only increases frequency of limbs defects in patients with dup 4q31q32, but also shifts the mode of action from the affection of preaxial structures to ectrodactyly or more severe limb underdevelopment.

P339. Two cases with Pentasomy X and 49,XXXXY

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Pentasomy X and 49,XXXXY are rare X chromosome aneuploidies which present with multiple malformations and delayed psychomotor development. We present two cases, a 49,XXXXX girl and a 49,XXXXY boy.
A 6 month old female patient was referred to the genetics clinic with cleft palate and atypical facies. Physical examination revealed telecanthus, epicanthus, broad and depressed nasal bridge, cleft palate, clinodactyly, generalized hypotonia, hypermobile joints and grade 1 systolic ejection murmur. Echocardiography revealed a small perimembranous ventricular septal defect. Cortical atrophy and hydrocephalus were noted on cranial MRI. Chromosome analysis revealed a 49XXXXX karyotype.
The second patient presented with a small penis and motor and mental retardation at age one. Hypertelorism, short and upslanting palpebral fissures, low and depressed nasal bridge, micropenis, fusiform fingers and hypotonicity were noted on physical examination. Cranial MR and echocardiographic findings were normal. A splenic cyst was seen in abdominal ultrasonography. Chromosome analysis revealed a 49,XXXXY karyotype.
The parental origin of the extra X chromosome in each patient was investigated by molecular analysis.

P340. Human meiotic studies by multiplex FISH (M-FISH)

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Meiotic studies have been carried out in testicular biopsies from two infertile men with obstructive azoospermia by multiplex FISH (M-FISH). Testicular biopsies were processed using the technique of Evans et al. (1964). The M-FISH procedure followed the manufacturer‘s instructions (SpectraVysion; Vysis). For each cell, both inverted DAPI counterstain image and M-FISH image were analysed. In the two cases studied, prophase I figures were apparently normal, with a sex vesicle and normal autosomal pairing. Of a total of 101 metaphases originally captured under phase contrast microscopy, 76 were analysable after M-FISH. Among 66 metaphase I cells found, 19.70% were abnormal (84.62% with numerical abnormalities, and 15.38% with structural aberrations). We also detected the presence of univalents for chromosome pairs 18 (1/66), 21 (4/66) and XY (11/66). Out of ten metaphase II cells observed, eight were normal and two were aneuploid. The M-FISH image allows us to identify each chromosome. However, due to the poor meiotic figures quality obtained by M-FISH, the inverse DAPI image is necessary to study chromosome morphology. Meiotic studies in spermatocytes by M-FISH will contribute to understanding the paternal origin of chromosome abnormalities and to detecting meiotic anomalies limited to germ cells.
This work received financial support from the Ministerio de Ciencia y Tecnología (project BFI2002-01193) and the Generalitat de Catalunya (CIRIT, 2001 SGR-00201), Spain. V. Murcia is a recipient of a fellowship (2002FI 00246) from the Generalitat de Catalunya, Spain.

P341. A highly complex chromosomal rearrangement between five chromosomes in a healthy female characterized by 24 color-FISH and multicolor banding (MCB)

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We report on a case of a de novo complex chromosomal rearrangement between 5 chromosomes found in a clinically healthy woman. The only indication for chromosome analysis was a planned ICSI; internal and external genitals, ovaries and hormone status were normal. Conventional cytogenetic analysis showed a rearrangement between chromosomes #3, #4, #7, #9, and #17 whereas karyotype analysis of the parents showed no cytogenetic abnormalities. Application of 24 color-FISH indicated the following aberrations: del(3), der(4)t(17;4;7), der(7)t(3;7), der(9)t(4;9), der(17)t(9;17). To concretize from which chromosomal arm the translocated material was derived, arm-specific probes were used where necessary and revealed that the derivative chromosome 3 had no simple deletion but contained a subtle translocation of material of chromosome 4. In the next step, multicolor banding (MCB) was applied to further characterize the exact breakpoints and to define the orientation of the involved chromosomal material. By this detailed analysis, the karyotype could be established as follows:
.ish 46,XX,der(3)t(3;4)(3pter-->3q22::4q34or35-->4qter), der(4)t(17;4;7)(17pter-->17p12or13::4p14-->4q34or35::7p12or13-->7pter), der(7)t(3;7)(3qter-->3q22::7p12or13-->7qter), der(9)t(4;9)(4pter-->4p14::9q13-->9pter), der(17)t(9;17)(9qter-->9q13::17p12or13-->17qter)
The rearrangement consisted of altogether 6 breaks and seemed to be balanced on molecular-cytogenetic level. Although the MCB-technique allows to determine exactly the breakpoints, submicroscopic deletions or duplications close to the breakpoints cannot be excluded. There are only very few cases reported with such a complex chromosomal rearrangement involving 5 chromosomes without clinical signs. As such cases are detected only by chance the frequency of them in the population still remains unknown.

P342. Studies on a repetitive sequence present in human chromosome 1q21, 1p12 and 1p36.1 lead to new insights into the evolution of chromosome 1 and its homologues in human and 4 ape species

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The phylogeny of human and ape chromosomes is not yet fully established as proven as well by the present study. Here a locus-specific probe (BAC b35B4) together with probes of the MCB1-probeset have been used. b35B4 derives from 1q21 and contains 143 kb of non-repetitive DNA, however, it produces three specific FISH-signals in 1q21, 1p12 and 1p36.1 of Homo sapiens (HSA). According to database search (NCBI) 123kb of b35B4 are present in at least 5 copies in 1q21, leading to a strong FISH-signal there. In 1p36.1 59kb of b35B4 with ~95% sequence homology are present twice in tandem order. Additionally, 21.5 kb with ~90 homology to b35B4 are located once in 1p12. Human chromosome 1 was studied in comparison to its homologues in Hylobates lar (HLA), Gorilla gorilla (GGO), Pan troglodytes (PTR) and Pongo pygmaeus (PPY). The results clearly indicated, that there was an up to present unrecognized pericentric inversion in the evolution between apes and HSA #1. Moreover, a duplication of the sequences homologous to human 1p36.1 could be detected in PTR. Finally, in HLA there is also a homologous region to HSA 1p36.1 and the region homologous to HSA 1q21/1p12 is split onto two different chromosomes. The present ZOO-FISH study using human BAC-probe led to new insight into the evolution of chromosome 1; details not recognized by M-FISH, chromosome bar-code or MCB could be detected. Supported by DFG (PO284/6-1) and INTAS (2143). Ape cell lines were kindly provided by Dr. Rocchi (Italy) and Dr. Hameister (Germany).

P343. Cytogenetic analysis of the frequency of chromosome nondisjunction in oocytes activated by injection of spermatozoa.

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Intracytoplasmic Sperm Injection (ICSI) is very effective procedure for overcoming male infertility. But questions remain about the influence of sperm injection on the spindle apparatus and non-disjunction of oocyte chromosomes. We used heterologous ICSI fertilization between mouse oocytes and human spermatozoa to assess the frequency and types of chromosome aberration. Mouse oocytes were recovered from superovulated hybrid females. Oocytes were activated by intracytoplasmic injection of human spermatozoa. These oocytes developed normally *in vivo* to the first mitosis and were fixed for analysis. In total 277 oocytes were analysed. The overall frequency of chromosomal abnormality was 22.02%. Aneuploidy was observed in 44 oocytes (15.88%), consisting of 4.33% hypohaploidies, 11.55% hyperhaploidies, also 5.4% oocytes were diploid. Structural aberrations observed in 2 oocytes (0.72%): one oocyte with translocation and one with two markers. The frequency of chromosome aberrations in our results is not different from the frequency of chromosome aberrations in mouse oocytes activated by ethanol and unfertilized human oocytes undergoing IVF. This suggests that ICSI does not elevate the frequency of chromosome nondisjunction and structural aberrations in mouse oocytes injected with human sperm.

P344. Investigation of chromosome imbalance in human embryos using Comparative Genomic Hybridization (CGH)

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Studies of cleavage-stage human embryos using fluorescence in situ hybridisation (FISH) for sub-sets of chromosomes have indicated that the incidence of chromosomal abnormalities is high. The use of whole genome amplification (WGA) and comparative genomic hybridisation (CGH) to investigate the full chromosome complement of a small number of human embryos has been reported. We have used WGA and CGH to identify genomic imbalance in individual blastomeres from human embryos and correlated the results with FISH analysis of sister blastomeres using appropriate probe combinations
Thirty-two embryos were analysed using WGA and CGH; 16 (50%) had a normal diploid karyotype, 14 (44%) were abnormal, with a chromosome imbalance in one or more cells and 2 (6%) had a chaotic chromosome complement. In 18 embryos, results were compared with those obtained after FISH on sister blastomeres. In 8 embryos (4 normal and 4 abnormal by CGH), FISH analysis was consistent with CGH results, in 4 embryos FISH results were inconclusive and in 5, FISH was unsuccessful. In only one embryo were the results between the two techniques discrepant; the embryo appeared trisomy 16 by FISH but normal by CGH. No embryos with full aneuploidies, indicating meiotic error, were found.
We conclude that abnormality rates in human embryos may be lower than previous estimates; it remains possible that errors in FISH or CGH may be inflating these estimates. However, our results show that chromosomally abnormal embryos are likely to be the result of cultural artefact or inadequate cell cycle surveillance, rather than meiotic error.

P345. Influence of parental origin on Ag-activity of individual NORs in human chorionic villus cells

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Inheritance of Ag-NOR activity was investigated in direct chromosomal preparations of CVS from 11 fetuses with normal karyotype (8-19 weeks of gestation) and in preparations of their parents' cultivated lymphocytes. FISH-signals (r-DNA probe) and Ag-

NOR-staining spots were evaluated on an arbitrary scale (0-3 a.u.) for each embryonic and parental chromosome. Parental origin of each NOR-bearing chromosome was defined according to heteromorphism of short arms and intensity of FISH signals. 81 out of 110 foetus's NORs (73,6 %) conserved their activity in chorionic villi comparable to these ones in parental lymphocytes (p>0.01, Mann-Witney test). However 29 out of 110 NORs (26,3 %) changed its activity in CVS of fetuses in comparison with parent's (p<0.01, Mann-Witney test): 27 changed NOR activity though FISH-signal was preserved; in 2 cases deletions of NORs in fetuses were found. In 13 cases Ag-NOR activity increased, in 16 decreased. Ag-NOR activity of chromosome 21 changed in 8 cases, of chromosomes 22 - in 6 cases, of chromosome 15 and 13 - in 6 cases for each one, of chromosome 14 - in 3 cases. It may be concluded that activity of some NORs may change in embryogenesis. NORs of chromosomes 13, 14, 21, 22 with changed activity were both of maternal and paternal origin, however NORs of chromosome 15 with changed activity were only paternal-of-origin. Obtained data suggest that Ag-activity of only maternal-of-origin NORs of chromosomes 15 is stably conserved.

P346. Our current experience in FISH diagnosis of marker chromosomes

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The poster deals with our current results in cytogenetic and FISH analysis of marker chromosomes. We present data from 37 patients that were examined in our laboratories using various locus-specific, centromeric and painting probes including the acro p-arm probe specific for the short arms of all acrocentric chromosomes. The major share in our group of patients represent extra structural markers derived from an acrocentric chromosome while the derivative 15 [especially psu dic(15)], 21 and 22 belong to the most common cases. Clinical data, genotype-phenotype correlation and its possible implications for prenatal diagnosis are discussed. Our work was supported by grants IGA 6912-4 (Internal Grant Agency, Ministry of Health, Czech Republic), Charles University Research Project No.111300003 and FRVS 2482/2002 G3 (Fund for the Advancement of Universities).

P347. Celiac disease in children with Down syndrome in Turkey

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The frequency of Celiac disease in patients with Down syndrome is higher compared to the normal population. In literature, the prevalence of Celiac disease is between 2.5% and 12.5% in Down syndrome patients. This is the first study undertaken to estimate the prevalence of Celiac disease in patients with Down syndrome in Turkey and to compare the sensitivity and the specificity of the serological tests used in the diagnosis of Celiac disease. 164 patients (83 female and 81 male) aged between 1-16 years followed as Down syndrome in Genetics Clinic of Cerrahpasa Medical Faculty, Department of Pediatrics were included in the study. All of the patients were screened using antigliadin antibody (AGA) IgG, antigliadin antibody IgA and antiendomysium antibodies (EMA). In 108 patients either antiendomysium or antigliadin antibodies produced positive findings and 51 of these patients underwent a small bowel biopsy. 5 of the patients who were biopsied manifested changes of celiac disease. This gives a frequency of at least 3% of confirmed celiac disease in our Down syndrome patients. EMA had a sensitivity of 80% and specificity of 77%, AGA IgA had 80% of both sensitivity and specificity and AGA IgG had a sensitivity of 100% and specificity of 65%.
In conclusion, we suggest the need for screening of celiac disease in all Down syndrome patients and recommend screening with both EMA and AGA IgA in order to decide which patients should be biopsied.

P348. The Palatine Bone in Prenatal Trisomy 21

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It is well known that the prenatal nasal bone is short in trisomy 21 and also prenatal osseous malformations have been described in the sella turcica region and in the hand.
Objective: The purpose of the present study was to analyse the horizontal part of the palatine bone in palates from human fetuses with trisomy 21. The goal was to improve the craniofacial phenotypic appearance of the genotypic anomaly, trisomy 21.
Methods: Material from 23 trisomy 21 fetuses ranged from 80 mm to 190 mm, corresponding to about 12 to 21 weeks of gestational age. The material was examined histologically.
Results and Conclusions: Histological examination demonstrated four different palatal phenotypes of the horizontal part of the palatine bone: Type I, palatine bone normal appearance; type II, the mesial region of the horizontal part of the palatine bone is lacking; type III, complete absence of the horizontal part of the palatine bone; and type IV, auxiliary bones in the region of the transpalatine suture. The finding shows four different types of malformations in the horizontal part of the palatine bone in human trisomy 21 fetuses.

P349. Celiac Disease in Children with Down Syndrome: The First Prevalence Study from TURKEY

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Introduction. The strong association between Down syndrome (DS) and celiac disease (CD) has been documented earlier. Many studies originating from different centers have reported an increased prevalence of CD in DS ranging from 4 to 17% . Studies from the United States have yielded different results, ranging from 4.8% to 10.3%. This study was performed to be the first prevalence study in Turkish DS population.
Material and Methods. The patients were currently followed in our department. A total number of 100 patients, older than 2 years age were screened using antiendomysium antibodies (EMA Ig A) and quantitative IgA. Routine physical examination and anthropometric studies were done.
Result. Only one patient (1%) was serologically positive for EMA IgA. The patient was a 10 years old boy with anorexia. The family refused intestinal biopsy. Abdominal distention was present in 13 (13%) patients, while anorexia in 9 (9%), vomitting in 7 (7%) and alopecia in 2 (2%) patients.
Discussion. The result of this serological study, yielding 1% EMA-positivity is the lowest yet determined among DS patients. Although not biopsy proven to be CD, recent data on the association between DS and CD show that most patients are determined among EMA-positive patients. Despite the lack of normal population figures we might have a lower incidence of CD among our DS population. However, we believe that asymptommatic screening and repeated screening od symptommatic patients should be considered. The most appropriate test is yet to be determined while EMA antibodies are probably the most convincing.

P350. Study of folic acid pathway genes alteration as maternal cause of Down syndrome

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Chromosome 21 nondisjunction event is responsible for more than 95% of down syndrome. The etiology of nondisjunction has not been very well described however some factors such as folate metabolism is recently considered as an important factor on chromosome nondisjunction event. This study was to evaluate the impact of abnormal folate metabolism induced by folate pathway genes mutation among mother of children with Down syndrome compared to the normal control mother. Common mutations of C677T and A1298C of MTHFR gene are reported to decrease folic acid

level in about 30 to 70 percents in heterozygous and homozygous forms respectively in blood samples. To evaluate the impact of these mutations, the parental origin of Down syndrome of all causes were determined at first place and mothers have been categorized into two groups according to the maternal or paternal origin of chromosome 21 trisomy. This study is unique compared to previous studies, since it determined and subsequently omits Down patients whose extra chromosome was of paternal origin at the first place to increase accuracy of obtaining results. Folic acid gene alterations were studied in 50 Down families and 60 normal controls. Till now our results have shown approximately 4 times higher risk of having Down syndrome child among mothers caring these mutations than normal control mothers or mothers with a paternal origin of Down syndrome in Iranian Down syndrome cases.

P351. A novel intronic point mutation in the Cystathione β-Synthase gene of Down's syndrome

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Cystathionine β-Synthase (CBS) is an important enzyme in the folate pathway which may play a critical role in disjunction during meiosis. The CBS gene is located on the Down's Syndrome Critical Region of chromosome 21. A preliminary study was carried out to detect for the presence of CBS gene mutation(s) in Down's syndrome patients. The study subjects comprised of 9 cases of Down's syndrome, 12 normal subjects that includes a patient's mother, a normal pregnancy woman and a Down's syndrome pregnancy case. Venous blood samples were obtained for genomic DNA isolation. Intronic primers were designed to amplify exons 1, 2, 3 and 8 of the CBS gene by polymerase chain reaction and analysed by denaturing high performance liquid chromatography (DHPLC) and direct sequencing. No mutations were found in any of the exons but a novel intronic point mutation was discovered on the sense strand (5'→3')of intron 1 (9231 A>C) of the CBS gene in all of the Down's syndrome patients, the mother of a patient and also in the normal subject with Down's syndrome pregnancy. Intron 1 is located after position 9151. A putative binding site of the regulatory motif for nuclear factor (NF)-1 element was found at the mutated position by computer assisted analysis. This study offers new information for exploring the mechanisms and the role of intronic mutations in non-disjunction of chromosome 21.

P352. Methylenetetrahydrafolate reductase enzyme polymorphisms as maternal risk for Down syndrome

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Advanced maternal age is the only fully accepted risk factor for trisomy 21, while most children with Down syndrome (DS) are born to younger mothers (<35 years). The relationship between chromosomal nondisjunction leading to aneuploidy and folate metabolism has drawn attention in the recent years. In this study, we examined the two polymorphisms in genes encoding the folate metabolizing enzyme methylenetetrahydrafolate reductase (MTHFR), namely 677C>T and 1298A>C. The prevalence of these variant genotypes in mothers of DS children (case mothers) (n=152) was compared with controls (n=91). Frequencies of MTHFR 677C>T genotypes (CC, CT and TT) and also combination of heterozygous and homozygous variant genotypes (CT or TT) (p = 0.28) demonstrated no difference between the case and control groups. Genotype frequencies of MTHFR 1298A>C (AA, AC, and CC) were similar among the case and control mothers. Variant genotypes of MTHFR 1298A>C (AC or CC) were also insignificant when compared between the two groups. This is yet the largest case control study conducted for MTHFR 677C>T and also the first to investigate a possible relation with MTHFR 1298A>C. The data presented in this study fail to support the relationship between MTHFR 677C>T and 1298 A>C polymorphisms and risk of having a child with DS.

P353. Sister chromatid exchanges in lymphocyte of severe chemical injuries exposed to sulfur mustard during Iran-Iraq conflict

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Sulfur mustard [HD] is a potent alkylating agent with mutagenic properties. It was widely used in the Iran-Iraq conflict.This study assessed the impact of this agent on the frequencies of sister chromatid exchanges (SCE) in the peripheral lymphocytes of severely injured Iranian combatants. Twenty five patients with severe lung and eye injuries and ten control subjects were included in the study. The subjects of control group were healthy volunteers matched for sex and age. The lymphocytes were cultured with conventional culture methods. At the end of the culture period and 48h prior to harvesting 20 µg/ml 5-bromo-2-deoxyuridine was added into the medium. Harvested cells were stained with Hoechst 33258, illuminated, and restained with Giemsa. A total of 25 well-spread metaphases were scored for each sample. We found that SCE frequencies in peripheral lymphocytes of patients were significantly higher than in the controls (p > 0.01). Since elevation of SCEs frequency has been proven in cancer and malignant disorders, studying this factor in severe chemical injuries may give us a good prognosis for evaluating the risk of malignancies in these patients.

P354. Altered Mode of Allelic Replication in Heterozygous Carriers of Ataxia Telangiectasia

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Ataxia telangiectasia (AT) is a rare (~1:4X10^4) and severe autosomal recessive disease, manifested in progressive cerebellar ataxia, ocultancous telangiectases, immunodeficiency, radiosensitivity, and cancer predisposition. There are controversial views regarding the phenotype of ATM heterozygous carriers, whose frequency in the population is questionable and suspected to be quite high (~1:100). Cellular studies claimed that heterozygous ATM carriers, display an increased sensitivity to ionization radiation and exhibit multitudinous modifications in gene expression compared to normal controls. As gene expression appears to be highly associated with DNA replication-timing, a parameter implicated recently with predisposition to cancer, we tested, in primary cell cultures of ATM heterozygous carriers, replication patterns of three genes, HER2 , RB1, and SNRPN . Using the fluorescence in situ hybridization (FISH) replication assay, we showed that all the three tested genes changed markedly their replication mode in cell samples of heterozygous ATM carriers. HER2 and RB1, which normally reveal synchrony in replication of allelic counterparts (as expected from biallelically expressed genes) when present in cells heterozygous for ATM display an asynchronous (allele-specific) mode of allelic replication. Also the imprinted SNRPN gene, which normally exhibits an allele-specific replication mode, (characterizing monoallelically-expressed genes), changes in heterozygous ATM cells its inherent replication mode and manifests loss of asynchrony in allelic replication. To conclude, DNA replication alterations, resembling those found in individuals prone to cancer, were observed in ATM heterozygous carriers .

P355. Cytogenetic follow-up study of complex structural chromosome abnormalities in ataxia-telangiectasia

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Ataxia-telangiectasia is a rare autosomal recessive disorder associated with abnormalities of both humoral and cell-mediated immunity; about one fifth of patients develop malignancies, frequently of lymphoreticular origin but some cases with solid tumors have also been reported. The gene is assigned to chromosome 11q22-23. Peripheral lymphocytes were cultured for 72 hours and conventional cytogenetic analysis with GTG-banding was performed on our investigated patients, aged between 7-14. Examination of G-banded metaphases revealed complex chromosomal abnormalities along evolution, including chromosomes 2, 5, 7, 10, 13 and 16, suggesting a malignant transformation. Although in ataxia-telangiectasia a high incidence of break-points clustered on chromosomes 14q32, 7p13-15 and 7q32-35 have frequently been described, our investigations

revealed other deletions such as: del (2)(pter25.24), del (2)(qter32.2), 13 acrocentric chromosome, deletions of 5q, 7q, 10q and 16p.
We observed that the amplification of structural chromosome abnormalities appeared among evolution, indicating the development of malignancy.

P356. Cytogenetic Evaluation of Egyptian Fanconi Anemia Families

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Fanconi anemia (FA) is a rare autosomal recessive disease characterized by congenital abnormalities, progressive bone marrow failure and cancer susceptibility. The aim of this study was to use of cytogenetic methods in the evaluation of FA patients and carriers. Our study included 50 cases suspected to have FA. According to the criteria of diepoxybutane (DEB) sensitivity 34 cases were classified as FA patients. The main clinical features were pancytopenia 90%, growth retardation 50% and limb anomalies 55%. Positive consanguinity was present in 97% of cases. All the cases showed the pattern of breakage characteristic for FA. In our studied cases 60% had 100% aberrant cells, three cases had less than 70% aberrant cells and probably had two cell lines. Mitomycin C (MMC) when added at the start of the culture proved to be a sensitive method for diagnosis of FA. No correlation was found between the number of congenital abnormalities and the sensitivity to DEB. By the use of DEB 90% of parents (obligate heterozygous carriers) revealed increased chromosomal breakage. Although this figure is statistically significant, there is overlap between the values of carriers and normal controls. Spontaneous breaks occurred at nonrandom sites and involved some oncogene sites, specially at 1q25, 3q21, 6q27, 8q24, 14q24 and 18q22. The diagnosis of FA can be made unequivocally by combining both the clinical data and the cytogenetic evaluation of chromosomal breakage induced by DEB or MMC.

P357. The Italian Registry of Fanconi Anemia: an update

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The clinical variability and the genetic heterogeneity are very high in Fanconi Anemia (FA), complicating the understanding of its pathogenetic mechanism, the research approach, even the diagnostic efficacy. In such a situation is very important to increase the knowledge of the illness phenotype, of its frequency, distribution and natural history. The easier way to get all this is through a specific illness Registry.
The Italian Registry of Fanconi Anemia (RIAF) was established in 1994 at the Genetics Department of the Elena d'Aosta Hospital in Naples. Its aim is to collect data regarding Italian FA patients and their relatives. Since FA is a rare genetic disease, the Registry is expected to benefit patients, improving the knowledge of this illness from the diagnostic, clinical, therapeutical and epidemiological viewpoint, and also supporting the laboratory and clinical research on FA aetiology, pathophysiology and therapy. Moreover, the Department of Genetics provides laboratory diagnosis through cytogenetic and biochemical tests and collects blood and tissue samples from diagnosed patients, their parents and siblings for genetic tests and research.
The Italian Association of FA Families (AIRFA) supports the Registry and the research on this pathology.
In these years, thanks to the families and the physicians collaborating all over Italy, we enrolled 118 patients, aged from 0 to 39 y, 61 males and 57 females; 65 are alive; 49 underwent bone marrow transplantation; 100 showed congenital malformations; 9 developed neoplasia.
The RIAF is participating to European research project.
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P358. Investigation of Clinical Heterogeneity and Chromosome Breakage in Some Iranian Patients referred for Fanconi Anemia

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Fanconi anemia (FA) is a rare autosomal recessive disorder with an extensive genetic and clinical heterogeneity. The lymphocytes and fibroblasts of FA patients show increased sensitivity to alkylating agents such as mitomycin C (MMC), generating increased chromosome breakage. In this study we have investigated 20 patients referred for FA both clinically and cytogenetically. Mitomycin C with 3 different
Concentrations (20,30,40 ng/ml) have been applied to the lymphocytes of the patients and their normal sex-matched controls.Our clinical and cytogenetic findings will be presented and compared with other similar studies.

P359. A new strategy in Fanconi Anemia diagnosis and characterization.

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Fanconi anemia (FA) is a rare genetic disease characterized by congenital anomalies, bone marrow failure and cancer proneness. Death often occurs for the complications of bone marrow failure. Diagnosis is based on hypersensitivity of FA chromosomes to DEB. Molecular diagnosis is time-consuming on unselected patients due to genetic heterogeneity. Five of the seven known FA proteins (FANC-A,C,E,F,G) bind together in a complex that functions upstream of a sixth protein, FANC-D2, which is activated by monoubiquitination. This recent finding has lead to the development of a new diagnostic tool based on the analysis of the FA proteins by means of immunochemical techniques.
This procedure is a precious support to cytogenetic diagnosis for border-line cases. Moreover, subtyping is mandatory for some clinical decisions, prenatal and preimplantation diagnosis and gene therapy. We performed protein study using highly specific antibodies against FANCA, FANCG, and FANCD2 in 10 FA patients diagnosed by DEB-test, 3 transplanted patients, and 10 controls. In 9 patients the FANCA protein was not detectable allowing us to classify them as A-group. In the remaining patient, FANCA, FANCG, and FANCD2 were expressed, suggesting that another gene, BRCA2 or one not cloned yet, is responsible for FA in this patient. As expected, the 3 transplanted patients and the controls showed normal protein pattern. The FA protein study seems suitable as a screening test, reserving the more expensive and time consuming complementation study to patients belonging to very rare groups.
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P360. *In vivo* Oxidative DNA Damage in Fanconi Anemia

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Extensive evidence has related Fanconi's anaemia (FA) phenotype to redox abnormalities. This study was to verify the occurrence of an *in vivo* prooxidant state in FA patients compared to their parents and to healthy donors, by measuring the oxidative DNA damage 8-hydroxy-2'-deoxyguanosine (8-OHdG). A total of 69 FA patients from 11 clinical centres, located in Italy and Turkey, were recruited; 21 of them were transplanted. Moreover, 77 FA heterozygotes (parents) and 100 unrelated controls (46 pediatric and 54 adult) were enrolled. Confirmatory FA diagnosis by diepoxybutane (DEB) test and clinical

overview were homogeneously carried out.
The levels of 8-OHdG were significantly increased in untransplanted FA patients (4.50 ± 2.84 µmol 8-OHdG/mol dG) vs. their parents and controls (2.88 ± 1.23 and 2.55 ± 1.24 µmol 8-OHdG/mol dG, respectively); p< 0.01. The levels of 8-OHdG in transplanted patients (3.62 ± 2.45 µmol 8-OHdG/mol dG) were not significantly different from 8-OHdG levels in untransplanted FA patients. Moreover, a significant excess of 8-OHdG levels was observed in female vs. male FA patients (p = 0.037).
The results showed that a significant excess in oxidative DNA damage is maintained in FA patients after BMT, consistent with the recognized excess cancer risk in transplanted FA patients, and suggesting that reactive oxygen species are generated in FA patients outside hematopoietic tissues. The excess of 8-OHdG levels in female vs. male FA patients may be related to the roles for androgens in modulating oxidative stress.
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P361. Individual sensitivity to micronuclei induced by styrene-7,8-oxide in vitro: role of epoxide hydrolase and glutathione S-transferase genotypes

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Styrene is a monomer of great commercial interest; its polymers and copolymers are used in a wide range of applications. In humans, styrene metabolism involves oxidation by cytochrome P450 monooxygenases to styrene-7,8-oxide (SO), an epoxide thought to be responsible for the genotoxic effects of styrene exposure. SO is detoxified by hydrolysis catalyzed by epoxide hydrolase (EH) or, to a minor extent, by conjugation mediated by glutathione S-tranferases (GSTs). The objective of this study was to investigate if genetic polymorphisms of *EH* (codons 113 and 139), *GSTP1* (codons 105 and 114), *GSTM1* and *GSTT1* modulate SO-induced micronuclei (MN) in humans. We studied lymphocyte cultures from 30 healthy donors treated with 50 and 200 mM SO. When *EH* genotypes were classified into low, medium and high with respect to the expected EH activity an increase was observed in induced MN frequency in EH low activity donors, consistent with the detoxifying activity of this enzyme. In addition, increases in MN frequencies for *GSTP1* *A/*B and *A/*C genotypes were detected with regard to the wild type homozygous *A/*A genotype. As for *GSTM1* and *GSTT1* genotypes, no clear results were obtained, probably due to the minor role that glutathione conjugation plays in styrene metabolism. This in vitro study suggest that polymorphisms in *EH* and, to a lesser extent, in *GSTP1*, may affect MN induction by SO.

P362. Somatic chromosomal mutagenesis in children from contaminated by radionuclides territory of Ukraine in delayed terms after Chernobyl accident

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Significantly elevated conventional and FISH cytogenetic effects have been observed in children from the agricultural regions of the Ukraine that were compulsorily evacuated, and in the model of chronic low intensity radiation exposure, and spontaneous chromosomal mutagenesis parameters have been established. Comparing these data with previous observations of children from the same region (1988-1995) it has been determined that the frequency of unstable chromosome aberrations remained relatively constant, but the frequency of stable chromosome injuries showed accumulation with time. The mean group rate of dicentrics and centric rings found under conventional staining analysis formed only 17 % of the total complete and incomplete translocations revealed by FISH. So, in spite of the long-term radiation exposure, nearly 83% of cells with unstable aberrations had been eliminated. The frequency of stable cytogenetic markers determined by FISH analysis differed significantly from the age standard, confirming the predominance of a radiation origin of chromosome mutagenesis in the observed children. We suppose that the increased level of chromosome aberrations in the exposed group can be induced not only by irradiation but by the modified action of some unknown environmental mutagens. The results obtained confirmed the negative influence of the regional environment upon the observed persons. Irrespective of the cause of the induced genotoxicity they must be regarded as a high risk group.

P363. Frequency unstable chromosomal aberrations in peripheral lymphocytes to victims after accident on the Semipalatinsk nuclear test site

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One of the reliable and widely used methods of medico-genetic testing of environmentally contaminated regions is the frequency of chromosomal aberrations in peripheral blood lymphocytes.
We tested a total of 49 individuals living in the village of Dolon, Sarzhal, a zone of extreme radiation risk, and 25 individuals in a control region; more than 25,000 metaphases were analyzed.
The frequency of aberrant cells was 3.18±0.43, almost 3 times the frequency in the control group 1.15±0.18 (t=5.24, p< 0.001). The frequency of chromosomal aberrations, 2.47 ±0.03, was significantly greater than in the control group, 0.6±0.1 on 100 crates (t=5.1, p<0.05). The increase is caused mainly by pair fragments, 1.3±0.2 compared to 0.2±0.06 in the control group on 100 metaphases (t=5.17, p<0.001). The frequency of dicentrics and rings in the zone of extreme radiation risk was 0.43±0.03, in the controls 0.01±0.05 per 100 cells (p < 0.05). This type of aberration was found in almost 96 % of the persons surveyed. Individual fluctuations were within limits of 0.2 to 3.0 on 100 crates.
Thus,the cytogenetic research carried out on peripheral blood lymphocytes of the population living in a zone of extreme radiation risk has shown the influence of ionizing radiation.

P364. 'The "rogue" cells in workers exposed to densely-ionising (high-LET) radiation'.

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The analysis of "rogue" (multiaberrant) cells was performed in 60 workers of Nuclear-Chemical Plant (NCP) who have various internal Pu-239 burdens (I group – 1,5-11 nCi; II group – 13-20 nCi and III group - more 25 nCi). As control 36 unexposed persons from ecologically clean village were observed. In average 300 cells were analyzed in each individual. Cells with 6 and more chromosome aberrations were considered as the "rogue" cells. At present the spontaneous level of lymphocytes with multiple chromosomal aberrations in blood culture is estimated as 1 per 10000. In our study the "rogue" cells in control and I group of NCP workers were not detected. In II and III groups of NCP workers we found 6 and 7 "rogue" cells from 5786 and 6567 metaphases (0.09±0.08 and 0.10±0.04 percent) respectively. Differences between II and III groups of SCP workers and control individuals were statistically significant (P<0.01). It should be noted, that "rogue" cells contain mainly of chromosome-type breaks. This data offer new indication that incorporated sources of alpha-particles may be involved in the origin of multiaberrant cells.

P365. Consistent gain of long arm chromosome 17 as a primary change in the karyotypic evolution of human embryonic stem cells.

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As part of experimental studies on human stem cells, we have monitored two human embryonic cell lines, H7 and H14, and their sub-lines, for karyotypic changes. A significant difficulty in the study of ES cells, and in realizing their long term potential as sources of normal differentiated cell types for use in transplantation therapies, is their tendency to undergo spontaneous differentiation. Following prolonged culture, we found that some human ES lines appear easier than others to maintain in an undifferentiated state and we have been able to culture sub-lines in the absence of feeder cells, or conditioned medium from such feeders. We found that the lines better adapted

to culture had acquired karyotypic changes, but were surprised to see that a primary change in the two unrelated and different sex cell lines, H7 and H14, involved effective gain of long arm chromosome 17 in both cases, one due to trisomy and the other to a der(6)t(6;17)(q27;q1). Furthermore, secondary changes in one cell line involved additional copies of chromosomes 1 and 12 including the appearance of an i(12p) in some sub-lines.
These changes are characteristic of human embryonal cell carcinomas, gain of 17q being well-documented in neuroblastoma, and i(12p) in testicular germ cell tumour. It seems likely that these chromosomes carry genes that play a key role in proliferation and differentiation. Further work on these cell lines using microarray technology may elucidate the molecular mechanisms responsible and aid in the development of human stem cell lines for therapeutic purposes.

P366. No relation between sperm aneuploidy frequencies of chromosomes 13, 16 and 21, their diploidy rate and the abortion rate after assisted reproduction

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Introduction: The use of reproductive techniques has allowed couples with severe infertility to reproduce. However, approximately 20% of the pregnancies resulted in miscarriages, showing often chromosomal abnormalities, most commonly a trisomy.
A significantly higher frequency of sperm aneuploidy in subfertile men suggest that the patient is at an elevated risk of producing offspring with numerical chromosomal abnormalities. The purpose of our study was to assess the relation between the rate of some specific chromosome abnormalities in sperm in the male partner of couples with recurrent pregnancy loss in an assisted reproduction program.
Material and methods: In this prospective study, 14500 spermatozoa out of 29 sperm samples of the male partners of couples undergoing IVF with two or more first-trimester spontaneous abortions were analysed. As an internal control we analysed 5000 sperms from 10 patients with normozoospermia undergoing successful IVF cycles with at least two deliveries and no abortions. Diploidy and aneuploidy rates were assessed for chromosomes 13, 16 and 21 in decondensed sperm nuclei using three-colour FISH.
Results and discussion: Chromosomal abnormalities (nullisomy, disomy and diploidy) in sperm samples of men from recurrent abortion couples were not significantly increased compared to that from our internal controls (3,24% vs. 2,78%).
In this study we did not observe the clear correlation between the frequency of chromosomal aneuploidy of chromosomes 13, 16 and 21 and the diploidy rate in sperm and the fertilization and/or abortion rates. This rate of chromosomal abnormalities do not appear to compromise the reproduction outcome.

P367. Intercellular distribution of chromatid exchanges caused by different types of chemical mutagens in human lymphocytes culture

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We present an analysis of quantitative laws governing the intercellular distribution of chromatid exchanges. Chemical mutagens with one (mitomycin C) or two (trenimon) active centers were used. Human lymphocytes (G1-stage) were treated with 6 doses of mitomycin C and 4 doses of trenimon at 72 h for 1 hour. To analyze aberrations in first mitosis after mutagen treatment cultures were harvested at 96 h. The frequency of all chromosome aberrations was scored and the intercellular distribution of chromatid exchanges was studied. Three distribution types (Poisson, geometric and negative binomial) were applied to the data. The results obtained for mitomycin C and trenimon both appeared to be in a good agreement with the geometric and negative binomial distributions. According to the mass service theory, a geometric distribution can be interpreted as a composition of a Poisson distribution of primary requirements (origin of lesions) with an exponential distribution of the time of servicing the primary requirements (repairing of primary lesions). And a negative binomial distribution appears to be a composition of Poisson and Erlang distributions. The intercellular distribution of exchange aberrations appears not to depend on the number of active centers of the mutagen. However, it differs from the Poisson distribution observed after exposure to radiation. Nevertheless, comparison of quantitative laws for chromosome mutations caused by different types of mutagenic agents can be useful in investigating and analyzing the process of formation of chromosome aberrations.

P368. Cytogenetic study of chromosome damages in children with chronic thyroiditis following the Chernobyl disaster

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After the Chernobyl disaster the frequency of chronic thyroiditis in chidren from areas of Ukraine that were contaminated by radionuclides increased significantly. To help understand the possible mechanism of the radio-induced transformation of thyroid we performed cytogenetic investigations on three groups of children living in the Rivne region of Ukraine with iodine deficiency in the environment (with chronic thyroiditis, risk of chronic thyroiditis and without thyroid pathology) who were exposed to the acute impact of radioactive iodine in childhood in 1986 and then lived in the territory contaminated by Cs radionuclides. Chromosome aberrations in peripheral blood lymphocytes were studied using G-banding. The mean frequency of chromosome aberrations in the chronic thyroiditis group (0.056 ± 0.008 per cell) and in the risk of chronic thyroiditis group (0.044 ± 0.007 per cell) were significantly elevated above the control group level (0.028 ± 0.006 per cell). Deletions (both terminal and interstitial) and translocations predominated among the chromosome injuries in children with thyroid pathology. The total mean frequency of dicentrics and centric rings were in the normal range in all observed persons. The random distribution of chromosome breaks involved in aberrations has been estimated according to the chromosome length. In the chronic thyroiditis group chromosome bands 1q32, 3q27, 5p15, 6p23, 7p13, 2p12 and 17p13 contained more than one breakpoint. A significant clustering of breakpoints at the telomeres have been found.

P369. The effects of thallium-201 on sister chromatid exchange and chromosomal instability in patients with atypical angina pectoris

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The purpose of the study was to investigate genotoxic effects of thallium-201 (^{201}Tl) that is used in myocardial blood flow imaging. Lymphocytes of 35 patients who received 111 MBq (3mCi) ^{201}Tl. were used in the study. Chromosomal aberrations (CA), sister chromatid exchanges (SCE), mitotic index (MI) and replicative index (RI) were measured before, and 24 and 72 hours after ^{201}Tl administration. In spite of the fact that CA and SCE values before and after 24 h did not change significantly (P>0.05), there were a significant increase 72 h after ^{201}Tl administration (P< 0.001). However, ^{201}Tl decreased MI and RI after 72 h of ^{201}Tl administration (P< 0.001). The results of the study suggest that clinical administration of ^{201}Tl to image for myocardial blood flow may induce genetic damage

P370. The association of SCE frequencies with and without HLA-B27 in ankylosing spondylitis

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The analysis of sister chromatid exchange is a cytogenetic technique used to show DNA damage due to an exchange of DNA fragments between sister chromatids. We investigated if HLA-B27 positive patients with ankylosing spondylitis (AS) were associated with higher SCE frequencies than patients without B27. In this study, lymphocytes from 38 patients with AS (15 females, 23 males) and 34 control subjects. Peripheral lymphocytes were cultured in darkness for 72 hours in BrdU added culture. Metaphase chromosomes were stained with a fluorescence plus Giemsa technique after a standard harvest procedure. The frequency of sister chromatid exchange was found to be increased significantly in patients with AS compared to

controls (p<0.001). Furthermore, the SCE frequencies in patients with positive HLA-B27 was much higher than ones with negative HLA-B27 (p<0.001). Additionally, difference between SCE frequencies has not been found to be significant in the control groups with and without HLA-B27.- The study results displayed strong association between HLA-B27 and frequencies of SCE in patients with AS.

P371. Micronucleus frequency in the acquired middle ear cholesteatoma

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Cholesteatoma of the temporal bone can be defined as keratinizing squamous epithelia growing in the middle ear to the mastoid cavity and surrounding bone. It can destroy the middle ear and mastoid cavity, and cause serious complications by erosion of the temporal bone, such as conductive or sensorineural hearing loss, labyrinthine fistula, facial paralysis, intracranial infections, brain hernia, and cerebrospinal fluid leakage. Cholesteatoma behaves like a low-grade neoplasm because of features such as migration, uncoordinated proliferation and recurrence. The pathogenesis of cholesteatoma has been debated for more than a century. The Micronucleus (MN) test is a biomarker that can show biological effects on the target tissue. The aim of the present study was to determine MN frequency in the acquired cholesteatoma tissue. Eighteen patients were diagnosed to have chronic otitis media with acquired cholesteatoma. They were divided into primary and secondary acquired cholesteatoma according to located lesions. For MN analysis, cholesteatoma tissue and normal tissue specimens of postauricular skin were taken from the patients by surgical operation.
MN frequency was analysed in all cholesteatoma and control samples. MN frequencies in the cholesteatoma and controls were evaluated by using paired t-test in SPSS for Windows. There was a significant difference between cholesteatoma tissue and controls (p<0.05).

P372. Chromosome constitution of persons environmentally exposed to depleted uranium

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During the last war in Bosnia and Herzegovina, radioactive anti-tank ammunition containing depleted uranium was used (1994/95). At the same time the rate of the cases with cancer pathology in Bosnia and Herzegovina is constantly increasing. This causes concerns that depleted uranium can lead to leukaemia or other health problems, known as- „Balkan syndrome“. Although potential threats were reported in 1999, the issue gained attention with the announcement that six Italian and some other soldiers who served in the Balkans died from leukaemia. The United Nations Environment Programme measurements revealed the presence of higher radioactivity and pieces of depleted uranium weapons at several examined sites. One of the places at which depleted uranium has been detected is tank repair facility and ammunition storage in the Hadzici area, close to Sarajevo. In this research, the human lymphocyte genome has been investigated due to environmental exposure to depleted uranium. The study includes individuals who spent the war and post-war period in municipality Hadzici and have been directly exposed to possible side effects of depleted uranium. Chromosome aberrations and micronucleus tests have been performed in order to compare their types and frequencies with those that normally occur in non-exposed populations. Preliminary results will be presented. Supported by Federal Ministry of Education, Science, Culture and Sport.

P373. A rapid diagnosis of Fragile X full mutation by polymerase chain reaction

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Fragile X syndrome, previously known as Martin Bell syndrome is the second most common genetic cause of mental retardation. The gene responsible for Fragile X syndrome, Fragile X mental retardation-1 (FMR1) contains an unstable repeat sequence of (CGG)n. The number of repeats varies in normal individuals as well as individuals with premutations and full mutations. In this study, the molecular diagnosis for Fragile X syndrome was optimised in which the method of Polymerase Chain Reaction (PCR) was utilized. The need for optimisation was due to the difficulties in amplifying the specific region caused by the abnormally high GC amplification at the FMR site. Trials involving the addition of PCRx Enhancer Solution (GIBCO BRL) in the PCR reaction succeeded in amplifying premutations as well as full mutations, instead of using alternative co-solvent such as 75% 7-deaza dGTP or DMSO. This would be the first report of a detection method for Fragile X syndrome using PCR without the additional step of Southern blotting. This technique can be applied in the diagnosis of Fragile X syndrome patients, as it is rapid, accurate and reliable.

P374. Haplotype analysis at the FRAXA locus in some Iranian fragile X syndromes

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Fragile X syndrome, the most common form of inherited mental retardation, is caused by expansion of a (CGG) repeat located in the FMR1 gene. The molecular factors involved in the mutation process from stable (CGG) alleles towards unstable alleles are not well understood. We have analysed the haplotype distribution of closely located microsatellite markers to examine the relationship of specific haplotype association with the expanded repeat region. We analyzed 50 unrelated control subjects and 21 unrelated fragile X patients using 4 microsatellite markers, DXS297, FRAXAC1, FRAXAC2 and RS461 close to FMR1 gene. The preliminary results have indicated the significant relationship between RS461 and FRAXAC1 markers haplotype frequencies between 21 fragile X males compared to 50 normal males. Significant linkage disequilibrium was found between specific marker alleles and the fragile X mutation and normal control in Iranian fragile X patients. Extending the data till the time of presentation will defined better the frequencies of different haplotypes and existence of chromosomes genetic relationship between Iran, Europe and Asia in regard to the tested markers.

P375. Population cytogenetics of Fragile X syndrome from the Indian subcontinent - A review

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Fragile X syndrome, a form of X-linked MR, registered a lot of importance in the last decade, because of its high prevalence among subjects with mental retardation and autism and acurate diagnostic tools available, right from cytogenetic evaluation of Fragile X manifestation (Fra Xq27.3) to PCR based DNA diagnosis and Southern blot analysis of FMR-1 gene. In the present study, we report herein results of 500 cases refered for Fragile X syndrome diagnosis through cytogenetic methods and confirmed by molecular techniques. A detailed report on the population genetic aspects based on the available literature in the Indian population will be reviewed and presented.

P376. Detection of FMR1 mutations in Fragile X syndrome in Iran

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Fragile x syndrome is the most common cause of inherited mental retardation.
The molecular basis of the disease is an expansion of trinucleotide

repeat (CGG) n in the 5‘ untranslated region of the *FMR1* gene. Affected individuals have 200 or more CGG repeats expansion; this is associated with methylation of the promoter region in the gene and correlated with gene inactivation.
We used a two step approach, cytogenetic method and molecular analysis (PCR and Southern blot) to investigate the *FMR1* gene in the suspected individuals and to validate our molecular analysis.
Over last three years we have analyzed 320 individuals who were referred to our center for diagnosis of FMR. After comprehensive genetic consulting, blood was taken from suspected individuals and subjected to analysis.
In the molecular approach we used a non- radioactive protocol, DIG-labeled probe STB12.3 (kindly provided by JL Mandel) for Southern analysis and silver staining for detection of PCR products.
Out of 320 individuals from 222 families with at least one mentally retarded child, 118 cases had a full mutation (FM), 21 had a premutation (PM) and 181 were normal (N) . Prenatal diagnosis was performed for 9 fetuses from these families. 3 normal males, 1 normal female, 3 full mutation males and 2 full mutation females were detected.

P377. The role of FEN1 in fragile X $(CGG)_n$ triplet repeat expansion

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Expansion of a $(CGG)_n$ triplet array within the promoter of the human FMR1 gene results in a length gene silencing, loss of the encoded FMRP protein and subsequent clinical features of the fragile X syndrome. Expansion prone $(CGG)_n$ arrays integrated into *S.cerevisiae* chromosomes exhibit length and orientation instability. These become further destabilised, with a 10-fold increase in the frequency of expansion, in *rad27Δ* cells, suggesting that the encoded FEN1 protein may lie in the expansion pathway. To examine this further, we have now examined the ability of the human FEN1 protein to function in yeast cells and studied its effect upon stability of $(CGG)_n$ arrays. Complementation of the *rad27Δ* mutation with human FEN1 gene suppresses expansion, restoring array stability to wild-type levels. Using FEN1 point mutants, we show that this ability is upon the nuclease activity of the FEN1 protein. In addition, elevated expression of the related yeast endonuclease gene, EXO1, which plays a role in mismatch repair pathways, also suppresses expansion. These results suggest that expansions of $(CGG)_n$ arrays can arise in cells deficient in nuclease activities directed toward branched DNA structures such as those which arise in replication and DNA repair. To analyse the function of FEN1 in human cells, ribozymes directed towards human FEN1 mRNA have been developed and are being examined for their effects upon stability of various expanded triplet arrays. The implications for human triplet expansion diseases will be discussed

P378. Fragile X syndrome: an expansion / contraction mosaic

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Fragile X syndrome is the most common form of inherited mental retardation and is almost exclusively due to an expanded CGG repeat in the first exon of the FMR1 gene. We describe the daughter of a female premutation carrier who, on PCR, showed two CGG repeat alleles within the normal range (28 and 31 repeats) but had 10% fragile sites on cytogenetic analysis. Southern blot analysis identified a methylated full mutation expansion. Haplotype analysis indicated that the daughter had inherited the full mutation expansion and the 31 repeat allele from her mother. This suggested that the expansion occurred pre-fertilisation with a subsequent somatic event early in development reducing the number of repeats on a proportion of the daughter’s X chromosomes carrying the expansion. Haplotype analysis also confirms that a grandson has inherited the contracted allele from his mother which upon sequencing was shown to comprise $(CGG)_8AGG(CGG)_{22}$. The repeat length is within the normal size range and the deletion/contraction does not affect the 5’ or 3’ flanking regions of the repeat. This configuration is likely to be associated with stable transmissions and provides no evidence for the grandson being affected with fragile X syndrome. This case reaffirms that individuals with a family history of fragile X syndrome should be investigated for the possibility of mosaicism.

P379. Lymphocyte expression of FMRP and correlation to clinical severity in three fragile X patients with mosaic pattern of CGG repeats.

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Fragile X syndrome is a common form of inherited mental retardation, affecting 1 in 4000 males and 1 in 8000 females. This was the first triplet repeat disorder discovered, with the CGG repeat polymorphism in the first exon of the *FMR1* gene. Since the discovery of the *FMR1* gene responsible for the fragile X syndrome, molecular diagnosis of fragile X is widely used, specially Southern blot analysis was found to be of great use in determining the mosaic pattern in the number of CGG repeats as well as methylation of CpG island. The mechanism leading to mosaicism of the CGG repeat is still elusive. We report three brothers with mosaic pattern of CGG repeats. In the first patient we report a mosaic pattern of normal, premutation and full mutation alleles. In other two we identified mosaic pattern of premutation and full mutation alleles. The mother of these patients was found to carry a premutation allele. We have done FMRP studies in these patients using antibody test. In the patient with mosaicism for the normal allele we observed expression of FMRP in 12 % of lymphocytes; in other two patients it was 5% and 1%. Clinically the patient with mosaicism for the normal allele was normal with borderline MR. Therefore the expression of FMRP in 12% of cells may be a necessary threshold for normal development.

P380. Preimplantation Diagnosis for Sonic Hedgehog Mutation Causing Familial Holoprosencephaly

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Sonic Hedgehog (SHH) gene mutations were demonstrated to cause the failure of cerebral hemisphere separation into distinct left and right halves, resulting in holoprosencepaly (HPE), which is one of the commonest developmental anomalies of forebrain and midface. Familial cases of HPE show clear autosomal dominant inheritance and a great intrafamilial clinical variability, which makes preimplantation genetic diagnosis (PGD) an attractive option for the at-risk couples. PGD was performed for a couple with two children with HPE, both carrying SHH mutation due to GAG>TAG sequence change, resulting in premature termination of the protein at position 256 (Glu256→stop). PGD cycles were performed using a standard IVF protocol coupled with micromanipulation procedures, involving blastomere biopsy from the eight□cell embryos. Single blastomeres were tested by multiplex nested PCR analysis, which included simultaneous specific mutation and the microsatellite linked DNA maker D7s550 testing. As SHH mutation was not found in either parent, a single sperm testing was performed, allowing identification of gonadal mosaicism for the mutation. Seventeen embryos from two PGD cycles have been tested, resulting in preselection and transfer of 3 mutation free embryos, which yielded a singleton unaffected ongoing pregnancy, confirmed to be mutation free by amniocentesis. This is the first PGD for SHH mutation, which may have practical implications for primary prevention of congenital disorders.

P381. Detection of recurrent trisomy 21 and uniparental disomy 21 in a family by STR analysis

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Objective: A 32-year-old pregnant woman was referred to our genetic counselling because of recurrent trisomy 21 in the family. Karyotype 47,XY+21 of the fetus was found at analysis of amniotic fluid cell culture. Methods: Karyotyping and molecular analysis was undertaken on the fetal and parental samples to determine the origin of the extra chromosome 21. Results: Both parents had normal blood

karyotype. Microsatellite marker analysis showed maternal origin of the fetal extra chromosome 21. As the mother showed homozygosity for all investigated markers on chromosome 21, we also tested her family. We detected the same homozygosity in some family members, which was consistent with isodisomy of the chromosome 21 caused by uniparental disomy (UPD). Conclusion: Here we report on a family, in which multiple aneuploid conceptions occurred with trisomy 21 and molecular analysis showed, that the euploidy of the investigated healthy family members is due to UPD21. This observation stresses the importance of prenatal cytogenetic and molecular analysis in case of parental UPD.

P382. Six years experience in prenatal diagnosis of cystic fibrosis in Yugoslavia

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Cystic fibrosis (CF) is the commonest mendelian disease in Caucasian populations. The molecular basis of CF in Yugoslavia is highly heterogeneous-19 different mutations accounted for almost 74% of identified CF alleles in CF patients. Prenatal diagnosis of CF has been performed in the Mother and Child Health Care Institute since 1996, in collaboration with the Department of Medical Genetics, University of Athens. Parental samples and the CF child were screened for CFTR mutations (prior to prenatal diagnosis) by heteroduplex analysis on PAGE (for the presence of F508del), agarose gel electrophoresis and restriction enzyme digestion for haplotype analysis. Samples negative for F508del were analysed by DGGE of PCR-amplified exons 1-24. Prenatal diagnosis was performed on 49 families, all having at least one CF child. Some of these families applied for prenatal diagnosis several times, so the total number of prenatal analysis performed was 64.
During these analyses, 9 different mutations were detected: F508del, 2907delTT, S466X, 457TAT>G, R75X, 2184insA, G542X, 621+1G>T and R1070Q.We identified 12 affected, 32 carriers and 20 healthy fetuses. DNA extraction was from chorionic villus samples in 45 cases, amniotic fluid cells in 18 cases and in 1 case from a fetal blood sample. Prenatal diagnosis was based on direct detection of the mutation in all cases.
It is important to develop a good diagnostic strategy and offer appropriate genetic counseling to families with a CF child,since it provides rapid,a ccurate and reliable prenatal diagnosis for the majority of couples.

P383. Prenatal Diagnosis of Supernumerary Marker Chromosomes

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Supernumerary marker chromosomes (SMCs)are relatively common in prenatal diagnosis and pose a difficult counseling situration as the clinical outcomes vary greatly. In an attempt to evaluate the pregnancy outcome of the marker chromosomes, we retrospectively reviewed the clinical and cytogenetic data of the prenatally diagnosed SMCs. A total of 37 cases with marker chromosomes were identified from approximately 29,000 cases referred for prenatal cytogenetic diagnosis. Among the 37 cases, twelve SMCs were originated from non-acrocentric chromosomes including chromosomes 4,5,7,8,16,18 and 19. Two cases had one acrocentric and one non-acrocentric SMCs. The majority (23 cases or 62%)were originated from acrocentric chromosomes with 11 of them from chromosome 15. None of the 11 SMCs from chromosome 15 contained the Prader-Willi/Angelman Syndrome critical region, and uniparental disomy was ruled out in 5/5 cases tested. Four pregnancies were terminated with normal or unknown phenotype and three cases were lost to follow-up. Clinical follow-up were obtained in the remaining cases ranged from birth to 20 months of age. Among the cases followed-up, a total of 3 cases (two non-acrocentric SMCs and one case with both a chromosome 17 and an acrocentric markers)resulted in abnormal phenotypes. Fetal abnormalities were detected by prenatal ultrasound examination in two of the three cases. In summary, our results are consistent with the previous reports indicating an overall lower risk for acrocentric SMCs and higher risk for non-acrocentric SMCs. In addition, our study shows that ultrasound examination is helpful in detecting some of the abnormalities associated with SMCs.

P384. Impact of prenatal diagnosis on the live birth prevalence of congenital anomalies

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The objectives of this study were to describe the impact of prenatal diagnosis on the birth prevalence of congenital anomalies during 22 years (1979-2000) in a well defined population.
The material for this study came from analysis of data from multiple sources on births and terminations of pregnancy after prenatal diagnosis of congenital anomalies in 279,917 consecutive pregnancies of known outcome. The study period was divided into 3 parts 1979-1988, 1989-1993 and 1994-2000.
Between 1979 and 1988 and 1993 and 1999 prenatal detection of congenital anomalies increased from 11.7% to 25.5% and to 32.8%. 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 66.0 vs 4.8, 7.3, and 11.2 respectively. The birth prevalence of Down syndrome fell by 80% from 1979-88 to 1994-2000. 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%)
In conclusion 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.

P385. Fetal sex determination with real time PCR of fetal DNA in maternal plasma

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Background: Non-invasive methods using maternal plasma and serum for molecular genetic diagnosis become an important field of interest in prenatal genetic diagnosis. Free fetal DNA in maternal plasma and serum has been shown to be useful for fetal gender determination, and seems to offer a new possibility to perform non-invasive prenatal genetic diagnosis. A possible application is fetal sex determination for couples at risk of X linked diseases. The aim of our study was to control the reliabilty and reproducibility of the real-time PCR amlification of the SRY region. **Methods:** Maternal serum before amniocentesis, and amnionic fluid samples were obtained from 30 pregnant women during the 10th to 15th weeks of gestation. Real-time PCR analysis of the SRY region was performed in order to determine fetal sex. Routine karyotyping of cultured amnionic cells was also performed on the samples. **Results:** We found male fetuses in 14 of 30 pregnancies by cytogenetic analysis. Real-time PCR of maternal plasma has been positive for the SRY region in the same patients. No false positive or false negative cases were found. **Conclusion:** Here we present our preliminary results with the real-time PCR method in the determination of fetal sex using maternal plasma. The real time PCR detection of fetal DNA in maternal plasma seems to be an easy non invasive method to determine the fetal sex at this gestational age. Our preliminary experience is promising in terms of the specificity and sesitivity of the method.

P386. Prenatal diagnosis of t(4;14),(p11;p11) inherited from mother had previous fetus with cranial anomalies.

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The risk for reciprocal translocation carriers of having a child with

abnormal phenotype shows variety considerably depending on the probability of the production of different types of unbalanced gametes and the probability of intrauterine survival of different types of unbalanced progeny. Meiotic segregation products of carriers with balanced translocation are important for assessing the risk of unbalanced forms, breakpoints of inherited balanced translocations and appropriate genetic counseling.
We report a fetus who had reciprocal translocation with 46,XX, t(4;14),(p11;p11) karyotype. The chromosomal abnormalities was initially detected on amniocentesis with GTG banding and was confirmed by Fluorescence In Situ Hybridisation (FISH). Ultrasonographic examination of fetus was normal. Peripheral blood samples were taken from mother and father for karyotyping. The mother had a balanced rearrangement involving the same region of these chromosomes. Their first fetus had had some serious cranial anomalies in ultrasonographic scaning and terminated, unfortunately there were no chromosomal analysis. The meiotic behaviour of structural rearrangement depends on the chromosomes involved in rearrangement, the morphology and length of chromosome fragments involved and on the presence or absence of aggregated heterochromatin and on the localization of breakpoints in the chromosomal bands. In our case it may assume that; submicroscobic chromosome loss, position effect, and breakpoint site mutations may be as plausible hypotheses to explain previous fetus anomalies.

P387. Cytogenetic component of reproductive losses

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Infertility and spontaneous abortions are a major medical-social problem in the conditions of demographic crisis existing in the Ukraine during the last decade. It is claimed that one in eight of all couples with reproductive problems needs cytogenetic analysis. Following an Order of the Ministry of Public Health of Ukraine, registers for the infertile marriages and spontaneous abortions have been set up for assessing the level of reproductive losses and risk factors among in the Kiev region since 1999. The objective of this study was to determine the frequency of chromosome variability in cases of reproductive loss.
Cases of sterile marriages and spontaneous abortions of unknown aetiology (190 couples) among married couples were selected from the registers for research. Cytogenetic features of peripheral blood lymphocytes cultivated according to standard techniques on PBmax medium for 72 hours were studied. G and C-banding were used. At least 29 metaphase plates were analyzed, and 100 when markers or mosaic forms were detected. As result of the study 82 cases of karyotype changes (21.5% of all cases) were revealed, including both chromosomal anomalies and chromosomal variants. The frequency of chromosomal anomalies among couples investigated for reproductive disorders was 3%. Chromosomal variants (polymorphisms) were seen in 18,5%. The changes found were significantly more frequent than population levels mentioned in the literature and could testify to an effect of these cytogenetic features on the reproductive process.

P388. A chromosome 21-derived minute marker in a mosaic trisomy 21 background: implications for risk assessments in marker chromosome cases

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We report a prenatal case of a chromosome 21-derived minute supernumerary marker, found as a mosaic along with a trisomy 21 cell line at amniocentesis. Follow-up analysis of other fetal tissues confirmed the mosaicism and also disclosed a normal cell line. It is likely that the marker reflects a mutation event that resulted in trisomy rescue early in embryonic development. Had the trisomy 21 cell line not been found at amniocentesis, a low risk of an abnormal phenotype (approximately 5%) would have been assigned. We suggest that the risk associated with minute non-euchromatic marker chromosomes should be revised to account for the possibility of mosaicism with potentially aneuploid populations and/or uniparental disomy. The finding of any marker chromosome should prompt a thorough investigation for aneuploid cell lines. In the case of small markers with no euchromatin the given risk of adverse phenotypic effects should not be associated with the marker per se but with the possible presence of a cryptic aneuploid cell line.

P389. Impact of prenatal diagnosis on neural tube defects in northeastern france 1979 - 2001

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The objectives of this study were to examine the total prevalence and the prevalence at birth of neural tube defects (NTD) from 1979 to 2001 in Northeastern France.
Using data from our registry of congenital anomalies all cases of NTD were identified including live-births (LB), stillbirths and termination of pregnancies (TOP) during 3 periods of time : 1979 - 1986, 1987 - 1994 and 1995 - 2001. In our country an ultrasound scan in the mid-trimester of pregnancy is routine part of antenatal care since 1979 as well as a triple test including alpha-fetoprotein since 1997. There is no upper limit for TOP. There was no food fortification with folic acid in the area under investigation and the uptake of periconceptional supplementation was low, around 20% during the third period of the study.
The total prevalence rates of anencephaly, spina bifida, encephalocele and all NTD in the area under investigation were not significantly different during the 3 study periods. However there was a dramatic decrease in the prevalence rates of liveborn with NTD. No anencephalic child was born since 1988 whereas the prevalence rate per 10,000 of LB with spina bifida dropped from 4.6 to 1.5, and 1.1 during the 3 periods of the study, respectively. The decrease of the prevalence rate of LB with encephalocele was lower.
In conclusion this study demonstrate that the impact of prenatal diagnosis on the prevalence of NTD at birth was very high for anencephaly, high for spina bifida and low for encephalocele.

P390. Correlation between pathological ultrasonographic findings and chromosome abnormality

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Ultrasonographic (USG) examination, being a non-invasive technique is routenly used for every pregnant women in order to follow up the pregnancy. The pathological findings are important as they are generally reported to be correlated with chromosomally abnormal fetus.
In this study, 104 women who underwent prenatal diagnostic test with various pathological findings were investigated. The pathological findings were; intrauterine growth retardation (IUGR) (n:16), oligohydramnios (n:13), polyhydramnios (n:10), cystic hygroma (n:4), ambigious genitale (n:3), choroid plexus cyst (n:4), hydrops fetalis (n: 6), corpus callosum agenesis (n:1), short femur length (n:4), neural tube defects (NTD) (n:19), polycystic kidney (n:8), abnormal placenta (n:2) and other abnormalities (n:14).
Among these, 20 fetuses had two of these findings, 15 cases had multipl anomalies and the rest have only one isolated pathological finding.
The ages of the women ranged from 17 to 41. The gestational ages were between
11 to 36 weeks.
Consanguinity was present in 33 (31.7 %) of the couples.
Amniocentesis, cordocentesis and chorion villus biopsy (CVS) was performed to 31, 70, 3 of the patients respectively.
In the obtained karyotypes, 6 cases were found to have chromosomal abnormality, giving a pathological rate of 5.7 %.
There was no correlation between special fetal malformations with spesific chromosomal abnormalities.
It was concluded that, pathological USG results were highly correlated with chromosomally abnormal fetus. For that reason, in cases with abnormal USG findings, fetal karyotyping is important in epidemiologic, cost-benefit, counseling and pregnancy management implications.

P391. The Late Replication of Chromosomes of Human Fetal and Chorionic Villi Cells

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Technique of immunofluorescent localization of BrdU with monoclonal anti-BrdU antibody was applied for the investigation of the pattern of late replicating metaphase chromosomes of chorionic villi cells as well as of tissues fragments from five human embryos at 7 and 12 weeks gestation. 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 and 9. 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 in embryos of 7 week of gestation, while in embryonic cells they replicated earlier than pericentric heterochromatin. However, these segments replicated simultaneously with pericentric heterochromatin of chromosomes 1, 9, 16 in both tissues at 12 weeks of gestation. Thus there are some changes in the replication pattern of heterochromatin regions in chorionic cells in embryos of 7 - 12 weeks of gestation. This may be reflect that changes in the replication pattern of heterochromatin regions probably reflect differences in their functional status in embryonic and extraembryonic tissues at different stages of embryonic development.

P392. Unbalanced (1;17) translocation resulting in 1p36 monosomy in a fetus with intra-uterine growth retardation

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1p36 monosomy is a newly delineated subtelomeric deletion syndrome. It includes constant mental retardation, postnatal growth delay, craniofacial dysmorphy, frequent sensory impairment and inconstant cardiac defect or minor brain anomalies. Its incidence is approximately 1/10,000 but is probably underestimated because this microdeletion is difficult to detect on karyotype and requires FISH techniques. To date, only four cases have been ascertained in the prenatal stage because of sonographic abnormalities.
We report here on a fetus with an isolated intrauterine growth retardation at the third trimester of pregnancy. Karyotypes on fetal blood and amniotic cells demonstrated a de novo 17p12-pter deletion. Subsequent FISH analyses displayed an unbalanced translocation between 1p and 17p resulting in 1p36 monosomy. Parents opted for termination of pregnancy due to poor mental prognosis.
This observation demonstrates that fetal hypotrophy is one of the sonographic features to be considered in this syndrome ; moreover, it emphasizes the importance of FISH analyses in any structural abnormality in order to detect a cryptic rearrangement and enable accurate genetic counselling.

P393. Dilemmas of Pregnant Women Towards Prenatal Diagnosis in a Prenatal Clinical Care from a State Hospital of Rio de Janeiro, Brazil

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In Brazil, where abortion is only allowed in cases of rape and maternal health risk during pregnancy, prenatal diagnosis tests have been adopted in a daily routine basis as part of the prenatal clinical care protocols towards genetic diseases and congenital malformations. We investigated in the past three years (2000-2002) the psychological impact and the cultural meaning of the prenatal diagnosis tests in brazilian women assisted in an outpatient clinic situated in the metropolitan area of Rio de Janeiro city. The aim of our work was to delineate factors and perceptions among pregnant women that were submitted to genetic counselling or genetic tests during their prenatal follow-up, specially their coping strategies towards the risk situation and their dilemmas in all decisions involved during the gestation period. Our results revealed that prenatal diagnosis intervention added to the pregnancy an emotional overload interfering with the women's affective life and its repercussion in theirs reproductive project. In their decisions to medical non-invasive or invasive procedures the emotional, moral and religious factors were more decisive in their decisions than the rational understanding of the medical facts, evidences or the calculated risk factors involved with their main clinical referral. Its necessary to create favorable psychological conditions in pregnant women seeking prenatal diagnosis for their understanding of all informations explored during the prenatal care sessions and to secure that the patient completes all the prenatal process culminating with her return to the genetic counseling appointments if that will be the case.

P394. Prenatal Diagnosis of a Partial Monosomy 7q11®q31 in a Fetus with Split Foot

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Prenatal Diagnosis of a Partial Monosomy 7q11→q31 in a Fetus with Split Foot
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We report the prenatal diagnosis of partial monosomy 7q11→q31 in a fetus with split foot. A 27 year-old woman was referred for genetic counselling at 26 gestational weeks due to sonographic findings of intra-uterin growth retardation, Dandy Walker malformation and lower extremity abnormalities. Chromosome analysis after cardiocentesis showed an abnormal karyotype with a structural abnormality of the long arm of chromosome 7. Both parents' chromosomes were normal; thus, the fetal karyotype designation was 46,XX,del(7)(pter→q11::q31→qter) de novo and skin biopsy was taken to confirm the karyotype result, which was identical. The pregnancy was terminated. Autopsy revealed syndactyly between first and second right toes, hypoplasia of the right third toe. Left split foot malformation, syndactyly between 1st and 2nd toes, and 4th and 5th toes of the left foot, and facial dysmorpic features.

P395. Identification of supernumerary marker chromosomes (SMCs) in prenatal and postnatal human ontogenesis

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Five cases of SMCs (2 prenatal, 3 postnatal) were analyzed. In all cases, the karyotype was investigated using conventional cytogenetic analysis followed by QFH-, RBA- and in some cases Ag-NOR and C-banding. Chromosome painting using FISH with different centromere-specific and chromosome-specific DNA probes in 3 of 5 cases demonstrated that 2 SMCs (1 postnatal, 1 prenatal) were 14/22 of origin, and one SMC (postnatal) was i(18p). Two other cases were analyzed using two alternative methods: forward and reverse painting. For reverse painting microdissected probes, specific to marker chromosomes, were used. For the first case (postnatal) using both methods we demonstrated the marker chromosome to be derived from short arms of chromosome 15. In second case we revealed 46,X,+mar karyotype in chorionic villus cells (CVCs), but 47,XX,+mar in fetal lymphocytes. The forward painting showed that SMC in CVC contained the material of centromeric region of chromosome X. However, this was not confirmed for SMC from fetal lymphocytes. Using microdissection with degenerate nucleotide-primed polimerase chain reaction we constructed four DNA-libraries

specific for the same marker chromosome from fetal lymphocytes. All of them painted pericentromeric region of chromosome 7, including small euchromatic segment of 7q, but only one painted both pericentromeric region of 7 and centromeric of X. This suggests that SMCs from CVCs and from fetal lymphocytes differed in origin. SMC from lymphocytes could be of a compound origin or fetal lymphocytes could be present by two clones of cells with different SMCs. Possible origin and evolution of SMCs are discussed.

P396. Trisomy 2 mosaicism at amniocentesis and birth of a normal child

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We report a case of prenatally detected trisomy 2 mosaicism followed by the birth of a normal male infant.
A primagravida woman presented for amniocentesis at 17 weeks gestation due to an elevated risk of Down syndrome of 1:102 on serum screening.
2/40 amniotic fluid cells were trisomic for chromosome 2 while 38/40 revealed a normal male karyotype. 1 trisomic cell was detected in each of 2 independent cultures, representing level III mosaicism.
A male infant was delivered by caesarian section at 40 weeks gestation. The birth weight was 3900g. Apart from a very small patch of skin with white hair on the left temple the child appeared normal. Samples of blood, placenta and placental membranes were forwarded for cytogenetic analysis. 60, 40 and 44 cells were examined from each sample respectively and no evidence of the trisomic cell line was found.
Subsequent assessment at 16 weeks indicated normal development with age appropriate tone and reflexes.
There are few reported cases of prenatally detected trisomy 2 mosaicism and therefore there is little information to help in the counseling of such cases. Of the 3 previously reported cases of low-level trisomy 2 mosaicism, 2 resulted in still-births while 1 resulted in the birth of a normal child.
Low-level trisomy 2 mosaicism, found at amniocentesis, may be associated with a normal outcome.

P397. STR germline mutations rate is not increased in spontaneously aborted human embryos

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Comparative study of the STR mutation rate in families with early embryonic losses and in healthy families was carried out. DNA was extracted from fetus sack tissues (abortuses) and peripheral blood (liveborn individuals). We used polyacrylamide gel electrophoresis following PCR amplification of 14 tetranucleotide loci for 8 different human autosomes (D2S1242, D9S768, D11S1983, D11S1304, D16S2624, D16S685, D17S1113, D19S601, D19S394, D20S161, D20S168, D21S11, D21S1435 and D21S1413) in 100 families with karyotypically normal spontaneous abortuses and 70 families with healthy children (control group). No evidence of deviation from Hardy-Weinberg equilibrium was observed for all loci studied. Using of many highly polymorphic DNA markers allows to exclude the cases of non-paternity and children in care with great accuracy. Combination of probability of paternity/maternity more than 99,999% with single STR mismatches interpreted as an indication of a germline mutation. Overall 4019 events of allelic transmission from the parents to offspring were investigated. Average rate of STR germline mutations was 4,9 x 10-3 per locus/gamete/generation in families with miscarriages and 5,2 x 10-3 per locus/gamete/generation in families with normal reproduction, without significant differences of mutation rates between samplings ($\chi 2 = 0,04$; $P = 0,85$). This result is contradicts the report of Spandidos et al. (1998) about increased germline mutation rate in spontaneous abortions. Thus, STR mutations in gametes of parents with early embryonic losses and STR mutations in gametes of parents with healthy children appear with the same rate, and are transmitted to the next generation without considerable influence on prenatal ontogenesis.

P398. Antenatal Diagnosis Of Multiple Pterygium Syndrome Associated With Klinefelter's Syndrome

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A non-lethal form of multiple pterygium syndrome (MPS) was diagnosed prenatally at 16 weeks of gestation with an association of Klinefelter's syndrome in the same fetus. Ultrasound findings were remarkable for cystic hgygroma, hypertelorizm, micrognathia, low set ears, flexion contractures of upper and lower extremities, rocker bottom foot. Genetic amniocentesis performed upon those findings revealed a 47, XXY karyotype. After genetic counseling, parents decided to have a therapeutic abortion. We presented this case for the purpose of further describing the early ultrasound findings and clinical features of multiple pterygium syndromes. Also what makes our patient unique was the coincidental presence of Klinefelter syndrome with MPS. To our knowledge, this is the first case in the literature in which a 47, XXY karyotype was found in a fetus with multipl pterygium syndrome. The importance of delineating exact subtype of MPS, and making a precise differential diagnosis becomes critical during the process of evaluation of patients with MPS.

P399. Prenatal genetic diagnosis of fetal RhD antigen by nested- PCR

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The rhesus blood group antigen system is important in transfusion and clinical medicine, being involved in hemolytic disease of the new born, transfusion reactions and autoimmune hemolytic anemia. Despite the widespread use of rhesus immunoglobulin prophylaxis in rhesus D (RhD)-negative mothers, rhesus isoimmunization still occurs. In those cases in which the father is heterozygous for RhD, knowledge of the RhD status of the fetus is important in the clinical management, because no further diagnostic or therapeutic procedures are necessary if the fetus is RhD-negative. RhD antigen can be detected using a sensitive PCR-based assay. It was shown that RhD negative individuals lack the RhD gene. We obtained 5 ml blood samples from thirty RhD positive and negative blood donors as controls and thirty chorionic villus samples (CVS) from pregnant women at 8 to 12 weeks of gestation. DNA was extracted from CVS by a standard phenol-chloroform procedure and nested PCR was carried out with appropriate primers. PCR products were analysed on an agarose gel and by sequencing. With further improvement in diagnostic accuracy, this assay may have implications in the management of RhD-sensitized pregnancies in women whose partners are heterozygous for the RhD gene.

P400. Prenatal and preimplantation Diagnosis of sex using amelogenin gene by nested-PCR

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Sex determination has many applications in identification, criminology and archaeology. But its most important application is to determine fetal sex in X-linked genetic diseases. The aim of this project in clinical aspects is to establish prenatal and preimplantation diagnosis of fetal sex.
We obtained 74 chorionic villus samples (CVS) from pregnant women at 9-12 weeks of gestation, sixteen samples from human embryos which were in different cellular stages (8- 16 cells) as well as

peripheral blood from thirty male and female blood donors as controls. DNA was extracted from all samples by standard boiling methods. Nested PCR specific for a region of the amelogenin gene was used for sex (X/Y) determination. Results suggest that identification of fetal sex in CVS and single cells is posible. The system sensitivity increased up to amplification of a single cell and it was validated by amplification of DNA from fertile oocytes. We randomly followed up ten families and the sex of all ten newborns agreed with our PCR results. In single cell PCR, to prevent amplification of only a single allele or preferential amplification, we increase the initial temperature of denaturation to prevent the occurrence of allele dropout.

P401. Prenatal screening of aneuploidy by interphase FISH

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This study was a prospective clinical trial with fluorescent in situ hybridization (FISH) for prenatal detection of the most common aneuploidies. In the period between March and December 2002 FISH with multicolor, commercially available, specific probes (Vysis) for chromosome 13, 18, 21, X and Y have been routinely performed on uncultured cells of amniotic fluid samples taken from 157 consecutive cases refered for fetal karyotyping. Karyotypes by standard cytogenetic analysis of cultured amniotic fluid cells were compared to the FISH results. Out of 157 cases, the hybridization reaction was informative in 135 (unsuccess in the beginning was explainable by methodological failure). In the 135 samples, one trisomy 21 and one monosomy X were detected and confirmed by karyotyping. There were no false positive or false negative result. Two cases of chromosomal rearrengement, not detectable by FISH, were found by karyotyping. Maternal blood cell contamination, even in macroscopically clear amniotic fluid, was found in seven cases what could be clarified by the analysis of cultured cells. Our conclusion is that rapid FISH analysis in interphase cells is an accurate and sensitive method for prenatal screening of the most common aneuploidies.
However, special attention should be taken to exclude maternal cell contamination. Before interphase FISH might be considered as a screening approach alone, further studies are needed to analyse the cost-benefit relation and the validity of the results.

P402. Chromosome aberrations in congenital heart disease detected prenataly

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Prenatal cytogenetic examinations of 93 fetuses with congenital heart disease (CHD) are presented. The indication for prenatal diagnosis was abnormal result of USG and echocardiography of the fetus at 16–36 weeks of gestation. AVSD and VSD were the most common defects in fetuses with chromosomal aberrations. Extracardiac abnormalities were found in 24 of 29, and in 24 of 64 cases with abnormal and normal karyotype respectively. In 29 fetuses the karyotype was abnormal. Aneuploidy was diagnosed in 23 cases: trisomy 18 in 17, trisomy 21 in 7 cases, trisomy 13 in 1 case. In 5 cases other chromosomal abnormalities were detected: triploidy in 1 case, addition (fragment of chromosome 2) on chromosome 15 in 1 case, der 22 due to balanced maternal translocation in 1 case, and 22q11 microdeletion in 2 cases. FISH for 22q11 del was performed in 19 cases with otherwise normal karyotype. 15 pregnancies were terminated, in 13 fetuses intrauterine fetal death was reported, 26 newborns died perinatally and 19 newborns were alive. In 14 cases the pregnancy outcome was unknown.
Based on USG, echocardiography and cytogenetic results all families had cardiologic and genetic counseling.

P403. An association study of idiopathic generalized epilepsy with single nucleotide polymorphisms in two glutamate transporter genes

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Idiopathic generalized epilepsy (IGE) is a common neurological disorder consisting of a spectrum of several syndromes. Twin and family studies indicate a strong genetic component, demonstrating a complex pattern of inheritance. We are performing association studies to identify polymorphisms which predispose to IGE, using a candidate gene approach. Our current cohort consists of 279 probands (including 133 trios with both parents) and 241 ethnically matched controls collected from Kent and Canterbury Hospital. We are expanding our sample by collection elsewhere.
EAAT1 (Glast) and EAAT2 (Glt-1) are two of the five high affinity sodium-dependant excitatory amino acid transporters responsible for glutamate removal from the synapse and prevention of excitotoxic levels in the extracellular space. Studies have suggested that these transporters may contribute to epileptogenesis: elevated levels of plasma glutamate in children with absence seizures and homozygous mice deficient in EAAT2 exhibiting lethal spontaneous seizures.
In this study we examined the association of variants of EAAT1 and 2 with susceptibility to IGE, using case-control comparison. A single nucleotide polymorphism (SNP) present in intron 7 of EAAT2 showed significant association (p=0.011). Clinical analysis of the sample showed that this effect was spread throughout the sample and not limited to a specific IGE subtype or seizure type. A second SNP (a silent G/A change at Pro201 in exon 5) was not significantly associated (p=0.30), although the two SNPs are in strong linkage disequilibrium (R=0.82, D´=0.84). No association was found for either of 2 EAAT1 SNPs examined: in intron 2 (p=0.09) and intron 5 (p=0.16).

P404. Clinical application of fetal DNA in maternal plasma: Non-invasive prenatal diagnosis of Huntington Disease.

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The prenatal diagnosis of some inherited genetic diseases such as Huntington disease (HD) is very frequently required by the affected families due to the severe prognosis and lack of treatment.We have tested the possibility of performing prenatal diagnosis of HD in early gestation using maternal plasma and semiquantitative fluorescent PCR (QF-PCR).
We studied five cases of pregnant women attending our unit for a prenatal diagnosis, whose husbands were affected by HD. Before a chorion biopsy was performed at 10-14 weeks of gestation we took 10 ml of peripheral blood in EDTA with informed consent. Results from QF-PCR showed two healthy fetuses, two-affected fetuses and in one case we could detect fetal DNA. We confirm our results with the DNA analysis of the chorion villus sample (CVS).
In the present study we have successfully performed four prenatal diagnosis of HD in a non-invasive way. In the fifth case the lack of fetal DNA detection could be due to the low age of pregnancy (10 weeks).
The QF-PCR technique combines superior precision an thousand-fold increased sensitivity. The most important benefit of this technique is its applicability, in the detection of a wide range of dominantly and paternally inherited disease in late first trimester of gestation. The advances is this field would be very important to avoid the risk that invasive technique entail.

P405. A case of prenatal diagnosing partial trisomy 7q and partial monosomy 9p resulting from family carrying reciproct translocation t(7;9)

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A pregnant woman of 26 was referred to the center for an ultrasound screening examination. Her obstetric history showed two previous pregnancies broken by spontaneous abortions at 8-10 weeks. She was considered to be at risk of producing a fetus with congenital or hereditary disease. She and her husband were karyotyped. The husband‘s karyotype was normal; the woman‘s karyotype was 46,XX,t(7;9) (7pter–cen::9 pter; 7qter–cen::9qter).
To exclude a chromosome abnormality in the fetus, the woman had cordocentesis carried out at 21-22 weeks. The fetal karyotype is 46,XX, t(7;9)(7pter–cen::9pter; 7qter–cen::9qter). The USE showed no anomalies, the pregancy developed.
Three years later the woman returned to the Centre at 20-22 weeks of pregnancy because of concern over the future child‘s health. The USE
at 20-22 weeks showed numerous congenital developmental defects: double artery outlet from the widened right ventricle, microphthalmia, ventriculomegalia, nose hypoplasia, tower-formed skull, club hand and foot, peculiar form of fingers and toes, polyhydration, 0.5 cm cervical-occipital fold. Considering the USE results and the mother‘s karyotype, cordocentesis was performed. The fetal karyotype was 46,XY,der 9 (7qter-cen-9qter).
The pregnancy was aborted due to medical reasons. The post-abortion examination confirmed the prenatal diagnosis. Numerous congenital development defects were noted including pseudohermaphroditism.
Conclusion: the case presents interest as combinations of phenotypical signs and inner organs defects of the trisomy 7q syndrome and monosomy 9p are observed.

P406. Prenatal diagnosis and deletion screening of Duchenne musculardystrophy in Iranian families.

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Duchenne muscular dystrophy (DMD) is one of the commonest X-linked genetic disorders seen in children. Mutation in the DMD gene, coding for the dystrophin protein, causes severe muscle wasting disorders. We performed PND for Iranian families with clinically diagnosed DMD using multiplex PCR, RFLPs and also microsatellite polymorphic marker analysis.
During 5 years, we have studied 60 families for deletion screening and linkage analysis. Initially three sets of multiplex PCR were used for screening 20 exons in the dystrophin gene, followed by three intragenic RFLPs (pERT 87-15/ BamHI, pERT87-8/TaqI, pERT 87-15/ XmnI) and two CACA repeats (5’- Dys MSA and 3´ Dys MSA). Deletions were observed in 33 affected boys (55%). The most common deletions were in exons 49 and 50; no deletions were identified in the promoter (Pm) region. In 45 families these three intragenic RFLPs were used and in 30 families one or more of these RFLPs were informative (70%). The most informative RFLP in our population was BamHI (46%) and the least was TaqI (30%). In 30 families the 2 microsatellite repeats were used to identify the mutant alleles and in 12 families 5’-Dys was informative. Prenatal diagnosis was performed for 15 families (10 by CVS and 5 by amniocentesis). 10 fetuses were male, of which 8 were normal and two affected. 5 fetuses were female including one normal and 4 carriers.

P407. Multiplex Polymerase Chain Reaction Assay for Gender Determination from a Single Cell

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Preimplantation Genetic diagnosis (PGD) is a new technique that allows the selection of unaffected embryos of high-risk couples prior to implantation. On of the major indication for PGD or prenatal diagnosis is the risk of X-linked disorders. For the majority of these disorders, the underlying causes and gene mutations are not yet known. Thus gender determination could be useful in couples at risk for X-linked recessive traits.
For gender determination of preimplantation embryos we performed a multiplex polymerase chain reaction assay from a single cell. This assay which co-amplifies X (DXZ1) and Y (DYZ1) specific repeat sequences, yields 308 bp band in females and two bands of 154 and 308 bp in males.
We have studied 16 single cell after biopsy of embryo and compared the results. No allele dropout in single-cell PCR analysis was observed.

P408. Parental origin of the two additional haploid sets of chromosomes in an embryo with tetraploidy.

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We report on the molecular findings performed for an embryo with tetraploidy. Tetraploidy is a rare ploidy abnormality, which is invariably lethal. In the majority of cases spontaneous abortion occurs in the first trimester, although rare cases have been documented of liveborn neonates.
The proband in this study was an embryo spontaneously aborted after 8 weeks of gestation. The karyotype was 92,XXXY. We performed molecular analysis in order to determine the parental origin of the two additional haploid sets of chromosomes. Microsatellite markers mapping to pericentromeric chromosome regions and to more distal regions were used. Our results show a maternal origin of one additional set of chromosomes due to the incorporation of the polar body in meiosis I and a paternal origin of the second additional set of chromosomes due to dispermy.
The karyotype 92,XXXY is rather unusual, indeed the majority of cases with tetraploidy have the karyotypes 92,XXXX and 92,XXYY. To the best of knowledge this is the first case with 92,XXXY, for whom molecular investigations have been performed.

P409. Prenatally diagnosed trisomy 9 mosaicism and paternal uniparental disomy 9 in the child.

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Mosaic trisomy 9 (47,XX,+9[25]/46,XX[34]) was detected in two parallel amniotic fluid cell cultures from a 24-year-old woman because of IUGR and ASD/VSD at 29 wks of pregnancy. Birth was induced 3 weeks before term. The child had slight facial dysmorphology, low set ears and VSD, but no other abnormalities. Two separate lymphocyte cultures and a skin biopsy revealed no trisomy 9 in 120 cells. Molecular analysis of parental and child DNA showed paternal uniparental disomy (UPD) for chromosome 9 in the child. Trisomy rescue seems to have been responsible for the UPD 9. At one year of age she is psychomotorically delayed with growth retardation and hypotonia. As far as we know, this is the first report of paternal UPD 9 detected prenatally with follow up post-term.

P410. Prenatal testing for uniparental disomy in Robertsonian translocation carriers: Risk estimation and diagnostic management

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Uniparental disomy (UPD) is defined by the inheritance of both homologous chromosomes from only one parent resulting in an imbalance of the expression of imprinted genes. While the clinical consequences of UPD of chromosomes 15 are well established (Prader-Willi or Angelman syndrome), the association of UPDs of chromosomes 6, 7 and 14 and a clinical picture is obvious but needs further genotype-phenotype correlation analysis. Acrocentric chromosomes involved in Robertsonian translocations (RT) are

particularly prone to be affected by malsegregation events possibly resulting in UPD. While UPDs of chromosomes 13, 21, and 22 have no clinical consequences and therefore have no diagnostic impact, prenatal testing for UPDs 14 or 15 is demanded more and more often. Based on own data from molecular testing in 30 prenatal RT cases and on findings published in the literature, we delineated a risk of 0.3% for a UPD with clinical consequences for prenatally detected carriers of a non-homologous RT. Prenatal UPD testing is not associated with any additional risk for the pregnancy once invasive prenatal testing has been carried out. It can easily be performed on cytogenetic cell cultures and is therefore recommended to the parents. However, the possibly conflicting consequences in case of a prenatal UPD identification should be discussed in advance. Furthermore, risk figures in specific clinical cohorts such as couples prior to ICSI as well as questions of prenatal diagnostic management will be discussed.

P411. FISH analysis on fetal cells from maternal blood: comparison of our results to those of the NIFTY trial.

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Our Genetics Department, which is integrated in a Hospital, has a Cytogenetic Prenatal Diagnosis Unit. We wanted to assess the real scope of the different techniques developed to perform a non-invasive prenatal diagnosis. We stablished what we thought was the most suitable method to put into practice in a diagnostic laboratory. The protocol to study the fetal cells present in maternal blood consisted in a double density gradient, a positive selection of the cells, a posterior separation in a magnetic field and the posterior identification of the fetal erythroblast and FISH analysis.
The National Institute of Child Health and Human Development Fetal Cell Isolation Study (NIFTY) is a prospective, multicenter clinical project to develop non-invasive methods of prenatal diagnosis. It compiles the data obtained in 9 different academic medical centers, using several study protocols.
We have compared our results with those of the NIFTY trial. We have obtained a higher sensitivity rate in fetal gender assesment as well as in aneuploidy detection with no false positive rate.
The advantages of our protocol are: 1) the blood sample was delivered directly to the laboratory, 2) the use of the MACS technique, 3) the use of direct labeled probes, 4) the whole study was performed by the same person
CONCLUSIONS: In our opinion our results seems to be slightly better due to the protocol used and the characteristics of the laboratory.

P412. The combined use of bi-dimensional, three-dimensional sonography and NMR improvesthe phenotypic definition of rare malformation syndromes.

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Development of three dimensional sonography may improve the assessment of foetal morphology and allow a better phenotypic definition of congenital facial defect and dismorfisms.
We studied two cases referred to our prenatal unit after an initial diagnosis of „abnormal foetal profile“ seen with two-dimensional ultrasound.
Patient 2 was a 33 year old woman, gravida 1, para 0, referred because of a severe foetal micrognatia at 22 weeks. Foetal karyotype 46,XY. With three-dimensional scan we identified several additional dismorphisms besides the marked mandibular hypoplasia : very low and posteriorly rotated ears, bilateral preauricular tags, high nasal root, triangular face, abnormal conformation of cervical vertebra. NMR of CNS showed only a dilated posterior fossa. The foetus was suspected to have a facio-auricolo-vertebral spectrum. The baby was born by spontaneous delivery at the 38th week and he underwent surgical correction of oesophagus stenosis with tracheo-esofageal fistula. Facial dismorphisms had all been correctly predicted ;diagnosis was confirmed.
Patient 1 was a 32 year old woman, gravida 1, para 0 referred at 30week gestation because of „bilateral cysts of lacrimal ducts“. Scan showed orbital hypertelorism, abnormal nose, bilateral cysts in the malar region. NMR confirmed the orbital dislocation due to bilateral cysts under the internal cantii and detected two more cysts in the nasal fossa. Three dimensional sonography demonstrated protrusion of the frontal lobes and bones, orbital hypertelorism and broad nasal root. Macrostomia was present. The diagnosis of Fronto-nasal malformation was suggested. The child was born at the 38 week and the diagnosis was confirmed..

P413. Prenatal chromosomal mosaicism detected by QF-PCR and karyotype analysis.

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QF-PCR assesses relative allele dosage at polymorphic loci. A one tube assay for chromosomes 13, 18 and 21 was used to test 4527 amniotic fluid (AF) samples and 1100 chorionic villi (CVS) samples. One (0.02%) AF and 6 (0.55%) CVS showed aneuploidy mosaicism on “direct” (uncultured material) testing by QF-PCR. The AF result was confirmed by FISH analysis, which indicated 11% mosaicism for a trisomy 21 cell line. Karyotype analysis of cultured material did not detect mosaicism for any of these 7 cases. QF-PCR analysis of cultured material from 5 of these cases was consistent with the karyotype result and in a sixth case, the QF-PCR result still indicated mosaicism.
Karyotype analysis detected mosaicism in 2 (0.04%) AFs where this had not been detected by direct QF-PCR. In the first case, a 15% trisomy 21 cell line and in the second case a 6% normal cell line against a trisomy 21 background had not been detected. These results demonstrate the heterogeneous cell populations present in uncultured prenatal samples; cloned cultured cell populations from CVS are closer to the embryonic cell lineages, and mosaicism detected only by direct QF-PCR is likely to represent confined placental mosaicism. However, the diagnosis of trisomy 21 mosaicism in an uncultured AF sample indicates that QF-PCR is detecting potentially clinically significant imbalance not detected by karyotype analysis of cultured cells. In conclusion, QF-PCR is a sensitive technique for the detection of chromosomal mosaicism; levels of mosaicism as low as 11% were detected.

P414. Autosomal dominant Alport may not be distinguishable from the X-L dominant form: consequences in prenatal diagnosis.

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We present here a case of a 31 year-old woman affected with Alport syndrome (ATS), who asked for prenatal diagnosis. She had microhematuria, without proteinuria, since age 13. Her blood pressure and renal function was normal. Her father was transplanted at the age of 52 years. Linkage with COL4A5 markers showed that the male fetus received the grandfather’s haplotype. On the hypothesis of an X-linked transmission, this finding predicted an affected fetus. The mother gave birth to a male baby who at 9 months showed microhematuria. The hypothesis of an autosomal dominant form of ATS was also indicated. After birth of the baby, direct mutation analysis of COL4A4 resulted in the identification of a mutation (p.G957R), that was present in the proband, her father and the newborn baby. Extension of segregation analysis to a paternal uncle with microhematuria and slightly impaired renal function by the age of 59 and to a paternal healthy aunt demonstrated that the mutation was present in the first and absent in the latter. These results demonstrated that an autosomal dominant instead of an X-linked form of ATS segregates in this family .Our results confirm that autosomal dominant forms of ATS are less rare than thought and that prenatal diagnosis in ATS by linkage is unadvisable, because large clinical and genetic heterogeneity makes inheritance unpredictable in small families. This conclusion is critical because about 20-50%

of interviewed people found abortion acceptable in cases of an ATS affected fetus.

P415. First-trimester pregnancy scanning as a screening tool for high-risk and abnormal pregnancies in Kazan, Tatarstan

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This study set out to evaluate the feasibility and acceptability of routine early ultrasound (12- 14 weeks) within a city for identifying high-risk and abnormal pregnancies.The study involved 9852 women who presented clinically pregnant before 12 weeks' gestation between Jan 2000 and Dec 2003. Fetal nuchal translucency thickness (NT) and maternal age was performed for this program. 152 pregnancies (1.5%) were diagnosed as abnormal or having high risk of abnormality at the early scan. For 142 of them were performed prenatal karyotyping by chorion villi samples. Any kind of chromosomal abnormality was found in 23 (16.2%)cases.

P416. Maternal isodisomy 22 in a fetus with malformations

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We report on a fetus with malformations and maternal isodisomy 22. Karyotyping of chorionic villi was performed in the 11 + 4 week of gestation due to nuchal translucency of 2.4 mm (adjusted risk for trisomy 21 – 1:5 and for Trisomy 13/18 - 1:8) and maternal age (45 years). Chromosome analysis from placental tissue revealed non mosaic trisomy 22 while subsequent amniocentesis at 14 + 1 week of gestation showed a normal 46,XX female karyotype. Microsatellite analysis using DNA from cultured amniocytes and the parents lymphocytes showed maternal isodisomy for chromosome 22. Detailed ultrasound examination in the 19 +1 week of gestation revealed a distinct subcutaneous oedema (5 mm) at the back of the head in terms of a hygroma colli which was visible from the 11 + 1 gestational week on. Fetal measurements were accordant to gestational week. After detailed genetic counselling the parents elected for termination of pregnancy, which was performed at 19 + 5 weeks of gestation. Pathological examination of the fetus showed a distinct hygroma colli and deep set back rotated ears. Microsatellite analysis from a skin biopsy from the fetus confirmed maternal isodisomy 22. Uniparental disomy 22 is a rare condition. Normal phenotypes in previous reports have suggested that maternal UPD 22 has no impact on the phenotype. Mechanisms of development of maternal isodisomy 22 and its possible impact on the phenotype in our case are discussed.

P417. Cytogenetic and molecular analysis of a father's triple translocation and a fetal balanced double translocation.Case report.

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A childless couple was reffered for cytogenetic examination because of their reproductive problems; after nine years period of sterility and two spontaneous abortions without recognizable gynecological causes, we assumed a possible chromosomal anomalies,as the etiological factor of recurrent pregnancy loss.
We performed cytogenetic analysis of both partners using convencional banding techniques.The wife had a normal karyotype 46, XX; but the husband was found to be a carrier of a balanced triple translocation: 46, XY, t (8;16;17) [(8;16) (q 22 - p ter) (8;17) (p12-q ter)].
In their third pregnancy we performed amniocentesis in the sixteenth week of gestation.The routine ultrasonografic examination of the fetus was normal.Cytogenetic analysis of amniotic fluid was showing karyotype: 46, XX, t (8;16) (q 22 - p ter).
The results from the comparative genomic hybridization (CHG) analysis showed balanced kariotype of the fetus. However, we knew that the limitations of the method were 10 Mb and that the microdeletions could not be excluded.By 24 - color FISH we confirmed cytogenetic finding both in the father and the baby.
The explanation of this cytogenetic finding is probably a mosaic of the father's constitutive karyotype or a gonadal mosaicism: 46, XY, t (8;16) (q 22 - p ter)/ 46, XY, t (8;16;17) [(8;16) (q 22 - p ter) (8;17) (p12-q ter)].
However, other similar reports would be necessary to support this hypothesis.Aspects of genetic counselling will be discussed.

P418. Specific preimplantation genetic diagnosis for Duchenne muscular dystrophy

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Specific preimplantation genetic diagnosis for Duchenne muscular dystrophy
Girardet A, Hamamah S, Anahory T, Déchaud H, Coubes C, Hédon B, Demaille J, Claustres M
Duchenne muscular dystrophy (DMD) is a common X-linked recessive lethal disease with a worldwide incidence of 1 in 3 500 male births. Preimplantation genetic diagnosis (PGD) of DMD is often based upon gender determination followed by the transfer *in utero* of only female embryos, thereby avoiding the birth of potentially affected males. We here report a single cell polymerase chain reaction assay, allowing the multiplex amplification of four exons of the dystrophin gene together with ZFX and ZFY genes for gender assignment. In order to test the reliability and the accuracy of this 5-plex protocol, we performed preliminary experiments on more than 200 single lymphocytes isolated from male and female individuals. As a result, ~87% of the single cells amplified for the five sequences, and sex diagnosis was obtained in about 98% of the cells. A couple underwent a PGD treatment cycle, as the woman was heterozygous for a 14 exons-deletion in the dystrophin gene. Two exons located in the deleted region were analysed, together with two exons non-involved in the deletion, and ZFX/ZFY genes that allowed sex assignment. PGD has been successfully applied and the couple proceeded to embryo transfer, but unfortunately, no pregnancy ensued. The obvious advantage of this protocol is to transfer to the mother's uterus unaffected male embryos in addition to females embryos.

P419. Pecularities of biochemical markers fluctuation in 130 Down syndrome cases

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Pecularities of biochemical markers fluctuation in 130 Down syndrome cases.
Kascheeva T., Vakharlovskiy V., Baranov V. , Vokhmyanina N.
Ott's Inst. Obst. and Gynec., City Medical Genetic center, Saint - Petersburg, Russia.
Biochemical screening of pregnant women in St.Petersburg was performed since 1989, initially as a single serum marker (AFP) and since 1993 as a double test (AFP & HCG). The report summarize the data of biochemical tests carried out during 1993-2002 years in Saint-Petersburg. Over 150000 pregnancies were tested. Proportion of population covered in 2000-2001 was more than 84 %. Homemade software was used for risk calculation. Altogether 130 cases of DS were revealed during this period. 97,2 % cases fetus with DS were detected in women 39 ages and over, 85,7 % - from 35 to 38, and 50,7 % - in women younger than 35. Average detection rate was 71,2 %. Average AFP in pregnancies with tr21 was 0,78 and HCG was 2,78 MoM. False positive rate was 6,7 %. Serum markers for Down's syndrome in women after in vitro fertilisation were quite differed from this one in common pregnancies. AFP and hCG values in Down syndrome screening after IVF should be adjusted to avoid high screen positive rate. Pilot study of maternal serum markers (HCG, PAPP-A) at the 1st trimester of pregnancy is ongoing .

P420. Prenatal Diagnosis of an X-linked retinoschisis

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X-linked juvenile Retinoschisis (XLRS)is a progressive inherited retinopathy consisting of intraretinal splitting due to degeneration of the retina.
The gene associate with this disease (XLRS1)is localised on Xq22.2-22.1.The 6 exons identified in this gene encode a protein of 224 aa which it is only expressed in retina and contains a highly conserved motif implicated in cell-cell interaction and possibly active in cell-cell adhesion processes.
We have performed a molecular prenatal analysis of XLRS in a chorion biopsy. We already know the status of the grandmother, mother and brothers. They have the Arg 213 Gln mutation in the XLRS-1 gene.
Molecular analysis was carried out by different techniques:
• Indirect studies
Haplotype analysis using 3 short tandem repeats markers (DXS9911, DXS999, DXS989).
• Direct studies
Automatic sequencing of exon 6 of the XLRS-1 gene.
Indirect studies showed a female foetus, and the X-chromosome inherited from the mother was different from the one inherited by the affected brothers. These results were confirmed by automatic sequencing, in which the absence of the mutation was observed.
Genetic studies of macular dystrophies are very important in order to determinate the status of carrier female and affect male, since there is not any treatment for this kind of degenerative diseases.

P421. Hyperhomocysteinaemia and abruptio placentae: molecular genetic analysis of 100 consecutive patients at Tygerberg Hospital, South Africa

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Introduction
Abruptio placentae, the premature separation of the placenta from the uterine wall, is a devastating pregnancy complication resulting in severe maternal and fetal morbidity or mortality. Maternal hyperhomocysteinaemia (modulated by folate status, vitamin B6 and B12 concentrations and genetic mutations) is a risk factor for abruptio placentae. Hyperhomocysteinaemia is also associated with the hypertensive pregnancy condition pre-eclampsia. The purpose of this study was to investigate homocysteine levels, genetic polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) gene and haematological parameters in 100 consecutive patients with abruptio placentae and to correlate this with clinical outcome.
Methods
100 consecutive patients with abruptio placentae and 100 matched controls were recruited after delivery. Fasting homocysteine, genetic status of the C677T and A1298C polymorphisms in the MTHFR gene and full blood count at delivery were determined.
Results
30% of patients with abruptio had raised homocysteine levels (above 11mmol/l) versus 11% of the control group (OR 2.36, 95% CI 1.05-5.35). The mean homocysteine level in the abruptio group (8.6mmol/l) was significantly higher than the control group (4.8mmol/l), p<0.001. The mean homocysteine level was significantly higher in study patients where the pregnancy was complicated by pre-eclampsia as well (9.6mmol/l vs 6.3mmol/l; p<0.001). The maternal and fetal allele frequency (respectively) of the mutant T allele of the C677T polymorphism was not different between study and control groups (0.11 and 0.17 vs. 0.08 and 018).
Conclusion
Abruptio placentae is only associated with hyperhomocysteinaemia when there is concurrent pre-eclampsia and is not associated with known variations in the MTHFR gene.

P422. Prenatal Diagnosis Of Cystic Fibrosis:the Experience Of The Milan Centre

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Over the last ten years prenatal diagnosis (PND) of Cystic fibrosis (CF) using DNA based methods has moved from linkage analysis to direct mutations detection.
Prenatal diagnosis of CF started in our Centre in 1987.
Molecular analysis performed in 624 CF patients followed at the regional CF Centre of Lombardia showed a great heterogeneity of CF molecular defects. In 83% of chromosomes 67 mutations were detected. In the remaining 17% of CF chromosomes the mutation was not detected and prenatal diagnosis could be performed using linkage analysis by intragenic microsatellite markers.
To date 199 PND in family at risk 1:4 of CF has been performed using increasingly specific techniques to study foetal DNA extracted from chorionic villi. Since 1992 the genetic study protocol, which previously covered only the molecular segregation of the gene responsible for the pathology in question, has been expanded to forensicmarkers.
Among 199 PND: 45 were affected, 127 heterozygous and 27 normal.
In 2 cases of affected foetus the parents decided to continue the pregnancy.
Among the 199 PND performed in 182 cases the couples were already parents of an affected child, in 9 couples one subject was carrier already known (family history) and the second was identified as carrier during to DNA analysis at our Centre, 6 cases had as a positive marker for hyperechogenic bowel, 2 couples were identified at risk tank to neonatal screening program and in the last period two cases of PND were carried to check the result of preimplantation genetic diagnosis.

P423. Unexpectd result in an abnormal fetus with Cystic Hygroma

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An ultrasound scan at 17 weeks of gestation (fl 23mm) revealed an abnormal fetus with several ecographic anomalies: cystic hygroma, anasarca, anydraminos, ascytis, pleural effusion, no bladder was found and the umbilical cord had two vessels.
It‘s a young, health, non-consanguineous couple, with a normal obstetric and familial history (the first pregnancy resulted in a normal male).
At the prenatal counselling, the couple understood the situation and elected termination of pregnancy and once there was no amniotic liquid, we opted to do the punch in the cystic hygroma for cytogenetics study of the fetus.
After the termination of the pregnancy, blood, skin and placenta, were taken to cytogenetics studies. Unfortunately it was not possible to achieve a result with skin and placenta. The blood and cystic hygroma karyotypes were 45,X and 46,XX/ 47,XX,+20 (10-90%) respectively.
Whenever we have hygromas we attempt to make comparative studies between blood and cystic hygroma`s results. This case was the first that we found a difference between them.

P424. Changes in indications and methods of prenatal diagnosis of chromosomal aberations in last 5 years.

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Prenatal diagnostic cases account for more than 1/3 of all cases at our department of clinical genetics. The respective numbers of diagnosed cases of chromosomal aberations during last 5 years (1998-2002) follow:
trisomy 21: 21
trisomy 13: 6
trisomy 18: 6
X0 and mosaics: 7
XXX and mosaics: 3
XXYand mosaics: 6

XYYand mosaics: 4
unbalanced translocations: 2
balanced translocations: 8
marker chromosome: 4
others: 19
The total number of patients investigated during these 5 years was 3125.
Several trends can be observed in our prenatal diagnostic routine within the last years:
- wider range of indications for prenatal diagnosis (with differing frequencies of various chromosomal disorders / predictive values of the various indication schemes)
- increasing number of pregnant women entering the investigation because of their age (reproduction at older age)
- wider range of diagnostic procedures offered (and a shift towards less invasive methods).
Evaluation of prenatal diagnostic cases has been supported by VZ FNM 64203.

P425. "Cytotrainer" a interactive simulator of the cytogenetic analysis process for educational use

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At the medical faculty of Graz University a new Curriculum has been developed for the studies of human medicine. The general objective of the Virtual Medical Campus Graz is the realization of an Information-system to make the new curriculum digitally accessible. In the field of cytogenetics our aim was the development of a interactive Learning Object . Cytogenetic analysis is an essential part of diagnostics in dysmorphology, syndromology, prenatal and developmental diagnosis, reproductive medicine, neuropediatrics, hematology, and oncology. Currently cytogenetic analysis is represented in the curriculum in a practical exercises unit. We use photographic material of microscope G-banded metaphases. Each student is given a photograph of a normal as well as a pathological metaphase and has to cut out the chromosomes manually to rearrange them in the form of a karyotype In routine analysis this manual procedure has been replaced by computer based analysis tools. These analysis tools have been designed to fulfil the complex requirements of routine analysis and are therefore not practical for educational use. Thus the idea emerged, to develop an Internet enabled simulation tool in the form of a Learning Object, which is reduced to the parts of didactic functionality. In the new curriculum we will subsequently retain the manual procedure for the normal karyotype as an introduction to the topic, but for the pathological cases we utilize a web based simulator of a cytogenetic analysis tool. The developed simulator called „cytotrainer" provides both a full featured visual and functional reconstruction of a cytogenetic analysis process.

P426. BRCAPRO is useful in Spanish breast cancer families

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Accurate risk assessment in breast (BC) and ovarian cancer (OC) is important because genetic testing has important health and cost consequences. BRCAPRO is a Bayesian model, based on US family data, that predicts the probability of detecting a **BRCA** gene mutation. ***Aim****:* To measure the performance of the BRCAPRO model in a Spanish Genetic Counselling Unit. ***Methods*****:** Estimated *BRCA* genes mutation probabilities for 144 Spanish pedigrees were calculated by the BRCAPRO model. Inclusion criteria: (**I**) three or more first-degree relatives with BC or OC; (**II**) Early onset of BC (< 35 yr); early onset of bilateral BC (< 40 yr), or BC and OC in the same patient, all of them in the absence of family history; and (**III**) Two first-degree relatives with BC or OC plus any of the following: onset before 50 yr, both cases of OC, or BC in a male. *BRCA1/2* genes were fully sequenced. Areas under receiver operator characteristics (ROC) curves were calculated. Sensitivity, specificity, positive and negative predictive values were all calculated based on a greater-than-10% probability.
Results**:**

	Criteria I (n=70)	Criteria II (n=33)	Criteria III (n=41)	Criteria III without OC (n=28)	All families (n=144)	Families with BC only (n=111)
Area under the ROC curve	0.751	0.937	0.579	0.792	0.747	0.818
Sensitivity	0.86	0.75	0.55	0.83	0.75	0.86
Specificity	0.53	0.82	0.5	0.41	0.6	0.59
Positive predictive Value	0.44	0.37	0.28	0.28	0.39	0.33
Negative Predictive Value	0.9	0.96	0.75	0.9	0.88	0.95

BRCAPRO performed very well in patients fulfilling criteria **II** (high risk individuals without family history) and adequately in patients fulfilling criteria **I** (strong familial aggregation). However, BRCAPRO is not sensitive when moderate familial aggregation is present (criteria III). Exclusion of OC results in a significantly improved performance.
Conclusions**:** The BRCAPRO computer model is a useful counselling tool for determining BRCA gene mutation probabilities in our setting provided that OC is excluded from moderate familial aggregation cases.

P427. Knowledge of genetics relevant for daily practice among nearly graduated MDs

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Objective To investigate knowledge of genetics relevant for daily practice among nearly graduated medical doctors (MDs) in the Netherlands.
Methods A genetic computer exam was designed by Clinical Geneticists (CGs) and Educational Specialists consisting of 215 questions divided over 194 case-histories and divided into three categories: a) necessary, b) desirable and c) useful for the daily practice. By assigning one point for each correct answer a genetic exam score was calculated, expressed in the percentage correctly answered questions. Three sub-scores were created for the above mentioned categories (a-c). After a validation study among 11 Non-Medical students, 11 nearly graduated MDs, and 10 CGs, showing ascending scores as expected from these groups, this exam was completed by a study sample of 291 nearly graduated MDs from 7 different Medical Schools.
Results The study sample of MDs scored 62% (SD 6.9; 95% CI 61-63%) correct on the total exam score. In the necessary knowledge category, 93% of the MDs scored at least 60% correctly. Only 15% of the MDs scored over 80% correctly and none of them scored more than 90% correctly in this category. Almost a quarter answered 60% of the desirable knowledge questions correctly. Moreover, half of the students scored less than 44% correctly in the useful genetic knowledge category.
Conclusions The results suggest that nearly graduated MDs lack genetic knowledge necessary for daily practice. It is desirable that the genetic knowledge of MDs is upgraded in their postgraduate training. Future MDs' training programmes should focus more on this topic.

P428. A practical course in counselling skills for professionals in genetic counselling

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Psychological support of clients is an integral component of genetic counselling. The ability to support a client safely and non-judgementally is a learnt skill, rather than a natural attribute. The need for training in counselling skills is acknowledged by professional groups representing genetic counsellors in many countries. However, counselling skills content in courses (such as the Master's degree in genetic counselling) varies, and practitioners who enter genetic

counselling from other professional backgrounds (e.g. nursing or medicine) may not have had significant training in practical counselling skills. To address this gap in education, a dedicated course in counselling skills for genetic counsellors was developed in the UK under the auspices of the AGNC. This course draws on generic counselling and psychotherapeutic models and theories, but all practice work and professional issues relate to clinical genetics. There are 4 main course components: 1) development of practical counselling skills achieved through practice in a safe environment 2) development of personal awareness in the counsellor (vital for effective counselling) 3) discussion of relevant counselling theories appropriate for use in the genetic setting and 4) enhancement of professional competence and ethical practice through case discussion.
The certificate course consists of 160 learning hours delivered in 4 residential blocks, to aid access by students from throughout the UK. Feedback from the first 2 student cohorts indicates this is a highly relevant course that enhances clinical practice. Students regard the opportunity to develop skills and practice scenarios specifically related to genetic counselling as valuable.

P429. Intentions to undergo predictive genetic testing for dementia

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Molecular genetics provides diagnostic tests for familial cases of dementias with autosomal dominant inheritance and predictive genetic tests for risk factors. Genetic testing might allow more accurate diagnostic procedures and provide the opportunity to develop adequate plans for later life and, eventually, to take advantage of innovative therapeutic interventions. However, providing genetic diagnoses to a healthy individual who could be at risk for dementia raises ethical concerns.
In our study, we have been looking for the presence of potential predictors of test intentions, in order to establish a more accurate approach and future guidelines for genetic counselling in dementias. Our sample includes 134 subjects (57% women) recruited in families with 2 or more demented patients; their mean age is 47.5 ± 11.1 years and mean education is 11.3 ± 4.2 years. Seventy-five percent are married and 84% employed; 72% have children and 87% are a first-degree relative. The assessment deals with the following domain areas: psychological, social, socio-demographic, pathological, dementia attitudes, beliefs and experiences.
We investigated the selection variables for the prediction of test intentions through a multinomial logistic regression analysis, which allowed us to isolate four variables; the model predicts category membership with 52% accuracy. The predictors are two specific beliefs about the cause of dementia (concerning the relative importance of brain functioning and of taking preventive medications), decisional balance (difference between benefits and risks) and a dummy variable contrasting the status of housewife with any other profession.

P430. The Role of Genetics Professionals in Public Debate – results of a survey of British Society of Human Genetics members

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The public debate on genetics and its social and ethical implications will accelerate in the post-Human Genome Project era, when new developments may have far reaching repercussions.
The British Society of Human Genetics (BSHG) is committed to contributing to the public debate. With the Wellcome Trust, we conducted an electronic survey of BSHG members, asking them 23 questions concerning their attitude to and experience of engagement with the lay public.
The response rates of members from the different constituent groups of the BSHG varied considerably: with clinical geneticists, nurse/counsellors and molecular geneticists nearly twice as likely as cytogeneticists to respond.
97% respondents agreed that the public needs to know about the wider implications of scientific research.
95% respondents thought that genetics professionals have a duty to communicate their work to the public, although, only 63% would like to spend more time on this. The remaining 37% include those individuals who already do much work with the lay public.
173 (68%) respondents considered they were well equipped to communicate the scientific facts of genetics to the public while 133 (52%) felt they were equipped to communicate the social and ethical implications.
Only 45 (18%) respondents had received any training in communicating with the public.153 (60%) respondents thought genetics professionals should obtain assistance from professional communicators when communicating with the public.
In summary, there is support amongst genetics professionals for public engagement. The data gained from this exercise will be used to inform future initiatives in the BSHG.

P431. Challenges to Genetics in Brazilian Nursing Education: students and professors' perspectives

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In Brazil, only one nurse is PhD in genetics and nursing professionals and students have insufficient information about their real role in genetics field. Knowledge continues to expand but nursing curricula programs have continued essentially the same. Not every nurse needs to be an expert in genetics, but independently of the specialty, this professional have a role in delivering genetic health care and managing genetic information. The purpose of this study was to evaluate, by a questionnaire, beliefs about genetics, nursing and the actual curriculum in a group of undergraduate nursing students (66), the Maternal-Child Nursing Department's professors (13), and their interplay with the opinions of the Genetics professors (9). Data analyses involved quantitative and qualitative methods. In the student group, 88% answered that genetics is important, because it has application in the nurse's quotidian, in the comprehension of the basic mechanisms of inheritance, diagnosis, planning, developing, and implementing an individualized plan of care for people with genetic disease. Six percent have abstained, because they have no idea about the correlation between genetics and nursing. In general, the students (83%) expressed the necessity of changes relating to the course content and its length of time, including the new and exciting instructional modalities to assist in teaching constantly changing genetics content. This study reinforces the needs to extend this work to other nursing school, and we are performing an on-going national research project to assess the type and amount of genetics content in the graduate nursing programs in our country.

P432. The interactional framing of moral dimensions in genetic counselling discourse

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Interactions between healthcare professionals and patients have been characterised within sociolinguistic and communication research as a tension between the medical and the social. According to the stronger version, the voice of medicine inhabited by the medical experts is juxtaposed to the voice of the lifeworld inhabited by the patients. The weaker version holds that the medical and lifeworld voices are dispersed among the healthcare `experts' and the `lay' patients for their preferred social agendas.
In the context of genetic counselling for predictive testing, it is suggested that the two concepts – `medical' and `social' – need to be reassessed along the moral dimensions (to include is/ought formulations, attributions of responsibility/blame, maintenance of professional neutrality etc). The different components such as laboratory test results, clinical symptoms, family inheritance which make up one's current genetic status assume moral significance with regard to consequences for other family members including the yet

unborn, lifestyle choices and so on. Conflicting demands may thus arise from the imperatives of patient autonomy and confidentiality and the wishes and rights ('to know or not to know') of other family members. Using the methodology of interactional and thematic mapping of 50 audio-recorded and transcribed genetic counselling sessions (part of a larger Wellcome Trust-funded project), we present findings across different genetic conditions. Our analysis focuses on how various participants (counsellor, nurse, client and family members) selectively orient towards individual and/or family concerns while foregrounding issues of responsibilities, motives and rights (not) to know vis-à-vis disclosure of genetic information.

P433. Strategies of risk relativisation in counselling for predictive genetic testing

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The activity of risk communication in healthcare settings is contingent upon the associated notions of uncertainty, normality and decision making. Focusing on genetic counselling for predictive testing, we point out that because there is `medical' uncertainty surrounding such testing, the discourse of risk assessment concerning an unwanted event is articulated in terms of likelihood (in probabilistic language) and relative aversiveness (in subjective, emotive language) of the different possible test results. Based on transcribed audio-recorded clinic data from counselling for HD, HNPCC and FBC (part of a larger Wellcome Trust-funded project), a distinction is made between the risk of the genetic disorder manifesting and the risk of problems that might arise from undergoing genetic tests. In a given counselling session, both types of risk – the strictly genetic risk of disease, and the more contextual risk of 'knowing' – become conflated, and in fact the risk of disease is understood in the light of the other, external risk factors. We suggest the analytic notion of relativisation to capture this dynamics and identify six discourse strategies (abstraction, reformulation, externalisation, localisation, temporalisation, agentivisation). Although it is not possible to determine the exact valency of these discourse strategies and plot them into a continuum, we argue that they are selectively and cumulatively drawn upon by both counsellors and clients to escalate or de-escalate the risks under discussion. These manoeuvres serve to manage the pragmatically informed decision-making process, while simultaneously attending to the relevant epistemological levels of uncertainty.

P434. The Genetics and Medicine Historical Network

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This web based resource has been constructed to encourage the documentation, preservation and wider knowledge of the important historical aspects of genetics in relation to Medicine. Among the initial activities being developed are:

1. Identification of key workers in different fields and countries, recordings of oral history and location and preservation of their records
2. Documentation of the evolution of the main areas of human and medical genetics and the special contributions of different countries
3. Recording of events of particular importance or controversy with associated images

A newsletter and email list now exists for all who wish to be involved or associated (harperps@cardiff.ac.uk).
The poster presents some of the early results
(Supported by Wellcome Trust).

P435. Predictive testing for hereditary breast cancer and Huntington's disease: the opinions of general practitioners, nurses and midwives in Flanders

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Questionnaires were sent to 356 general practitioners (GPs), 881 nurses and 119 midwives to assess their opinions on predictive testing for (1) hereditary breast cancer (HBC) in an adult, (2) Huntington's disease (HD) in an adult, (3) HD in a child at the parents' request and (4) HD in a 16-year old adolescent at his/her request. Rating scales and open-ended questions to explain their rating were used.
The response rate was 60% for GPs, 58% for midwives and 33% for nurses. The acceptability ratings for predictive testing for HBC and HD in adults and for HD in adolescents were predominantly favourable. However, predictive testing for HD in a child at the parents' request was also judged favourably by about half the respondents.
Predictive testing for HBC in an adult was rated as more acceptable than testing for HD, mainly because prevention and treatment are unavailable for HD. The GPs' ratings for testing for HBC were less favourable than the nurses' and midwives' ratings. Prophylactic mastectomy was considered as a controversial risk reducing option by half the respondents.
Favourable ratings for testing an adolescent were mostly explained by respect for personal autonomy. More negative opinions about predictive testing for HD in a child at the parents' request were motivated by the child's right not to know, and by psychological and medical arguments. The practical implications of our results will be discussed.
The research was funded by the Flemish Interuniversity Institute of Biotechnology (VIB).

P436. Genetic education in Europe for non-genetics health professionals (GenEd); survey of existing policies and practices

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GenEd is an innovative 3-year EU funded collaborative project aimed at gathering data from 11 countries. We present phase 1 results. Available website documentation was reviewed backed up by direct contact with professionals from the 5 countries in which GenEd started. First data from medicine and midwifery show that the structure of non-genetic health professional training differs between countries, with wide variation in content and duration of genetic education. France and Germany have a nationally agreed undergraduate medical curriculum but with minimal genetic education, mainly confined to basic science courses and often not immediately visible. In Sweden, the Netherlands and the UK to a large extent the content is at the discretion of individual universities. Evidence from UK and France indicate that genetic professionals are influencing the genetic content of curricula. Some specialties have adopted specific genetic educational requirements but many lacked any. We show that within each country many organisations may have nominal responsibility for setting, assessing and implementing education. Phase II of this study is concerned with needs and will survey European health professionals' knowledge, attitudes and skills in regard to using genetic information.We propose (http://eoi.cordis.lu/dsp_details.cfm?ID=29944) the creation of a European Coalition of Health Professional Education in Genetics (ECHPEG) to facilitate collaboration between e.g. European Health Professional Organisations and Clinical Geneticists (EUMS), patient groups (EAGS), educationalists (ESHG Educational Affairs Committee), policy makers (The Wellcome Trust/ Department of Health UK Strategy) and international groups (NCHPEG). Research funding and

additional collaboration is actively being sought for this new venture.

P437. What do patients with Steinert's Myotonic Dystrophy know about transmitting the disorder to their children?

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Steinert's Myotonic Dystrophy (DM1) is an autosomal dominant disorder which, of great clinical importance, also shows marked anticipation. The purpose of our study, supported by an INTAS grant, was to find out if DM1 patients with clinical signs were aware of the risk of transmitting the condition to their children. The study was performed in Bashkortostan Republic (Russia). A short questionnaire was mailed to 38 men and women of reproductive age (15 – 40 years) registered in our database as having DM1. We received 29 completed questionnaires. The results were as follows: 24 (83%) patients knew of other cases of DM1 in their family. Most of the respondents (72%) did not plan to have children. One-half of the respondents (52%) were well informed about the risk of transmitting the disorder to their offspring and knew that the risk of having an affected child was 50%. There were 7 patients (24%) who were sure that they will not transmit DM1 to their children: 6 of them did not plan to have children and 1 of them did. Two patients (7%) recalled being told that the risk of having an affected child was 100% and they therefore decided not to have children. Three other patients underestimated the risk and 2 patients' reply was 90%. We found out that 12 (41%) patients had never heard about anticipation or the congenital form of the disorder . The results of our study show that more genetic education and counseling are needed in our region.

P438. Proxy consent for children: Parent and child research participants' perspectives on the use of genetic material in longitudinal, epidemiological research.

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This paper will address the issue of proxy consent in relation to the continued use of genetic material within longitudinal, epidemiological research. This paper is based on data derived from the Wellcome Trust funded EPEG project [Ethical Protection in Epidemiological Genetic Research: Participants' Perspectives]. The EPEG project consisted of focus groups and 1-1 interviews with both children [aged 9-11 years old] and adults, some of whom take part in a long-standing epidemiological study which includes the use of environmental, social and genetic information about children, mothers, and fathers.
The EPEG project explored how participants considered ethical protection (taken in its widest sense) in longitudinal, genetic epidemiology.
We found that: 1) Children currently underestimate the amount of control that they have with regard to their participation in research. 2) Children and parents' views of risk differed in such a way as to call into question the ability of adults to give genuinely informed proxy consent for their children. 3) Questions were raised about the ‚right' of parents to consent to the long term use and re-use of data/biological material/genetic information in the future, when the person from whom such information had been collected could feasibly be of age to consent for themselves.
This paper will present data and findings from our research, and discuss their implications for the protection of children's interests in research. Our results have implications for both researchers and policy makers concerned with children's participation in biomedical research.

P439. Frequency of congenital development defects, chromosome anomalies and hereditary syndromes in fetuses of high risk.

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The Medico-genetic Dispensary of the town of Astrakhan serves a population of 500,000 people. During the last two years 2647 pregnant women at high risk of producing children with congenital or hereditary diseases have been examined at the second level. They included:
1 Women with US markers of congenital or hereditary diseases (CHD) in the fetus - 32,3%;
2 violations of reproductive function 17,9%;
3 prediction of healthy progeny 12,9%;
4 family history of CDD and CHD 8,2%;
5 teratogenic impact on the fetus 7,8%;
6 age above 35 -7,5%;
7 previous perinatal or infantile death 5,9%;
8 combination of indications 4,8%;
9 consanguineous marriage 2,2%.
Perinatal examination results have been studied in risk groups. Congenital development defects (CDD), chromosome anomalies (CA) and hereditary syndromes (HS) in the perinatal period were considered as unfavourable outcomes.These occurred as follows:
I Suspicious US finding: CHD 13,4%: CDD 11,1%, CA 1,5%, HS 0,8%;
II. Family hisotory of CDDor CHD: 7,8%: CDD 6,4%, CA 0,9%, HS 0,5%;
III. Ageof the pregnant woman above 35: 7,5%: CDD 5,5%, CA 2%;
IV. Perinatal and infantile death: 4,4% : CDD 3,2%, CA 0,6%, HS 0,6%;
V. Violations of reproductive function 1,7%: CDD 1,3%, CA 0,2%, HS 0,2%.
In the other high risk groups we observed only CDD: consanguineous marriage: 3,4%, combination of indications: 2,3%, prediction of healthy progeny and teratogenic impact on the fetus : 1% each.

P440. Future provision of genetics education: views of staff in a UK regional specialist genetics centre.

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Recent advances in genetic science have resulted in a rapid expansion of clinical genetics. Much has been written about the resulting need to improve knowledge and understanding of genetics across society as a whole and within the health care professions in particular. In the UK and beyond, there is an expectation that specialist genetics centres will provide this additional education. A Centre for Education in Medical Genetics was therefore founded within the West Midlands Regional Clinical Genetics Service (WMRCGS) in summer 2002.
As part of its initial work, the centre has carried out a qualitative survey of the views of thirty one WMRCGS staff concerning genetics education and training. All clinical staff and senior laboratory staff were asked what genetics education WMRCGS should ideally be providing and to which groups. More than twenty different groups were identified, falling broadly into the following categories: doctors (non-genetics), other healthcare professionals, primary care, the public and genetics staff.
A number of issues were also raised including: the difficulty of prioritising such a large task; the failure of other professionals to engage with genetics as relevant to their own practice; the need for a wide range of educational opportunities to cater for everyone; the question of who will deliver this additional education; the opportunities for delivery provided by new technologies (eLearning); and the importance of ensuring the quality of education provided.
The results of this study and the implications for the provision of genetics education from a UK regional specialist centre will be discussed.

P441. Psychological and social sequelae of therapeutic abortion in second trimester of pregnancies

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Rapid development of prenatal diagnosis is proceeding among several fronts.One of them is psychosocial consequences of therapeutic abortion.
We report follow-up study of 108 women who underwent termination of second trimester pregnancies.A total of 108 woman were studied (age,maritial state, social class, education).When fetal aneuploidy is diagnosed prenatally, parents must choose between continuation and

termination of the pregnancy.It is always a great sadness for couple to have a child affected by inherited disorders but the abortion is also stressed.The fetus with chromosomal aberations was detected by amniocentesis and cordocentesis.Genetic counselling before and after intervention is very helpful.Follow up examinations were carried out by means of detailed, inteviews at three months, fiftheen months and two years after termination.We analysed psychiatric symptoms, guilt feelings and adjustment maritial and other interpersonal relationshep (sex,work).Also,we compared sequelle of abortion beetwen amniocentesis and cordocentesis.
The results from this study show that the legal abortion in second trimester accompanied by counselling carried risk of psychological and social sequelae up to two years afterwards.It means that termination of the pregnancy at first and second trimester have some differencies.The same time,we noticed that the sequelae of amniocentecis and cordocentesis have some differencies.

P442. Women's sociodemographic profile and motives for attending an oncogenetics service in a Brazilian Cancer Center

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An ever increasing number of women from families with breast cancer have been attending at cancer genetics services looking for counseling. It is important that the oncogeneticists optimally and efficiently recognize the informational needs that are essential to these people. Our principal goal was to examine whether sociodemographic and medical characteristics influence the different motives to seek genetic counseling in Brazil, in order to identify patients with specific informational demands. For this purpose, 1168 patient records from October 1999 to December 2002 were evaluated as part of a continuing study on risk evaluation and cancer prevention. We selected 432 families with familial breast cancer, 116 of them with Hereditary Breast and Ovary Cancer Syndrome, according to a rigorous criteria previously proposed by our Department. Thirty affected probands were interviewed and information about educational level, having children, motives for attending the consults, cancer's causes among other topics was collected. The mean age at diagnosis was 45 years (34-61ys), most women were married (67%), had children (80%) and university degree (57%). The main motive for seeking counseling in the case of women with children was to be informed about children's risk, whereas those without children are more concerned about their own risk (43%), and other relatives' risk (40%). Smokers and liquor drinkers did not mention alcohol or tobacco as cancer's causes. Some medical and sociodemographic characteristics might determine special interests, and communication during the counseling process has to be more tailored to suit the individual person.
Supported by Capes and FAEPA.

P443. The impact of Presymptomatic Testing for Huntington's Disease on marital relationships and partners.

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Huntington's Disease (HD) is an adult onset autosomal dominant neurodegenerative disorder usually developing between the ages of 30 and 50. Three classic clinical features are associated with HD: movement disorder, personality changes and cognitive impairment. One distressing factor is that it strikes in the prime of life, when social responsibilities and personal and financial possibilities are greatest. Individuals have often married and had children before they had any knowledge of the family history of HD or their own risk.
Individuals with HD exhibit a high degree of personality and behavioural changes. The spouse has to cope with their deteriorating partner's physical needs and disabilities and also with slowly losing a companion and life long partner. Intimacy, friendship and decision making deteriorate to the point when many spouses feel that the individual they are caring for is no longer the person they married. Accurate presymptomatic testing has been available since 1993, but studies tend to focus on the person at risk, rather than their partner. The partner is sharing the life of the person at risk, and must be empowered during the decision making process and given care and support after the result is given. We have investigated effects of having kept secret a family history of HD, and the effects of presymptomatic testing for HD on the marriages and intimate relationships of 39 couples. Good communication with the person at risk and the clinicians dealing with them are essential regardless of whether the people are given favourable or unfavourable results.

P444. PEG feeding in end stage Huntington's disease:the views of the families

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Huntington's Disease (HD) is an adult onset, autosomal dominant neurodegenerative disorder which is progressive and incurable. Clinical features include movement disorder, personality changes and cognitive impairment. Deterioration progresses until excessive weight loss and uncoordinated swallow with risk of aspiration occurs. Feeding using percutaneous endoscopic gastrostomy (PEG) has been suggested as a means of providing additional calories.PEG feeding raises a number of ethical issues because it is a form of artificial feeding, and it does not remove the risk of aspiration. It will not cure HD, but it may sustain life. There has been little debate about withholding or withdrawal of such feeding which some view as treatment and others as basic care, yet the dilemma occurs frequently as the HD patient deteriorates. We surveyed the views of HD patients, relatives, carers and healthcare professionals regarding a patient's right to choice with regard to PEG feeding in the end stage of HD. The minority of doctors encouraged patients to discuss issues of end life care and very few doctors sought their patient's opinion regarding PEG feeding early in the natural history of HD despite 93% of respondents stating that the patient should be allowed to make the decision. We suggest that clinicians should discuss the prospect of PEG feeding early in the course of HD, when patients are competent and able to communicate their wishes. Their views should be recorded. Respondents agreed that guidelines on how this could be achieved would be acceptable to patients and healthcare professionals.

P445. Quality assessment, Division of Medical Genetics, Geneva University Hospitals : Evaluation of patient/client satisfaction related to Medical Genetics ConsultationSessions.

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Purpose : To assess the degree of client satisfaction, draw a sharper image of the activity of our group and identify fields of possible improvement.
Patients and method : From July 2001 to July 2003, 330 single or multiple consultations were performed by 6 MDs specialized in Medical Genetics. Approximately one month later they were sent a 55 item questionnaire for prospective analysis of satisfaction related to 6 aspects : - precounseling status – professional/technical skills - human competence - administrative procedures - process of decision making - general satisfaction.
Results : 70 % returned questionnaires. Despite substancial preconsultation anxiety in 30 %, expectations were satisfied in > 85 % clients and global satisfaction expressed in > 90 %. The consultation clearly helped 83 % to deal better with their situation. For 90 % the amount of given time was appropriate. For > 89 % the MD completely understood what was preoccupying them. > 90 % received all the information hoped for. Satisfaction rate for explanation of inheritence amounted to nearly 96 % and the way ethical problems were dealt with to > 95 %.
Conclusions : The high return rate allows reliable conclusions. Global satisfaction > 90 % attests an adequate response to the demand of the population. Specific fields for future improvement have been identified.

P446. Professor Francis Albert Eley Crew from Birmingham as a creator of the Polish School of Medicine at the University of Edinburgh in the dark days of 1941

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Geneticists from the past have provided a great cultural inheritance, not only in the scientific field. We would like to come back to the dark days of 1941, recalling the "Magnanimous Gesture" of the University of Edinburgh in founding the Polish School of Medicine for soldier-students in the Polish Forces. Lt. Col Professor F.A. E. Crew, born and educated in Birmingham, was a medical geneticist who, as Commanding Officer of the Military Hospital at Edinburgh Castle at the time of the greatest misfortunes of the Second World War, conceived the idea of a Polish medical school as part of the University of Edinburgh. The School was a Scottish-Polish academic enterprise, unique in the history of universities and a venture in international academic co-operation never previously attempted. Created as an experiment it was successful and ceased in 1949. Owing to the post-war situation in Eastern Europe over 200 graduates did not return home and, equipped with their precious diplomas, dispersed world-wide. However, the Polish School of Medicine lives on spiritually in the form of the Polish Historical Collection in the Erskine Medical Library in Edinburgh and Memorial Fund for Scholarships. Coming to Birmingham for a European meeting of human geneticists, we wish to honour Professor F.A.E. Crew, the famous son of this region, our Europe and our genetic society.

P447. Learning from history - The geneticist Hans Nachtsheim, human experimentation in the National Socialism, and functional genomics

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The historical study I present deals with (human) genetics in Germany and asks how normal science can be become criminal. It is commonly believed that only ‚pseudo-science' is compatible with moral offence. This argument derives to some extent from the perception of science under National Socialism. Back then, German geneticists and medical geneticists participated deliberately in forced sterilisation, racial politics and criminal human experimentation. After the war this was seen as due to ideological deformation of "normal" science. An equation of "normal" and morally "good" science was thereby emphatically implied. But besides Nazis, anti-Semites or racists, apolitical or even liberal thinking scientists practising "normal" science were actively involved in criminal action. Therefore, it is an urgent task to differentiate and understand the processes leading to transgression of moral boundaries. The case of Hans Nachtsheim (1890-1979), for example, is alarming. Nachtsheim was an internationally well-regarded integrative figure of post-war German human genetics. Though he was always opposed to anti-Semitism and racial politics, for many years nobody knew that he had used children from a sanatorium for dangerous experiments in 1943. Clearly, the participation of scientists in the holocaust makes their crimes incomparable. But worse, even skilfully performed and freely practised (experimental) science has its own dangerous tendencies. In this aspect, Nachtsheim's case is relevant to research in functional genomics today.
His example shows why we should be aware that the dramatically increasing need for clinical data essential to functional genomics might lead scientists to transgress moral boundaries.

P448. Counseling And Ethical Issues In Families With Child With Chromosomal Defects

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Genetic counseling of patients with chromosomal diagnoses must provide precise information about the disease, based on the biology of chromosomal defects and the reproductive risks they may entail.
We have followed 90 families who had a child with a chromosomal defect in order to follow and analyze how parents decided to raise a child with a chromosomal diagnosis.
All 7 children with gonosomal aberrations have been accepted into their families, and parents reasonably accepted all the information provided. Parents of 83 children with autosomal aberrations have had difficulties in understanding and accepting all the medical facts provided by the counselor. 35 couples decided to send their child into an institution immediately after the diagnosis, 15 couples made this decision after two years of giving care in the family, and 32 couples decided to raise their child in the family. 8 children had multiple malformations and died in hospital. Fathers more frequently had problems with understanding and accepting the facts, and they were more radical in decisions to send child to an institution rather than raise the child in the family. Younger and childless couples more frequently decided to send child in some institution, than those with one or more older children.
The counselor has to respect the autonomy of the parents and it is generally agreed that counseling should be nondirective. It is the skill of the counselor that enables clients to reach a decision that is right for them.

P449. Impact of Orphanet, a web based information system, on the uptake of health care services

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The objective was to assess the influence of an Internet-based information service (www.orpha.net) in the area of rare diseases on the referral to appropriate health care services and to detect any harmful effect. A survey was sent to the 500 French clinics and the 350 French laboratories which are referenced in the database. The survey aimed at assessing the impact of Orphanet through three variables: the number of professionals effectively using the web service in their daily practice, the reasons why they were using it, and the estimated impact of the information on referrals. The data collection took place during spring 2002. The response rate was 68% for the clinicians and 75% for the biologists. The clinicians dealing with rare diseases are using Orphanet on a regular basis (80%). The reason for using Orphanet are the following: to identify a diagnostic lab (70%); to refer a patient to an other clinician (65%); to give information on support groups to patients (50%); to know more about a rare disease (49%); to identify a researcher (45%). Furthermore, 9% of the clinicians claim that orphaned is having a significant impact of the number of patients which are referred to them. The biologists are also heavy users of Orphanet (86%). Furthermore, 29% of them said that Orphanet is having a significant impact on their referrals. This survey shows that the availability of information on highly specialized health resources may contribute to empowering consumers and to improve right use of these resources.

P450. Human genetics research in the EU's Sixth Framework Programme

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Human genetics research will be a major part of the EU's Sixth Framework Programme (2002-2006) under Thematic Priority 1 *Life Sciences, Genomics and Biotechnology for Health*.
This thematic priority comprises two areas:
Advanced genomics and its applications for health, where the emphasis will be on multidisciplinary research to generate new technologies and new knowledge in fundamental genomics of all organisms to underpin applications to human health;
Combating major diseases, emphasising research aimed at bringing basic knowledge through to the application stage, to enable consistent and coordinated progress at the European level in medicine and improve the quality of life.
Europe has outstanding research capacities in human genetics but is not able to perform as well as some of its international competitors. This is due to structural and organisational differences between national research systems and a general lack of coherence among national and European efforts. The European Commission's (EC) policy initiative to realise a European Research Area (ERA) is aimed at addressing such structural problems in European research. Under the Fifth Framework Programme (1998-2002), the EC is funding

projects that address the need for better cooperation and coordination between population genetics programmes (e.g. GenomEUtwin, Cogene, Biobanks for Health). In the Sixth Framework Programme, the EC aims to strengthen Europe's competitive position in human genetics and to encourage the spreading of research excellence and progress towards the ERA policy objectives. In this presentation, current and future aspects of EC support for human genetics will be reviewed.
Ref: Framework Programme 6 website www.cordis.lu/fp6

P451. Living with BRCA1/BRCA2 test results: Women's post-test experiences

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Introduction: Since the introduction of BRCA1/BRCA2 tests into routine medical care in the late 1990's, more and more women at increased risk undergo predictive genetic testing. Little is known about women's post-test experiences. Methods: At the Department of Human Genetics, UKM, Germany, women at increased risk are offered BRCA1/BRCA2 testing. Pre- and post-test counseling is mandatory. Out of 50 women who underwent testing the last 2 years and who have obtained test results at least 6 months ago were asked to participate in a follow-up interview. 46 women agreed to participate. A standardized questionnaire addressing questions such as: communicating risks to family members; conflicts with family members because of test results; positive and negative experiences; decision regret; availability of preventive surveillance; etc. was administered by telephone interview. Results: 58.7% report that their main reason to undergo testing was to know more about their personal risk; 60.1% wanted their children to have certainty about a hereditary risk; 23.9% wanted to know their risk in order to optimize personal future plans. 23.9% reported that they only underwent testing because other family members asked them; 52.2% wanted to optimize prevention. More than 98% consulted with family members and all informed, albeit, selectively other family members about the result. 13% expressed decision regret and 87% would undergo testing again, 60.9% would recommend the test to other women at increased risk. Discussion: Few women who undergo predictive BRCA1/BRCA2 testing after a pre- and post-test counseling protocol express decision regret after a ≥ 6 month period.

P452. Preconceptional screening for carriers of haemoglobinopathies and/or cystic fibrosis, dependent on ethnic background. Feasibility of a combined offer in the Dutch population.

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Objectives: Cystic fibrosis (CF) and haemoglobinopathies (HbPs) are autosomal recessive disorders occurring in different sections of the Dutch population, defined by ethnic background. Preconceptional carrier screening allows couples at risk to arrive at informed reproductive decisions. In this project desirability and feasibility of a combined offer of preconceptional CF and HbP carrier screening are studied. The following research questions will be addressed: 1) Will it be possible to develop a valid instrument to support invitees for preconceptional carrier screening in deciding which disorder could be screened for in their particular case (only CF, only HbPs, both or none)?
2) How will people in the Dutch multi-ethnic society react to a combined offer of preconceptional CF and HbP carrier screening, making use of the above instrument ?
Methods: After pre-testing, 150 people are asked to read educational material and arrive at a decision by following our decision support material. Then they are interviewed about the origin of their ancestors to see whether their decision through our instrument was correct. The validated instrument will be used during a screening study, in which people aged 20-35 years will receive the educational material and decision instrument from either their general practitioner or the Municipal Health Services. Those who come forward for testing will complete questionnaires before and after testing and at six months follow up. Questionnaires will focus on determinants of participation, reproductive intentions and unwanted side-effects.
Results: First results will be available at the conference.

P453. Colorblindness and car driving

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151 colorblind subjects and 302 orthochromatics of comparison coming from Cosenza province (Calabria - Southern Italy) completed a psychosocial questionnaire regarding information about trouble linked to anomalous color vision in car driving. The use of the car is significantly less diffuse among the colorblinds than among the orthochromatics (93.7% to 98.4%, respectively; p=0.0242). Orthochromatics state that they have no preference for daytime driving over nighttime driving (38.4% to 6.7%, respectively; p<0.0001). At night, colorblind subjects have more difficulty identifying the lights of road reflectors compared to the orthochromatics (4.0% than 2.8%, respectively), and the lights of the car that precedes them (1.6% to 1.2%, respectively). 4.8% of the colorblinds to 2.0% of the orthochromatics have more difficulties identifying the colors of the traffic lights when these change their positions. Colorblinds have some difficulties stopping at red traffic lights. The frequencies of road accidents are the same comparing colorblinds to the orthochromatics (18.3% to 19.8%, respectively).The authors are in agreement with the literature and consider that a psychological compensation and more vigilance by colorblinds in driving explains why their accident rate is the same as that of orthochromatics.

P454. Arrhythmogenic right ventricular cardiomyopathy (ARVC)in Newfoundland: complex ethical challenges as genetic research findings progress to clinical application.

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In thirteen large kinships with autosomal dominant arrhythmogenic right ventricular cardiomyopathy (ARVC) from Newfoundland, segregation of the condition is linked to a highly conserved founder haplotype at 3p25. Clinical diagnosis is often difficult, but research is gradually defining the natural history and genetic epidemiology of the condition. 50% of males at high genetic risk have sustained a fatal cardiac event by age 40 years, compared with 5% of high-risk females. The risk of sudden death can be managed by implantable cardiac defibrillator.
Haplotype information from research studies within families allows more accurate definition of those at high genetic risk, and can be used to direct diagnostic and treatment procedures. This use of research information for clinical management presents a number of ethical challenges related to patients'/research participants' rights to know or not know their genetic status, and the researchers'/clinicians' duties of care to individuals at risk. Should different standards of disclosure, confidentiality, and duty of care apply because the information and samples originally collected through research now have clinical application?
How should we respond when an affected parent with a defibrillator, declines to burden his sons with knowledge of their genetic risk? Conversely, where does the researcher's duty lie when children decline to hear their genetic information, and pursue careers that may consequently put the public at risk? The future application of research findings should be considered carefully by all those designing genetic research projects.

P455. How should preconceptional cystic fibrosis carrier screening be provided? Opinions of potential providers and the target population

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Introduction: Since the identification of the cystic fibrosis (CF) gene, population-based CF carrier screening has become possible. One possible target group are couples planning a pregnancy (preconceptional screening), providing a maximum number of reproductive options and a minimum time constraints.
Aim: To identify obstacles to the implementation of a national preconceptional CF carrier screening programme, to find out how potential providers and the target population think the screening should be implemented, and to determine whether potential providers think they are able to provide the screening programme.
Methods: A survey was conducted among 200 general practitioners (GPs), 134 Municipal Health Service (MHS) workers and 303 recently married couples.
Results: In general, potential providers and the target population had a positive attitude towards CF carrier screening. Preferred methods of informing the target population were: using leaflets (77% of GPs, 89% of MHS workers), during a GP consultation for those people seeking advice before pregnancy (62% of GPs and the target population), and sending a personal invitation to people of reproductive age (53% of the target population). Potential providers believed that they would be able to provide the screening programme. Important perceived obstacles were the absence of a preconceptional care setting, high workload, and lack of financial resources.
Conclusion: Different intervention strategies will be necessary to overcome the obstacles in the implementation. The positive attitude towards preconceptional CF carrier screening in combination with the willingness of the potential providers to participate in the screening programme, will make it easier to overcome the obstacles.

P456. Information as a healthcare intervention. Adaptation of information and terminology to meet the need of the European Community in the management of hemoglobinopathies

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Access to health care for ethnic minorities in the multi-ethnic populations of Northern Europe is unsatisfactory. Awareness is often insufficient among the the population and in the first line of local health systems. It is essential to develop effective channels of communication, and careful consideration of culture-based elements is necessary to improve effectiveness and usefulness information in multi-ethnic societies. Haemoglobin disorders, which are common in ethnic minority groups but are rare in the autochthonous population of Northern Europe are largely neglected and need special attention. Based on existing experience in several countries, we have studied the effect of information and communication on the management and prevention of haemoglobin disorders. We report the present situation, existing tools, and proposed actions agreed during a recent meeting of a North European Working Party on Haemoglobin Disorders.
At present there is wide variation in the quality and accessibility of information, “state of the art” patient care, carrier detection and prevention in non-endemic countries . We propose a multi-disciplinary joint effort exploring the communication elements and channels that can be adapted to the situation of each country. This will lead to more efficient delivery of information to health professionals and to the multi-ethnic community.

P457. External quality assessment schemes illustrate the need for better validation of used methodology for genetic testing

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DNA-based testing for genetic diseases has developed from nothing into a general part of laboratory medicine. Testing for genetic disorders is usually carried out in molecular genetic diagnostic laboratories. However, as commercial kits become available, molecular genetic testing for more common inherited disorders such as cystic fibrosis is increasingly being carried out in general pathology laboratories and commercial centers.
External quality assessment (EQA) schemes (or proficiency testing) for cystic fibrosis have been organized over the past six years with 135 to 206 participating diagnostic laboratories. A significant improvement of the quality of genotyping results was obtained during subsequent QA schemes, coming from 65% of laboratories without errors in 1996 up to a more or less constant level of approximately 90% since 1999. During these years new commercial kits and methodology became available and were being applied in many diagnostic laboratories. However, the use of a commercial kit alone does not ensure high accuracy of mutation analysis: results of the EQA schemes demonstrated that laboratories which implement a new commercial kit or methodology for mutation analysis of CF (e.g. OLA, INNO-Lipa, Elucigene, DHPLC) made more mistakes during the implementation period. Nevertheless, these methods were already being used for mutation detection in their routine laboratory.
External quality assessment schemes and the use of reference materials or certified test samples are possible routes to help the laboratories to improve their quality. Laboratories should be aware of the importance to implement quality assurance systems that include validation of the used procedures.

P458. How accurate is your DNA sequencing? Results of a pilot external quality assessment scheme for DNA sequencing.

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Genetic testing is now a routine part of laboratory medicine. Many different technical approaches are used but DNA sequencing is widely regarded as the ‘gold standard’ for mutation detection. But how accurate is DNA sequencing, and can we be sure we are getting the correct result 100% of the time? External Quality Assessment (EQA), or Proficiency testing, is one approach to ‘independently’ quantifying the accuracy of the sequencing output from testing laboratories and sequencing facilities. The European Molecular Genetics Quality Network (EMQN), which runs EQA schemes for different genetic disorders, set up a pilot scheme for DNA sequencing in 2002. The scheme aimed to test the ability of participants to sequence DNA samples containing heterozygous and homozygous mutations. Six samples were sent out to each participant; they were asked to report back to EMQN with both the interpreted sequence and the raw data. Thirty-two laboratories participated including 2 commercial core-sequencing facilities. The scheme design (including the associated problems) will be discussed and examples of results and errors will be presented.

P459. Evaluation of MLPA for Use in the DNA Diagnostic Laboratory

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Gene dosage abnormalities account for a significant proportion of the mutations in genes tested in DNA diagnostic laboratories. Detection of these changes has proved a challenge as the methods available to date are time consuming or unreliable. The Multiplex Ligation-dependent Probe Amplification (MLPA) is a new technique allowing relative quantification of up to 40 different nucleic acid sequences in a single reaction (Ref 1). We evaluated MLPA for potential use in the diagnostic setting.
We used commercially available (Trisomy, HNPCC, and BRCA1) and prototype (CMT, VHL, MEN1 and SMA) kits manufactured by MRC-Holland following the protocol supplied. Analysis was carried out using an ABI 3100 with Genotyper results exported to an Excel spreadsheet. Over 400 assays were performed. Assay time was

under 24 hours, with less than 2 hours hand-on time, including data analysis.
For each locus, peak heights of the test and control samples were compared using the dosage quotient method of Yau et al. Over 90% of samples gave reproducible results, although dilute or low-quality DNA samples gave poor results. The results obtained in normal and mutation controls were consistent with other techniques, and single exon deletions were confirmed by PCR.
In conclusion, we have found that MLPA compares favourably with other available techniques based on cost, time, sensitivity and reproducibility. We propose that it represents a realistic option for gene dosage analysis in the diagnostic laboratory.
1) Schouten et al., 2002 Nucleic Acids Research 30 e57
2) Yau et al., 1996 Journal of Medical Genetics 33 p550-558

P460. DNA diagnosis of single gene disorders outside Europe: experience from Kuwait

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Knowledge of mutations underlying single-gene disorders in particular populations is essential for implementation of DNA-based diagnosis. The data on mutations identified in patients from Kuwait with the following diseases will be presented: cystic fibrosis (CF), spinal muscular atrophy (SMA), phenylketonuria (PKU), G6PD deficiency (G6PD-D) and some others. All but one of the mutations identified were already known. The only novel mutation was the C128R (514 T->C) substitution in exon 4 of the CFTR gene, associated with severe clinical phenotype. Three common CF mutations (deltaF508, W1282X and S549N) accounted for 34% of CF chromosomes. Importantly, five CF mutations (I1234V, 1548delG, 3120+1g->a, I560T, C128R) accounting for 28% of CF chromosomes are not covered by the commercial kits widely used for diagnosis of CF mutations. All our SMA patients had SMN1 deletion. Interestingly, the dual deletion of NAIP and SMN1 genes was much more frequent among Arabs than in Europeans (85% in SMA type I and 40% in SMA type II/III). Nine mutations were identified in PKU patients: 1066nt11g->a (35%), K363fsdelG (15%), IVS4nt5g->t in (15%), IVS2nt5g->t (11)%, G352fsdelG (8%), and DelE2-IVS2nt1, P281L, E208K, I224T (4% each). Before our study the I224T and IVS4nt5g->t mutations had been reported in a single family each. Five mutations were identified in G6PD-D males: 563C->T (73%), 202 G->A (14%), 1003 G->A (7%), 143 T->C (1%). The (TA)7 allele of the UDPGT1 gene promoter, which influences the severity of jaundice in G6PD-D patients, was observed in 60% of cases. The data on other single-gene disorders will also be presented.

P461. Development of a Service for Mutation Screening of the Cartilage Oligomatrix Protein Gene (*COMP*) in the Skeletal Dysplasias Pseudoachondroplasia & Multiple Epiphyseal Dysplasia.

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Introduction: Skeletal dysplasias are a common group of genetically heterogeneous disorders with over 200 individual phenotypes characterised clinically & radiograhically. Pseudoachondroplasia (PSACH) is a skeletal dysplasia presenting with clinical variability, however, >99% of PSACH cases result from mutations in the *COMP* gene. MED is a similar but more variable skeletal dysplasia. Between 25 & 30% of MED cases also result from mutations in *COMP* and show a similar distribution to those found in PSACH.
Methods: *COMP* mutations in genomic DNA samples from patients referred with known or suspected diagnosis of PSACH, MED, or other skeletal dysplasia were determined by PCR amplification of exons 8-19 of *COMP*, bi-directional fluorescent DNA sequencing and computer software analysis of the DNA sequence data using the Staden sequence analysis package.
Results:

Detection rates		
Initial clinical diagnosis	Number of patients	*COMP* mutations
PSACH	22	18 (82%)
MED	31	9 (29%)
Other skeletal dysplasia	3	0

Mutational class		
	Missense	Deletion / Duplication
Previously described	3	5
Novel	14	5
Total	17	10

COMP mutations were detected in 27 out of 55 patient samples tested by our laboratory. In patients where no *COMP* mutation was detected, radiographs were expertly reviewed to ascertain independently whether possible misdiagnosis had occurred. After revised clinical diagnosis, mutation screening of further genes known or suspected to be involved in the pathogenesis of PSACH & MED is currently being undertaken to offer targeted, precise molecular diagnosis. We have identified *COMP* mutations in 100% of clinically confirmed PSACH cases. In summary we have developed a molecular diagnostic service for a range of disorders affected skeletal development.

P462. Automated detection of common mutations and polymorphisms in the human Cystic Fibrosis gene using a PCR/OLA assay and Genemapper™ software

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Cystic Fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. We have developed an assay that is based on multiplex PCR amplification and subsequent probing of the various alleles by the oligonucleotide ligation assay (OLA). The assay detects the mutant and normal alleles for S549R, S549N, R553X, G551D, V520F, delI507, delF508, 3876 delA, 1717-1 G->A, G542X, R560T, 3120+1 G->A, R347P, R347H, I148T, W1282X, R334W, 1078 delT, 3849+10 kb C->T, R1162X, N1303K, 3659 delC, 3905 insT, A455E, R117H, 394 delTT, 2184 delA, 2789+5 G->A, 1898+1 G->A, 621+1 G->T, 711+1 G->T, G85E in a core panel. The mutations I506V, I507V and the intron 8 polymorphisms 5T, 7T, and 9T are detected using reflex panels. Future carrier or newborn screening programs for CF mutations will require reliable, automated high throughput detection systems. As a potential platform, we implemented the assay on the ABI PRISM® 3100 Genetic Analyzer. A full plate with 96 samples can be processed in 4 hours with unattended operation. The resulting electropherograms were analyzed using a prototype version of the Applied Biosystems GeneMapper™ software in an automated fashion. The software presents genotype data along with quality control flags. It also allows for sorting of samples by the detected normal genotype or by heterozygous and homozygous mutations for final review by the investigator. The assay and analysis system correctly detected all mutations present in the Coriell reference panel MUTCF, demonstrating the feasibility for automated CF genotyping.

P463. A Novel Lys130Gln Mutation of the TAU gene.

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The Tau gene on chromosome 17 is crucial in the pathogenesis of some neurodegenerative disorders like the Frontotemporal Dementia and Parkinsonism linked to chromosome 17 (FTDP-17),characterized clinically by a presenile dementia with rigidity and postural abnormalities, and pathologically by hyperphosphorilated tau deposits in neurons and glial cells.We presented a novel mutation at

codon 130 (exon 5)leading to a Lysine to Glutamine substitution in the tau protein. The proband is a Calabrian 56 years-old female. At the time of the molecular evaluation (disease duration 4 years) she complained a slight bradikinesia in the left limbs. Cerebral Magnetic Resonance Imaging scans was normal. Concerning the family history, her sister showed similar disturbances and her mother (who died at age of 88 years) had presented cognitive decline during her last 2 years. The mutation was detected by analysing the entire Tau gene with the Denaturing High-Performance Liquid Chromatography (DHPLC) technology (Transgenomic)- an automated method for detecting unknown DNA sequence variants- and confirmed by sequencing the selected exon 5 with the ABI PRISM 377 Genetic Analyser (Perkin Elmer). We also performed the same analysis in selected 50 healthy controls derived from the same geographic area but they did not show any mutation.

P464. Impact of wheat flour fortification with folic acid (FA) on the frequency of Neural Tube Defects (NTD) in Chile: preliminary results.

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Introduction: The protective effect of FA over NTD frequency is widely accepted. In Chile, starting January 2000, the Chilean Ministry of Health legislated to add FA to wheat flour to reduce the risk of NTD. This policy resulted in a mean FA additional supply of 427 µg/d. Serum folate and red cell folate increased significantly in fertile women after fortification
Objective: To measure the impact of this fortification on the NTD frequency. **Methods**: all births live births (LB) and stillbirths (SB) with birth weight ≥ 500 g of the 9 maternity hospitals of Santiago from 1999-2000 (pre-fortification) and 2001-2002 (post-fortification) are included; corresponding to 60,000 births/year. In each hospital, a contact professional (medical doctor or midwife) examines and registers each LB and SB with NTD; then information is confirmed by the research team through different sources and registers. This information is monthly reviewed and rechecked periodically. **Results**: The NTD rate for the pre-fortification period over a total of 120,636 NB was 16,99/10000 births (13,28 LB and 501,09/10000 SB). The NTD rate for the post-fortification period (January 2001 - June 2002) in 88.358 births, was significantly reduced 41%, to 10,07 (8,8 LB and 214,3/10000 SB). For the pre-fortification period rates of anencephaly, encephalocele and spina bifida were 5,97; 2,49; and 8,54/10000; and for the post-fortification period were 3,85; 1,7; and 4,53/10000 respectively.
Conclusions: Fortification of wheat flour with FA in Chile is apparently effective in the reduction of NTD.
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P465. Social, Ethical and Legal Aspects of Storage of Dried Blood Spots

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Blood spots collected from newborns during the first few days of birth, are tested for a number of treatable diseases and then stored to ensure proper follow-up. Although dried blood spots (DBS) are mainly used for diagnosis and prevention, they also served for quality assurance of screening programs and for the development of improved assays. When stored, DBS make up an extensive genetic database as well as an easily accessible resource for researchers. Indeed, requests for DBS have accrued as scientists, law enforcement agencies, persons wishing to establish paternity and others have realized that blood spots are a valuable source of genetic information. With the availability of such a vast amount of genetic information, certain questions arise as to the social, ethical and legal questions pertaining to the storage and secondary use of DBS. Fallen into the wrong hands, identifiable blood samples can threaten the privacy of the child or of the child's family. To ensure that good intentions do not lead to undesired adverse consequences, model consent forms and educational materials, addressing length and purpose of storage and access to and destruction of stored samples are required. Policies are also needed to regulate the utilisation of bloodspots for research use, for law enforcement, in government DNA databases, and even by health insurance companies. Our poster presentation will provide a comparative review of international, national and regional official statements that address these issues and present possible recommendations for data storage and research use.

P466. Understanding different uptake rates in women from different ethnic groups: a case of uninformed choice?

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Background: Uptake of antenatal screening for Down syndrome (DS) differs across ethnic groups. Debate continues around whether this reflects a difference in informed choice (reflecting differences in attitudes towards undergoing the test) or a difference in uninformed choice (reflecting differences in knowledge about the test or differences in acting upon attitudes towards undergoing the test).
Objective: To determine the extent to which different uptake of DS screening between ethnic groups reflect informed or uninformed choices.
Sample: 104 south-asian women and 1286 white women offered antenatal DS screening.
Results: Attitudes towards undergoing DS screening were similar between the groups (14.1 vs 13.9, t = 0.3, p= 0.8) but knowledge about the test was lower in south-asian than white women (3.5 vs 5.5, t = 10.5, p<0.001). Attitudes and knowledge were unrelated (r = 0.007, p = 0.8). South-asian women were less likely to act in line with their attitudes towards screening than were white women (64% vs 78%, chi-square =10.4, p=0.001), with 55% of south-asian women with positive attitudes towards having the test not having it compared with just 27% of white women (Difference 28%, 95% CI diff 15,41).
Conclusion: There is no evidence that the choice to forego DS screening in south-asian women reflects less positive attitudes towards undergoing the test. Rather the results suggest that lower uptake reflects lower rates of informed choice in women from south-asian. Rates of informed choice could be increased by first increasing knowledge and second helping those with positive attitudes towards the test

P467. Heritability of left ventricular mass and related echocardiographic parameters

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Left ventricular hypertophy (LVH), i.e. a left ventricular mass (LV mass) exceeding clinically defined threshold levels, is an important risk factor for cardiovascular morbidity and mortality. Age, blood pressure, and body weight are known determinants of left ventricular mass. We estimated the strength of the genetic component, i.e. the heritability of left ventricular mass and related echocardiographic parameters by analysing sibling correlation and by variance component analysis (using the program SOLAR).
The study population consisted of 218 normotensive individuals with LVH and their siblings. We found a substantial heritability of LV mass ($h^2 = 0.33$, p=0.0002) and LV internal systolic diameter (LVISD) ($h^2 = 0.50$, p<0.0001), which represents a measure of cardiac contractility. Whereas part of the heritability of LV mass is due to genes influencing body weight and blood pressure, LVISD seems to be more uninfluenced by these parameters. The relatively high heritability renders a search for genes involved in the determination of left ventricular mass and contractility (as measured by LVISD) promising. Knowledge of genes underlying these traits would be of great interest, since it could eventually lead to better preventive and therapeutical management of cardiovascular disease.

P468. Familial Hypertrophic Cardiomyopathy - Molecular Analysis in a large kindred family

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Familial Hypertrophic Cardiomyopathy (HCM) is an autosomal dominant disease characterized by left ventricular hypertrophy and myocyte disarray and is the most common cause of sudden death in otherwise healthy young individuals. Mutations in sarcomeric proteins have been identified as the molecular basis and also the cause of its clinical heterogeneity.
In the present study three families comprising 51 individuals have been screened for clinical and molecular evaluation of HCM. The 3 probands of these independently reported families were diagnosed to have obstructive HCM, hence the b-myosin heavy chain gene (MYH7) was screened for mutations.
Of the 51, 29 individuals were screened clinically based on proband ascertainment, history of parental consanguinity and availability of members. Echocardiography revealed HCM in 7 of the 29 individuals, the rest were clinically asymptomatic. Pedigree information revealed 3 sudden deaths at an early age.
DNA analysis was carried out on 29 individuals by Single Strand Conformational Polymorphism to detect mutations, followed by sequencing. 13 samples have been completed, analysis for the remaining 16 is in progress. Hot spot regions of MYH7 were screened, which revealed a mutation in exon 22 (codon 870 – rod region, Arg to His). Of the 13 samples screened so far, 2 were homozygous, 7 were heterozygous for the mutation and 4 were normal for exon 22.
Predictive genetic testing revealed 2 presymptomatic and 3 asymptomatic individuals. Although the 3 families were ascertained independently, they were traced to a common ancestry.

P469. Angiotensin converting enzyme and angiotensin II type 1 receptor genes polymorphisms contribute to left ventricular hypertrophy in subjects with essential hypertension

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Left ventricular hypertrophy (LVH) is an important risk factor for cardiac morbidity and mortality. LVH in essential hypertension (EH) is a complex phenotype that results from a combination of genetic, physiologic, and environmental factors. In patients with hypertrophic cardiomyopathy (HCM) the phenotypic expression of LVH is variable, indicating a potential role of modifying genes.
The case/control study was designed to assess the contribution of two polymorphisms of the renin-angiotensin system: A2350G in exon 17 of the angiotensin converting enzyme gene (ACE) and A1166C in 3'UTR of the angiotensin II type 1 receptor gene (AGTR1) to the development of LVH in samples of Russian patients with EH (n=91) and HCM (n=29). Left ventricular mass (LVM) and LVM index were determined by echocardiography. It has been found that EH patients with LVH differ from patients without LVH by allele and genotype distribution for both investigated SNPs. LVM was increased with the number of G alleles of the A2350G polymorphism of ACE (225,81±84,43; 276,10±88,98; 315,56±136,06 g in EH patients with AA, AG, and GG genotype, respectively; P=0,023) and with the C allele number of A1166C of AGTR1 (243,97±88,82; 300,11±116,14; 305,14±99,87 in subjects with AA, AC, and CC genotype, respectively; P=0,038). The same pattern was observed for LVM index for both polymorphisms in EH patients. For HCM patients, association either with ACE or AGTR1 SNPs was not detected. These data indicate that the variants of ACE and AGTR1 genes contribute to the development of LVH in patients with EH.

P470. Electrophysiological phenotyping of sudden death and arrhythmias in a pedigree with strong familiar aggregation

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The genetic component explains many cases of sudden death (SD) in young people with structurally normal heart (SNH). We studied a pedigree (20 consanguineous members, Table). I) Affected members: i) polymorphic ventricular arrhythmia (PVA) after effort, i.e., ventricular ectopics, non-sustained ventricular tachycardia, sustained ventricular tachycardia (SVT) with SNH (5); ii) SD after physical or emotional stress (2); iii) implanted cardiodefibrillator (ICD) after polymorphic SVT and malignant syncope (2). II) Non-affected members (11). Probable diagnosis: catecholaminergic polymorphic ventricular tachycardia (CPVT): familiar disorder due to calcium channel mutation, involving the ryanodine type 2 receptor (chromosome 1, hRyR2). Nevertheless, there are affected members with ECG alterations undescribed in CPVT patients, i.e., intermittent long-QT, incomplete right bundle-branch block (IRBB) with ST segment elevation (1mm) that might be part of some other genetic disorders sharing molecullarly the ionic channel malfunction (channelopaties), coincident with the arrhythmia described and the clinical expression. The phenotype variability points out the complex role of major and non-major genes in biological regulation, emphasising the fact that each ionic channel is modulated by other channels, the intracellular and extracellular environment, leading to an effect dependent on the quantitative balance of the mechanisms involved in electrical heart activity regulation. Conclusions: 1) familiar autosomic SD, without previous history and strong familiar aggregation; 2) the quantitative and qualitative heterogeneous ECG pattern in alive affected members does not provide a conspicuous phenotype to classify the disorder within the known hereditary arrhythmias; 3) an exhaustive phenotyping has been attempted as previous step to an expensive genotyping.

Pedigree Characteristics		
Negative Exercise Test & Aborts	----------	Non-Affected 9 & 2 (?)
Sudden Death	Affected 2	---------
PVA+syncope+ICD	Affected 2	---------
PVA (asymptomatic)	Affected 5	---------

P471. Molecular genetics of the Long-QT syndrome: screening for mutations in the genes KvLQT1, HERG and KCNE1 in Czech Long-QT families.

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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 (RWS), the autosomal dominant form of LQTs: KvLQT1 (11p15.5), HERG (7q35-36), SCN5A (3p21-24), KCNE1 and KCNE2 (both at locus 21q22). Mutations in KvLQT1 and KCNE1 also cause the Jervell and Lang-Nielsen Syndrome (JLNS), a form of LQTs associated with deafness, inherited in an autosomal recessive form.
We used mutational analyses to screen LQTs patients from 35 families for mutations in the **KvLQT1 , HERG** and **KCNE1**, genes encoding cardiac ion channel subunits. In 27 unrelated LQTs patients, single strand conformation polymorphism (SSCP) analyses identified aberrant conformers. DNA sequence analyses of these aberrant conformers determined three missense mutations of KvLQT1 gene, localized in the transmembrane domains S6 (G325R , G350A),or in pore region (T309I) and one missense mutation R534C, localized in S4 region of HERG channel. Two of them (G350A and T309I)were novel.
We also identified four different single-nucleotide polymorphisms

(SNPs) in KvLQT1 and two SNPs in HERG among the patients and wild-type controls.
The screening for mutations in the KCNE1 gene revealed rare amino acid variants G38S and D85N. The possibility of rare allelic variant to have a phenotypic effect cannot be fully excluded until functional analysis in a cell-based model system has been performed.
This work is supported by the Internal Grant Agency of the Ministry of the Health in the Czech Republic (IGA MZ 5718-3)

P472. Mutation detection in KCNE1 gene encoding cardiac channel subunit: first experience with using of SSCP analysis by capillary electrophoresis for maximize detection efficiency.

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The **KCNE genes** encode a family of small type I transmembrane proteins with one membrane spanning segment whose role in potassium channel regulation is emerging. Although they themselves do not form functional ion channels, they associate with a variety of voltage-gated K^+ channels and exert control over gating kinetics, voltage dependence, drug sensitivity and conductance. The importance of the KCNEs is reflected by the mutations within these proteins that are linked to human genetic disease steming from defective repolarization of cardiac (hereditary Long -QT syndrome), sporadic and drug-induced Long -QT syndrome. The first member of the family, **KCNE1** encodes the minK protein and associates with another Long-QT syndrome-linked protein, KvLQT1, to produce the slowly activating delayed rectifier K^+ current, I_{ks}.
Here we describe using of novel **SSCP** analysis adapted to the modern **capillary electrophoresis** (CE) system, which takes advantage of laser-induced fluorescence detection. SSCP system based on either slab-gel electrophoresis or capillary electrophoresis have been developed. Firstly, we optimized conditions for SSCP of KCNE1 gene by the slab-gel electrophoresis and then by the CE in standard ABI 310 genetic analyser.
Our first results show important advantages compared to slab-gel system: higher analysis speed, lower reagent consumption and maximization of detection efficiency. Because of it the automation of SSCP analysis by CE makes the method attractive for clinical genetic laboratories.
This work is supported by grant from the Internal Grant Agency of the Ministry of the Health in the Czech Republic (IGA MZ 5718-3)

P473. Genotype-Phenotype Correlation in Russian LQTS Families

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Congenital long QT syndrome (LQTS) is an inherited cardiac disorder characterized by a prolonged QT interval, syncope and high risk of sudden death. At least six genes, when mutated, produce this phenotype. One hundred and forty patients from 67 unrelated Russian families with different types of disease were available for DNA analysis. Using SSCP and DNA sequencing of 20% of the coding region of genes *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1* and *KCNE2* we identified genetic defects in 33 (~50%) probands. Three probands had two different mutations (one was inherited and second arose *de novo*). In all these cases the probands had a more severe phenotype than their affected parents. Only three mutations (G306R, G314S and A341V in *KCNQ1*) were detected more than once. The most severe clinical traits were seen in patients with A341V.
We analyzed corrected QT duration and other ECG-features in all genotyped patients. Three subgroups (LQT1, LQT2, LQT3) have very similar average value QTc, but LQT5 patients have shorter durations. Biphasic T-wave was detected only in LQT2 patients. We examined triggers associated with the onset of cardiac events (Table 1). We concluded that life-threatening arrhythmias in LQTS patients tend to occur under specific circumstances in a gene-specific manner.

Genotypes and triggers			
Subgroup	Exercise/emotion	Auditory stimul	Sleep/rest
LQT1	64.7%	0	3.7%
LQT2	25%	50%	25%
LQT3	25%	0	75%

P474. Loss of lamin A/C expression revealed by immuno-electron microscopy in dilated cardiomyopathy with atrioventricular block caused by LMNA gene defects

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Mutations of the *LMNA* gene encoding the lamin A and C nuclear envelope proteins cause an autosomal dominant form of dilated cardiomyopathy (DCM) with atrioventricular block (AVB).
The aim of this study was to investigate ultrastructural nuclear membrane changes by conventional electron microscopy and protein expression by immuno-electron microscopy in the hearts of patients with DCM and AVB due to *LMNA* gene mutations. Four immunohistochemical techniques were used: pre-embedding and post-embedding in Epon-Araldite resin and London Resin White (LRW), with and without silver enhancement (SE).
Conventional electron microscopy showed a loss of integrity of the myocyte nuclei with blebs of the nuclear membrane, herniations and delamination of the nuclear lamina and nuclear pore clustering. Post-embedding LRW was the most informative technique for morphology and immuno-labelling. Immuno-labelling was almost absent on the nuclear envelope of patients with *LMNA* gene mutations, but intensely present in controls. The loss of labelling selectively affected myocyte nuclei; the endothelial cell nuclei were immunostained in patients and controls. These findings confirm the hypothesis that *LMNA* gene defects are associated with a loss of protein expression in the selective **compartment** of non-cycling myocyte nuclei.

P475. Allelic Variation in Long QT Disease Genes Contributes to the Population Variation of the QTc Interval in the Surface-ECG

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Prolongation of QT on the surface ECG is indicative for repolarization disturbances leading to potentially fatal ventricular tachycardia.
In the general population the QT interval is a normally distributed quantitative trait. We investigated minor gene variants in Long QT Syndrome disease genes for their influence on QTc in the general population. We analyzed surface ECG recordings from the KORA population based epidemiological survey (n=4149). For genetic analysis ECGs only ECGs were selected, that showed no signs of any disease pathology (n=1035). Among all genetic variants investigated two SNPs in KCNH2, namely F513F (C->T, rs1805120) and K897T (A->C, rs1805123), which exhibited strong pairwise LD (D'= 0,914, r2 = 0,076), exhibited the most significant association to the QTc interval by ANOVA analysis. QTc interval was prolonged from 407,1 +/- 1,5 ms to 414,1 +/- 2,1 ms in probands heterozygous or homozygous for the rarer T-Allele of the KCNH2 F513F SNP (p=0,02) and was shortened from 410,9 +/- 1,7 ms to 405,0 +/- 1,9 ms in probands heterozygous or homozygous for the rarer C-Allele (T897) of the KCNH2 K897T SNP (p<0,01). Haplotype analysis gave similar results but with a loss of significance due to increased degrees of freedom in the statistical analysis. The genotyping of the full set of KORA S2000 probands or even larger sample sets will be required to significantly detect the even smaller contributions of other genes.

P476. The European Skeletal Dysplasia Network (ESDN) www.ESDN.org

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ESDN is an integrated Research and Diagnostic network of eight partner centres for skeletal dysplasias whose objectives are: to identify the cellular, molecular and genetic factors that can cause bone dysplasias; and to develop effective approaches for the diagnosis and treatment of bone dysplasias.
ESDN Research provides an integrated approach for studying the molecular genetics and cell-matrix pathology of skeletal dysplasias. This will be achieved through complimentary research programmes, which focus on identifying novel genes involved in human skeletal dysplasias (by EST screening, genetic linkage studies and positional candidate cloning), the identification and characterisation of susceptibility/modifier genes and mutations, an investigation of protein function and dysfunction through structural & functional studies and finally an investigation of the molecular cell pathology of skeletal dysplasias through proteomics.
ESDN Diagnostics provides molecular diagnosis for more than 28 skeletal dysplasias via a Pan-European Network, which encompasses efficient routing of samples, quality assessment, diagnostic service „best practice" guidelines and efficient dissemination of results and clinically relevant information. In addition, the ESDN Clinical and Radiographic Management Group can provide expert clinical diagnostic services. This diagnostic network aims to provide a means to improving access to services. In 2002, the first year of ESDN operation, a total of 98 skeletal dysplasia cases were referred to the ESDN hub in Manchester UK, and 12 cases were routed from Manchester to the ESDN partner centres around Europe.
Ultimately this integrated and multidisciplinary approach will promote the correct diagnosis and targeted treatment for many skeletal dysplasias.
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P477. Molecular study of Huntington disease in Iranian families.

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BACKGROUND: Huntington's disease (HD) is a neurodegenerative disorder with autosomal dominant inheritance. The genetic defect is a CAG trinucleotide repeat expansion at the 5' end of the IT- 15 gene on chromosome 4.In this study we present the first molecular data on the basis and the origin of Huntington disease in Iran.
PATIENTS AND METHOD: To obtain molecular data, genomic DNA from 51 patients and asymptomatic at risk members belongs to 20 affected families and 28 normal control were amplified in the involved region by polymerase chain reaction. The CAG repeat numbers varied from 40 to 74 (median: 52.6) in HD patients and asymptomatic carriers, while individuals of the normal control group had 7-34 CAG repeat numbers (median: 17.9). In our study age of onset varied from 17 to 56. We observed a significant increase of repeat size for paternal transmission of the disease and greater instability for paternally transmitted CAG repeats in the HD size range There were large CAG repeats (74 copies) in paternally transmitted HD case with early onset (age 17).CONCLUSION: Data generated from this study may have significant implications for the etiology, knowledge of the incidence, diagnosis, prognosis, genetic counseling and treatment of HD Iranian patients. Therefore, molecular confirmation of the clinical diagnosis in HD should be sought in all suspected patients, making it possible for adequate genetic counseling

P478. An alternative for FISH - detecting deletion and duplication carriers within 24 hours using MAPH

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When a deletion or duplication mutation has been detected in an index case, relatives may wish to be analysed for carrier status. The quantitative methods most commonly used for this purpose, namely Southern blotting, quantitative PCR and Fluorescent *in situ* Hybridisation (FISH), are either technically demanding, time consuming or not always applicable. Although relatively slow and expensive, due to its reliability FISH is usually the preferred method. We have previously described a method for the detection of deletions and duplications, applied to the analysis of Duchenne muscular dystrophy patients. Multiplex Amplifiable Probe Hybridisation (MAPH) scans all 79 exons of the DMD gene in two parallel reactions using capillary electrophoresis. Here we present an adaptation of this method for determining carrier status. Based on the mutation detected in the index case a series of probes are chosen within and outside the deleted/duplicated region, as well as several from unlinked loci. Following the MAPH hybridisation and PCR the amplification products are separated on Agilent's Lab-on-a-chip, which processes and analyses 12 samples in 30 minutes. As several independent probes are used, the method is not only fast but also reliable. To date we have unambiguously confirmed the status of 15 DMD carriers. We are currently testing the method in diseases where FISH is the diagnostic method of choice, including the Wolf-Hirschhorn, Sotos and Weaver syndromes. This simple method should facilitate genetic counseling in conditions where more difficult and costly analysis is not possible or where a fast diagnosis is important.

P479. Molecular genetic analysis of Czech Malignant Hyperthermia patients.

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Molecular genetic studies have shown that approximately 50% of causes of human malignant hyperthermia (MH) and/or majority Central Core Disease (CCD) are caused by a defect in the gene encoding the Ca^{2+} release channel of the sarcoplasmic reticulum (SR). This channel, known as the ryanodine receptor (RYR1), is hypersensitive to volatile anaesthetics in susceptible individuals. It is one of the largest known proteins with 2200kDa correspoding to 5000 aminoacids encoded by 106 exons making genetic screening very difficult.
To date more than 40 mutations are known in the gene RYR1. The majority of RYR1 mutations results in amino acid substitution in the myoplasmic portion of protein and appeared to be clustered in N-terminal aminoacid residues 35 -614 and the centrally located residues 1787 - 2458. A closter of RYR1 mutations identified outside of these regions were found in the highly conserved C-terminus of the RYR1 gene, which resides in the luminal/transmembrane region.
An occurence of RYR1 mutations only in this hot spot regions made us develop an effective RYR1 mutation testing service for MH/CCD susceptible patients. Chosen method is direct sequencing analysis of up to 6 overlaping cDNA fragments, which represent hot spot regions of RYR1 gene.
In this way we suppose to identify potencially causative mutations associated with MH/CCD.
This work is supported by grant from the Internal Grant Agency of the Ministry of the Health in the Czech Republic (IGA ND 6865-3)

P480. Genome-wide screening for chromosomal aberrations in mentally retarded patients.

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Submicroscopic chromosomal rearrangements play an important role in the cause of mental retardation. To facilitate the detection of these rearrangements we are implementing DNA-based whole genome deletion and duplication screening using Multiplex Amplifiable Probe

Hybridisation (MAPH). This is a PCR-based method that allows the parallel screening of up to 96 patients for copy number changes at many different chromosomal loci in one experiment.
Specific single copy sequences, (probes), containing identical ends are hybridized to immobilized genomic DNA. These probes are subsequently recovered, quantitatively amplified and analysed using a 96 capillary sequencer, resulting in a peak for each probe. Changes in probe yield correspond to changes in copy number of the genomic sequence analysed.
In total, we have designed 250 probes, corresponding to subtelomeric regions, genes on chromosome 22, XLMR genes, genes involved in microdeletion syndromes, and potentially interesting genes spread throughout the genome. Using these probe sets 200 mentally retarded patients were screened.
To date, we have detected 22 mutations, of which 18 have been confirmed by FISH. These results include both subtelomeric and interstitial rearrangements.
As MAPH has proven to be a quick and accurate method for detecting copy number changes, our ultimate goal is to develop a 3000-loci probe set covering the entire human genome with a one Mb spacing, and implement this for molecular karyotyping in a diagnostic setting.

P481. Use of free fetal DNA for non-invasive fetal sex determination

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Free fetal DNA (ffDNA) can be detected in maternal plasma during pregnancy and used to determine fetal sex. This is useful for managing pregnancies at risk of X-linked disorders and metabolic diseases affecting the external genitalia. Non-invasive assessment with ffDNA could potentially reduce invasive testing (amniocentesis or CVS) by 50% in these situations.
10ml maternal blood was collected from pregnant women at 8-29 weeks gestation and plasma extracted by two centrifugation steps at 3,000G. The supernatant was stored at –20°C prior to DNA extraction using a Qiagen DNA blood Mini kit. Fetal gender was determined using multiplex PCR for SRY and AMELB XY gene loci and the fetal sex was reported from gels stained with ethidium bromide. Results were compared to the sex determined by cytogenetic analysis.
56 women (27 female, 29 male fetuses) agreed to participate and 80 sample runs were carried out. 15 (19%) AmelB probe and 7 (9%) SRY probe results were uninterpretable. AmelB and SRY probes had sensitivities of 76% and 91%, specificities of 82% and 63% and positive predictive values of 85% and 78% respectively to determine the presence of a male fetus.
The SRY probe was more sensitive at detecting male pregnancies but was less specific and had a higher false positive rate than the AmelB probe that also provides a negative control by detection of the 'X' chromosome. Further work is necessary to improve the sensitivity and specificity of the test prior to clinical application. This may be achieved using real time PCR.

P482. A National Diagnostic and Advisory Service for Congenital Myasthenic Syndrome: Review of Molecular Genetic Analysis to date

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Congenital myasthenic syndromes (CMS) are a group of genetically heterogeneous disorders of neuromuscular transmission. Causative mutations may occur in genes encoding the subunits of muscle acetylcholine receptor (AChR), rapsyn, choline acetyltransferase (ChAT) and ColQ. Identification of an underlying mutation is important for clinical and family management. We have established a fully integrated diagnostic and clinical advisory service funded through the National Specialist Commissioning Advisory Group (NSCAG).
Mutations occur most frequently in the AChR epsilon-subunit gene, for which we offer full mutation screening. A similar service is under development for other implicated genes, which, at present, may be analysed for previously identified mutations. Screening is by dHPLC analysis of PCR-amplified products and bi-directional fluorescent sequencing of profile variants. Carrier testing is undertaken by restriction digestion of PCR amplicons. The system has been shown to be sensitive by analysis of 18 mutations previously identified by SSCP; all were correctly identified.
Between 01/04/2002 – 01/02/2003, 107 samples were referred (53 unrelated probands and 54 relatives), mostly for epsilon-subunit testing. Analysis has been completed in 57 patients (35 probands, 22 relatives). Mutations were found in all patients with previously known mutations, ~30% patients of unknown status and 96% of relatives. Twenty-two different mutations have been identified, three of which are novel. There has been one prenatal diagnosis for a family with a ChAT gene mutation.
This integrated diagnostic service for CMS is proving useful for the clinical management and genetic counselling of patients and their families. We review the molecular genetic results to date.

P483. The Italian national project for standardization and quality assurance of genetic testing: the first two year of experience.

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The Italian national project for standardization and quality assurance of genetic tests started in 2000 and its second year of activity finished on September 2002. The project, financially supported by the National Health System, is co-ordinated by D. Taruscio from the Istituto Superiore di Sanità. Laboratories have been enrolled, covering all Italian regions; they have been grouped in 6 inter-regional working Units (WU). Decisions are discussed by the steering commitee, including the WU co-ordinators and reference experts. Laboratories participate anonymously, identified by a code number.
The main activities of the Project are external quality control trials on :
1) cytogenetics (prenatal, postnatal including oncological)
2) molecular genetics (cystic fibrosis, beta-thalassemia, X-fragile syndrome, APC gene).
Two external quality control trials were performed during the two years on cytogenetics and molecular genetics, respectively.
In this second year a higher number of laboratories were enrolled (74 laboratories vs 68) and the percentage of response increased by 5% (from 88.23% in the first year to 93.24% in the second).
In this report, we take into account: a) a comparison between the results obtained by laboratories which participated both in the first and in the second trial; b) results obtained by laboratories which were enrolled during the second year of the Project. In particular, the same level of performance was mantained in postnatal cytogenetics diagnosis, cystic fibrosis and APC gene tests; whereas a significant improvement was observed for beta thalassemia. The main factors involved in the observed changes will be discussed.

P484. Certified Reference Materials in the field of genetic testing and *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 suitable reference materials. In order to receive CE marking for their products, IVD manufacturers are obliged to demonstrate this for test kits including kits for genetic testing. Most of the existing products circumvent the issue by stating „for research purposes only" on the label, but are broadly used in the genetic testing laboratories for diagnostic purposes for the investigation of human sample materials. At present, only a few certified reference materials are available, but development is making progress as reported by standard institutes, like IRMM and NIBSC.
It is evident that in the field of genetic testing 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.
IRMM in close collaboration with IFCC and IVD industry is contributing to various projects aiming at the development of reference measurement procedures and reference materials.
In this lecture, approaches towards standardisation of genetic testing using CRMs are discussed. The certification process including the GUM compliant estimation of uncertainty values for both, homogeneity and stability as well as the contribution of characterisation will be presented.

P485. Outsourcing DNA Purification to Streamline Genetic Analyses.

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Sample collection and purification is the first and often most important part of any genetic analysis. Without high purity and high quality DNA, downstream applications can be easily compromised and valuable samples and resources can be wasted.
Blood quality can vary widely dependent on method of collection, storage conditions, blood age and anti-coagulant used. As such, many automated and kit-based products for the purification of DNA from blood can often have difficulty in achieving reliable, high yielding and high purity DNA from the wide range of samples encountered. Manual methods are therefore the only choice for many sample types to achieve the required quality and recovery rates necessary for specific applications and long-term DNA banking.
Outsourcing the DNA purification is a simple and cost-effective method to guarantee high quality DNA. It enables researchers to concentrate on their core analyses with confidence. The GenXTrak[SM] Purification Service from Whatman is an established contract service for DNA purification from blood-based samples and has been operating for 5 years. The professional and flexible service is used by major Pharmaceutical Companies and some of the leading laboratories in the country. Data is presented here demonstrating the continuous quality and reproducibility of the service together with details of the processes in place to ensure complete sample traceability and integrity.

P486. Genetic discrimination - critical evaluation of official and internet resources

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Genetic tests reveal information which allows individuals and possibly their families to understand and control their inherited health risk. Quite a lot of human diseases have a genetic component that eventually will be relevant to disease prevention or early detection. Promoting the public's health will increasingly require the use of genetic data in practices that improve the health of the public.
This genetic information has the potential to be used out of medical context in ways that are contrary to the interest of the tested individual. It may be used by third parties, most notably by insurance companies and employers.
Over the past years the Internet has become an effective medium for reaching large numbers of health consumers and the general public. An analysis of English and German web sites with ‚genetic discrimination' as topic was performed using predefined criteria fo the evaluation of content.
Ministries of health and law in several relevant countries were contacted to collect information about awareness of the problem and legislation projects.
A lot of problems seem to exist with the information about genetic discrimination:
The definintion of the term genetic information is far from clear.
Genetic information gathered for one individual will willingly or not in most cases reveal information about others.
Even widely accepted medical procedures like prenatal diagnosis can be subsumed under the definition of genetic discrimination.
According to our analysis neither action of lawgivers nor information of general public via Internet are satisfying and appropriate for the magnitude of the problem.

P487. How a fully computerised general practice database enables the introduction of a genetic approach to primary care consultations.

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The computerised health care records of all patients registered with a South Manchester general practice were reviewed .
Total ascertainment of 4400 records were used to construct a comprehensive database of genetically relevant information recorded in the primary health care setting.Clinical areas reviewed were Mendelian and chromosomal disorders, antenatal and contraceptive care, fertility problems, cardiovascular disease , common cancers and self reported family history information.The practice is paperless , all consultations both face to face, telephone and home visits are recorded on computer by general practitioners, nurse practitioner, health visitor and administrative team. Individual patient records are problem based and include scanned referral documents and hospital notes within the patient record.Results include the number of patients in each clinical area reviewed and the value of a recall screen and use of protocols to ensure that patients with genetic and part genetic disorders receive appropriate management and preventive care.The paucity of Read coding for genetic disease on general practice computer systems and the inability to link family history data are limiting factors as is the variability in computer usage amongst primary care professionals in the UK.

P488. The first case of Hunter syndrome (MIM309900) in the population of Vyborg province

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Hunter syndrome (HS) is one of the mucopolysaccharidoses that result from the deficiency of one of the lysosomal enzymes required for glycosaminoglycan (GAG) catabolism. We report on the first case of HS in Vyborg province population. Proband is the 3rd child of healthy, non-consanguineous parents (24 year-old mother and 30 year-old father). He was born after the 7th pregnancy complicated with threatened abortion and toxemia by the 3rd precipitated delivery with umbilical cord repeatedly tightly winding round the neck and with asphyxia. His birth weight was 3250 g (50th centile), his length was 50 cm (25th centile).At 7 yr age his weight was 20kg (25th centile), his length was 109cm (3rd centile). Proband has coarse facial features, progressive joint stiffness and claw hands. He suffers from chronic obstructive bronchitis, recurrent inguinal hernia, sensorineural hearing loss, hepatosplenomegaly (+7cm, +4cm). He has no sings of mental deterioration. Laboratory feature was excretion of large amounts of GAG in the urine. To confirm diagnosis of HS we referred the family to Medical Genetic Research Centre, RAMS (Moscow): iduronate-2 sulphatase activity in white cells was found to be about 2% of normal range and urine GAG electrophoresis features were specific. Proband has two sisters, 14 yr and 13 yr aged. It is very important to realize DNA-molecular investigation to make correct genetic counseling for proband and his sisters. ACE polymorphism was investigated among all members of this family as a part of predictable genetic supplement.

P489. Molecular analysis of the ABCD1 gene in 11 italian patients with X-linked adrenoleukodystrophy

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X-linked adrenoleokodystrophy (X-ALD), the most common inherited peroxisomal disorder, is characterised by progressive demyelination of the central nervous system and ipoadrenalism. Different clinical forms have been recognised and phenotypes include an inflammatory cerebral form (cerALD), the milder, non inflammatory form adrenomyeloneuropathy, (AMN) and variants without neurological involvement. The pathogenetic mechanisms of the different clinical forms are still unclear and there is no apparent correlation between phenotypic presentation and genotypic abnormalities. Up to date

over 500 mutations in the ABCD1 gene have been identified showing different levels and activity of ABCD1 product, ALDP.
We analyzed the complete coding region of ABCD1 gene (10 exons) in 11 subjects (6 X-ALD patients presenting different clinical manifestations and 5 female carriers) from four unrelated families. DNA was processed by PCR amplification and directly sequencing on an ABI 377 automatic sequences. We detected 4 different mutations, each for every family, confirming the high genetic variability of the disease. While one mutation had already been reported (R554H), the 3 remaining changes are novel ABCD1 variants (C88W, Q332X, L503P). All missense mutations were confirmed by appropriate restriction fragment length polymorphism analyses and were absent in at least 100 control chromosomes. The correlation with residual activity of the gene product in cultured skin fibroblast lines obtained from the propositi is being explored.
Our study enlarges the spectrum of molecular variants in X-ALD, but does not show any clues as for genotype-phenotype correlation.

P490. Epidemiology of rare Lipid Storage Disorders in IRAN - A 10 year clinical experience

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Because of the high frequency of consanguinuous marriages in Iran, it seems that the incidence of rare Metabolic Disorders is very high in this population. Among the most common types of these disorders are Lipidoses and MPSs.
Over the past 10 years ago we have evaluate 141 families including 212 cases those were suspected to have inborn errors of metabolism in collaboration with the Genetics and Metabolic Department of Erasmus University, Rotterdam. We have been confronted in some instances with a very rare metabolic disorder which had not been previously reported in Iran.
Of the 141 families the final diagnosis in 54 (70 affected members) was of a mucopolysaccharidosis (MPS), while 54 families with 88 patients were suffering from lipid storage diseases. Prenatal testing was carried out in 34 families and showed that 7 (20.6%) of the fetuses were affected.
According to our data the most common lipid storage disease was Metachromatic Leukodystrophy, followed by Niemann-Pick, Gaucher's, Tay-Sachs, Mucolipidosis, Canavan's, Alexander's, and Sandhoff's diseases respectively.
Our study showed that rare metabolic disorders are very common in our population. For carrying out preventive measures, genetic counseling and detection of high risk families by detailed investigation of index cases and appropriate family members is mandatory. Based on this information detection of affected fetuses by prenatal testing would be possible.

P491. Establishing a highly-informative multi-step carrier detection test appropriate for Iranian phenylketonuria families

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Phenylketonuria(PKU), the most common disorder of amino acid metabolism, is an autosomal recessive disease caused by mutations in phenylalanine hydroxylase (PAH) gene. To date, hundreds of mutations leading to PKU have been identified in this gene. Therefore, direct carrier detection by identifying the causal mutation is impractical in many cases. Consequently, indirect carrier detection of PKU is performed by segregation analysis of polymorphic markers associated with PAH gene.However, it's not a successful approach in all cases because of non-informative meioses . In order to establish a highly- informative carrier detection test for Iranian PKU families, a combination of direct and indirect methods was studied. Direct carrier detection was performed in families identified to harbor one of the three major mutations in Iranian PKU population (IVS10nt546, IVS11nt1g->c and R261Q).In remaining families, indirect carrier detection was carried out by segregation analysis of a VNTR polymorphism located 3000 nucleotides downstream the last exon of PAH gene.Segregation analysis of an STR polymorphism in intron 3 of this gene was finally performed when the former steps were not informative. Carrier status was identified in 44 individuals among 46 unaffected siblings of Iranian PKU families under study. So the established multi-step test had a high informativity of 95% in our sample.

P492. L-dopa responsive dystonia due to sepiapterin reductase deficiency, a tetrahydrobiopterin deficiency presenting without hyperphenylalaninaemia

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Sepiapterin reductase (SPR) catalyses the last step in the biosynthesis of tetrahydrobiopterin (BH_4), the essential cofactor for the aromatic amino acid hydroxylases as well as glycerol-ether monooxygenase and the three isoforms of nitric oxide synthase. The first patients with this disorder were diagnosed in the last two years. Five patients are currently documented, and four missense mutations in exon 2 of the SPR gene have been identified in other populations. Molecular analysis of the SPR gene in 7 patients from 4 unrelated Maltese families led to the identification of a new splicing mutation at the acceptor site of intron 2. The new mutation, denoted IVS2 -2A→G, involves a nucleotide change at the highly conserved AG dinucleotide at the (3') intron acceptor consensus sequence. All patients were homozygotes. The mutation completely abolishes consensus splicing of exon 3. Activation of a possible cryptic splice site, identified 15bp downstream, would result in the deletion of 5 critical amino acids, but no frameshift.
The carrier frequency of the SPR, IVS2 -2A→G mutation was found to be 4.6% by restriction endonuclease digestion of a cohort of random Maltese neonatal DNA samples. Another mutation in the Dihydropteridine Reductase (DHPR) gene in the BH_4 pathway is also frequent among the Maltese (3.3%) raising the possibilities of complex interactions such as trans-heterozygosities and foetal-maternal genotype interactions that could influence mental development.
The high frequency of the two mutations in the Maltese is possibly due to a founder effect followed by a recent exponential growth of the population.

P493. Determination of mutations in G6PD gene in Northern parts of Iran

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Glucose –6- Phosphate Dehydrogenase enzyme is encoded by an x-linked gene with13 exons and its deficiency is one of the most common inherited Enzymopathy disorders of mankind in the world. Two important clinical manifestations of G6PD deficiency are hemolytic anemia and neonatal jaundice. Prevalence of this disease is high in Asia, South of Europe, Africa and Mediterranean regions. There are many G6PD deficient patients in Iran as this country is located in the Middle East. Prevalence of this disease is estimated 8.6 to 16.4% in the Mazandaran Province as people eat lots of Fava beans. In South and North East of Iran this rate is 12 to 22.8% because of high prevalence of Malaria.

The only study about the molecular mutations of G6PD in Iran is performed in Mazandaran by our group in which, 74 patients were examined and the following three genotypes were identified; Mediterranean (66.2%), Chatham (27%) and Cosenza mutation (6.8%).
Also, we extracted DNA from 176 blood samples of Gilan and Golestan (neighboring states of Mazandaran) and then analyzed them using PCR-RFLP with specific suitable restriction enzymes. The results show Mediterranean mutation occurring in 138 cases and Chatham mutation occurring in 21 cases. So, in total percentage 78.97% of individuals have Mediterranean, 11.93% of cases have Chatham and we have been studying other mutations in rest. Distribution pattern of these G6PD variants was more similar to Italian population rather than Middle Eastern countries.

P494. Splicing defects in propionic acidemia: underlying mutations and consequences.

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The effect of different types of propionic acidemia mutations affecting splicing of the PCCA or PCCB genes codifying for the two subunits of propionyl-CoA carboxylase have been analyzed using several experimental approaches. Using quantitative real-time PCR methods we have been able to detect correctly spliced transcripts in very low abundance in fibroblasts of homozygous patients with PCCA splice-site mutations IVS22-2A>G and IVS19+3del4, respectively. The barely detectable amounts of correctly spliced mRNA appear to be sufficient to permit the mild phenotype exhibited by the patients, which could not be explained by the predicted severe effect of the aberrant splicing observed in standard conditions. We have also set up minigene systems to investigate the potential inactivation of exonic splice enhancers (ESEs) by point mutations in the PCCA and PCCB genes. We have observed an aberrant splicing pattern for a point mutation identified in patients with propionic acidemia and initially classified as missense. This and other missense mutations are predicted to abolish binding to a Ser/Arg-rich (SR) protein, when analyzed with the ESE finder web interface (http://exon.cshl.edu/ESE/). The analysis of the consequences of this type of until recently unrecognized splicing mutations is important to gain a better understanding of the correlation between genotype and clinical phenotype and for the development of novel therapies.

P495. Methylmalonic Aciduria in Spain: biochemical and genetic studies of Mutase-deficient (mut) patients.

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The methylmalonic acidurias (MMA) are metabolic disorders resulting from deficient methylmalonyl-CoA mutase (MCM) activity, a cobalamin dependent mitochondrial enzyme. The aim of this work has been to perform the biochemical, clinical and genetic analysis of MMA patients from Spain resulting for functional defects in the MCM protein. Fifteen cell lines from 13 unrelated MMA families were assigned to the mut complementation group by measurement of MCM activity, 14C-propionate incorporation rate ± hydroxycobalamin and/or cell fusion assay. Ten MCM patients exhibited a severe form of the disease, and three presented a mild clinical phenotype. Mutation analysis has been done by RT-PCR and sequencing of the entire coding sequence of the MCM gene and/or sequencing analysis of genomic DNA. We have identified nine different substitutions; two of them are previously described, the nonsense mutation R228X and the recently reported frequent mutation N219Y. We have also found two previously described non-synonymous SNP (H532R and V671I). The new changes found include four missense changes (I69V, Q109R, A324T, L328P) located in the (a/ß)8 barrel domain and one (L617R) located in the cobalamin binding domain. With the exception of I69V, all missense substitution are not conservative changes and are located in highly conserved residues of the protein, being likely disease causing mutations. We have also found three novel frame shift mutations, (669ins8nt,1022-1023insA and 1929delA), all affecting essential ß/a flavodoxin-like domain that binds adenosylcobalamin. The structural consequences of point mutations have been explored using homology models, providing data to discuss the metabolic outcome of our patients.

P496. The phenotypic spectrum of hyperprolinemia: from schizophrenia to mental retardation

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The increased prevalence of schizophrenia among patients with the 22q11 Deletion Syndrome (22q11DS) led us to search for genomic rearrangements within the 22q11 chromosomal region in schizophrenic patients. Screening by Quantitative Multiplex PCR of Short Fluorescent Fragments (QMPSF) of 23 genes in 63 schizophrenic patients and 68 controls allowed us to identify, in a familial form of schizophrenia, a heterozygous deletion of the *PRODH* gene. The *PRODH* gene encodes proline dehydrogenase which ensures the conversion of proline to Δ -1-pyrroline-5-carboxylate, a precursor of two important neuromediators, glutamate and GABA. The schizophrenic patient carrying the *PRODH* deletion had hyperprolinemia (538 µmol/L, N< 290). In 6/12 children presenting a 22q11 DS and therefore an heterozygous deletion of *PRODH*, we also found a moderate hyperprolinemia (average: 355 µmol/L, extreme values: 295-532 µmol/L), which shows that the heterozygous deletion is not sufficient to induce an hyperprolinemia. We then identified several rare heterozygous *PRODH* nucleotide variations in schizophrenic patients with moderate hyperprolinemia. We found the same *PRODH* missense mutations and deletion, but at the homozygous state, in children suffering from the severe form of type I hyperprolinemia (MIM 239500**)**, characterized by seizure and mental retardation and associated with very high level of prolinemia (> 1000 µmol/L), which demonstrates that the severe form of the disease results from homozygous inactivation of the *PRODH* gene. All these data lead us to hypothesize that the cognitive and/or psychiatric disorders found in 1/3 of the patients presenting a 22q11DS could result, at least in part, from hyperprolinemia.

P497. Are there other genes or modifier genes involved in Cystinuria?

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Cystinuria (OMIM 220100) is an autosomal recessive aminoaciduria due to a disorder of renal reabsorption of cystine and dibasic amino acids that results in nephrolithiasis of cystine. The defective transporter belongs to a family of heterodimeric amino acid transporters made up of a heavy (HSHAT) and a light (LSHAT) subunit, linked by a disulphide bond. 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, mostly cause non-type I cystinuria. Up to now mutations have been found in 87% of the non-type I chromosomes, in 76% of the type I chromosomes and in 72% of the untyped chromosomes studied but there still are 23% of unexplained alleles. The existance of another gene or modifier genes for cystinuria could explain these alleles. In this sense two members of LSHAT, asc-1 and LAT-2 could be good candidates: 1) Asc-1 gene (SLC7A10) localizes at chromosome 19q13.1, near SLC7A9 in the critical region for non-typel cystinuria and shows high homology with SLC7A9. 2) LAT-2 (SLC7A8) is involved in trans-epithelial flux of cysteine and could modulate cystine reabsorption, is highly expressed in the epithelial cells of the proximal convulted tubule of the nephron, the region responsible of cystine reabsortion. We have found nucleotid changes in SLC7A8 and SLC7A10, in 39 Spanish patients in which none or one mutation had been found in SLC7A9 and SLC3A1, that could play a role in the cystinuric phenotype.

P498. Frequency of Z and S mutations in Alpha-1-Antitrypsin gene in Iranian childrenaffected by idiopathic liver dysfunction

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Alpha-1-antitrypsin (α1AT) deficiency is an autosomal recessive hereditary disorder resulting from a reduction of enzyme level in serum that predisposes individuals to the development of childhood liver dysfunction and pulmonary emphysema in early adult life.
The most prevalent types of deficient alleles associated with α1AT deficiency are Z and S , caused by two different single point mutations in α1AT gene.
In this research 43 unrelated families including parents and their affected children (100 individuals), referred to our center with idiopathic liver dysfunction and/or pulmonary disease were tested for molecular diagnosis of α1AT mutations Z and S using PCR-RFLP method. Results from molecular diagnosis (α1AT genotyping) were compared with α1AT concentration and its inhibitory activity (i.e.: elastase inhibition activity) obtained from Serum Radial Immuno Diffusion (SRID) and biochemical inhibition assay respectively. The association of α1AT genotype with serum concentration and inhibitory activity was also determined.
Low frequency of Z mutation among Iranian population is compatible to the previous reports. More investigation is being carried out using SSCP/HD and sequencing to identify the presence of other possible mutations in this population.

P499. Biochemical and molecular diagnosis of galactosemia in Iranian infants with galactosemic symptoms.

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Galactosemia is an inborn disorder of galactose metabolism that is inherited in an autosomal recessive manner.Classical galactosemia is caused by deficient activity of the galactose-1-phosphate uridyltransferase (GALT) enzyme that can result in galactosemia complications.
In this research for biochemical diagnosis of each case, urine was tested for the presence of reducing substance using urinary dipstick followed by thin layer chromatography of urinary sugars to establish the presence of galactose band. The diagnosis of galactosemia was confirmed by qualitative measurement of GALT activity in erythrocytes using the established Beutler enzyme assay procedure. Blood sample from patient‘s parents were used at the same time as well as controls for GALT activity. For the families with confirmed galactosemia, DNA was extracted from blood samples and molecular diagnosis of the three most common mutations of galactosemia (Q188R, K285N, and L195P) were performed using PCR-RFLP method.
6 out of 42 families referred to NRCGEB, with galactosemic symptoms, were diagnosed and confirmed by biochemical tests. Among them, 3 families with Q188R mutation and one family with K285N mutation were detected. More investigation is being carried out on Iranian patients with biochemically confirmed galactosemia, concerning determination of the most common mutations among this population.

P500. Clinical and molecular characterization of Gaucher disease in Saudi Arabia

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Gaucher disease is an autosomal recessive lipid storage disorder caused by mutations in the GBA gene that encodes for the lysosomal ezyme glucocerebrosidase. The disorder is both clinically and gentically heterogenious. There are at least three well recognized clinical variants of the disease: type1, chronic neuronopathic; type2, acute neuronopathic; and type 3, subacute neuronopathic. We have diagnosed twenty one patients with this disorder at our institue over the last 15yrs. Nine patients have lost follow up and mostly were before the introduction of enzyme replacement therapy (ERT. The diagnosis was confirmed by measurment of glucocerebrosidase in leucocytes. One patient had bone marrow transplantation and is doing well. Eleven patients were started on ERT. Among this group one died at a different institute of unclear cause. Two have lost follow up. Currently 8 patients are on replacement therapy with the recombinant enzyme Imiglucerase, and have regular follow up at our institue. The symptoms and signs of the disease in all patients started in infancy with hepatosplenomegally as the main manifestation. The full clinical presentation of these patients and response to therapy will be discussed. The underlying molecular defect will be highlighted with emphasis on genotype/ phenotype correlation.

P501. Caenorhabditis elegans as a model for lysosomal glycosidase deficiencies

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Pompe disease (a-glucosidase (GAA) deficiency), Fabry disease (a-galactosidase (a-GAL) deficiency) and Schindler disease (a-N-acetylgalactosaminidase (a-NAGA) deficiency) are common human lysosomal storage disorders. We have explored the relevance of C.elegans as a model for the study of molecular pathology of these disorders.
GAA, a-GAL and a-NAGA activity measurements in C.elegans mixed culture revealed high activities for all three enzymes.
BlastP searches for GAA, a-GAL and a-NAGA orthologs in the C.elegans genomic database, revealed four predicted genes with high GAA family homology (D2096.3, F52D1.1, R05F9.12 and F53F4.8) and a single predicted gene (R07B7.11) both for a-GAL and a-NAGA. Phylogenetic analysis of evolutionary relevant sequences supported common ancestral origin of mammalian a-GAL and a-NAGA.
Full lenght cDNAs for R07B7.11 and D2096.3 were amplified and sequenced including 5´ and 3´ UTR to confirm the in-silico prediction. Sequencing revealed SL1 trans-splicing RNA pattern for R07B7.11 and SL2 trans-splicing for D2096.3.
C-terminal GFP tagging was used to evaluate the expression pattern and to define functional promoter. 5kb extrachromosomal GFP expressing promoter D2096.3 construct showed broad expression pattern including intestine, pharyngeal and vulval muscle, coelomocytes and head and tail neurones during all developmental stages of C.elegans.
RNA interference (RNAi) method employing sequenced cDNA clones and genomic DNA as templates for dsRNA synthesis was performed to evaluate RNAi phenotypic presentation of D2096.3. Strong egg-laying phenotype was observed in at least 20% of the progeny of either N2 or rrf-3 injected worms.
Additional biochemical and molecular analyses are in progress to explore the relevance of the generated model.

P502. A case of ketoacidosis due to 2-Methyl Acetoacetyl - Co A Thiolase deficiency

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An 18-month-old female child was referred for metabolic studies with a history of vomiting, dehydration, and hypotonia, lethargy followed by coma. The arterial blood gas analysis at the time of coma revealed metabolic acidosis with partial respiratory compensation. Blood pH was 7.1, bicarbonate 3.0 mmol/l and base excess -23.9 mmol/l. Random blood glucose level is 86 mg/dl. Serum lactate was slightly high (32mg/dl) and pyruvate was normal (0.54 mg/dl). Urine was positive for DNPH and ketone bodies IV fluid therapy by glucose and sodium bicarbonate brought the pH to 7.5, bicarbonate to 36.4 mmol/l and base excess to 14.9 mmol/l. MRI findings showed bilaterally symmetrical hyperintensities involving the globus pallidus

and heads of caudate nuclei bilaterally. These findings followed by other clinical parameters suggest the diagnosis of a mitochnodrial encephalopathy. Urinary organic acid analysis by GC-MS showed normal lactate and methyl malonate; moderately high levels of 2-methyl 3-hydroxy butyrate and 2-methyl glutaconate; and elevated tiglyglycine. These findings indicate a block in isoleucine catabolism due to the deficiency of 2-Methyl Acetoacetyl - Co A Thiolase deficiency. Treatment included correction of acidosis with glucose supplementation and protein restriction, the child recovered and is put on carnitine supplementation with protein restriction. On follow-up, the mental, motor milestones were normal.

P503. Symptoms and signs of Fabry disease in patients below 18 years of age in FOS - the Fabry Outcome Survey

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Fabry disease is a rare X-linked lysosomal storage disorder. FOS – the Fabry Outcome Survey – is a unique database of the natural history of the disease and the effects of enzyme replacement therapy with agalsidase alfa. The database now contains demographic data from 35 children (18 boys and 17 girls) below 18 years of age with Fabry disease, confirmed by enzymology/mutation analysis. The median age at entry into FOS was 14.3 and 15.9 years in boys and girls, respectively. About 80% of all patients had neurological features of the disease, including acroparaesthesia, altered temperature sensitivity and dyshydrosis. Gastrointestinal symptoms (abdominal pain and altered bowel habits), tinnitus, vertigo, fatigue and angiokeratoma were also present in more than 50% of patients. These clinical features were noted in early childhood (< 12 years of age) with a similar frequency in both sexes. Contrary to the common view that females heterozygous for Fabry disease are asymptomatic, these results have shown a similar distribution of signs and symptoms in both males and females. This is the largest cohort of children with Fabry disease that has been studied. Documentation of the phenotype of this progressive disease in children is important. Misdiagnosis is common, and clinicians should be aware of the variety of signs and symptoms that even young children may present with, particularly as enzyme replacement therapy is now available.

P504. Haplotype background in gypsie patients with 985A→ G mutation in the *MCAD* gene.

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Medium Chain Acyl-CoA dehydrogenase deficiency (MCAD; MIM 201450) is the most common disorder of mitochondrial fatty acids beta-oxidation. The majority of the MCAD patients (*c.a.* 80%) are homozygous for the 985A→ G mutation, which has been always associated with haplotype 112. A high carrier frequency for the *G985* mutation (1/64-1/101) was observed in the northwestern European population, displaying a North / South gradient. In Portugal the 985A→ G mutation was identified only in MCAD patients of Gypsy ancestry.

In the present study, the haplotype background was investigated for the G985 mutation in the Gypsy MCAD patients (7 patients, including two siblings) diagnosed through biochemical studies and molecular characterization.

The study included six families corresponding to twelve independent alleles. The haplotype identification was ascribed by the evaluation of three intragenic MCAD gene polymorphisms (*Bam*HI, *Pst*I and *Taq*I).The achieved data showed that G985 alleles are in linkage disequilibrium with haplotype 112, suggesting the same origin as the one described in the European countries. In agreement with the migrational history of the Gypsies (Indian subcontinent) our results are consistent with the hypothesis that the 985A→ G mutation was brought into Europe by Indo-European speaking people. Studies are in course to determine the microsatellite polymorphism GT repeats.

P505. Clinical heterogeneity of Fabry disease in one family: Comparison of the phenotypic expression in affected female twins with their "asymptomatic" father

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Case reports: The index patient was a monozygotic female twin (20 years old) with a medical history of collateral ureteroceles that were surgically corrected at the age of 3 years. At the age of 17 years the patient developed increasing proteinuria of more than 2g/24 h with slightly diminished creatinine clearance of 77,2 ml/min/1,73 m² and serum creatinine levels of 1,09 mg/dl. Angiokeratoma were evident. There were no other symptoms of Fabry disease. Because of proteinuria a kidney biopsy was done, from which the diagnosis of Fabry disease was made. The molecular gene analysis confirmed the diagnosis.

The clinical presentation of the second female twin included only proteinuria of 230 mg/24h with a creatinine clearance of 101 ml/min/1,73 m² and serum creatinine of 0,84 mg/dl. She had angiokeratoma and recurrent burning pain in the fingertips. In the molecular gene analysis the same mutation as in the first twin was detected. There were no affections of the cardiac, gastrointestinal and visual-hearing system.

The molecular gene analysis in the father showed the same mutation in the α-galactosidase gene. Enzyme analysis confirmed the diagnosis. Angiokeratoma and an arterial hypertonia were the only pathological clinical findings. No other organ system involvement was seen.

Conclusion: The variability of clinical manifestation in this family especially in the twins proves the phenotypic heterogeneity in males and females with Fabry disease. Also in hemizygous males the range of clinical manifestation can vary from nearly "asymptomatic" to severe as we have already seen in females.

P506. Mutation analysis of isovaleric acidemia and identified three novel mutations

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Isovaleric acidemia (IVA) is a rare inborn error of metabolism caused by a deficiency of isovaleryl-CoA dehydrogenase (IVD). In the past 30 years, GC/MS was used to identify IVA by urine organic acid analysis. High-level isovalerylglycine was found in IVA patients' urine. Because of laborious and time-consuming sample preparation procedures and a long analytical time (40 -60 minutes), GC/MS cannot be applied to high throughput newborn screening for metabolic disease. From 1992, LC tandem mass spectroscopy(LC/MS/MS) was introduced for acylcarnitine analysis in blood, allowing detection of about 20 different metabolic disorders. LC/MS/MS allows rapid sample preparation and analysis andhas been applied in neonatal screening. In IVA patients the blood acylcarnitine profile reveals isovalerylcarnitine in high concentration. This compound can help us to identify IVA in newborn infants. During the past 2 years, two new IVA cases were found in our neonatal metabolic disorder screening. The concentration of isovalerylcarnitine in these two patients' blood was 2.91 and 2.37μM respectively, over four times our normal upper limit (0.51μM). In order to identify the gene mutation, the patients and their parents' *IVD* genes were amplified by PCR and subjected to direct sequencing. Three novel mutations were identified. The first patient had a homozygous mutation in exon 2 (G149A) that caused an arginine to histidine substitution in codon 50. The second patient's mutations were in exon 4 (A386G) and 12 (A1199G). These caused histidine to arginine in codon 142 and tyrosine to cystine substitution in codon 400 respectively.

P507. Validation of PAH genotype-based predictions of metabolic phenylalanine hydroxylase deficiency phenotype

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Phenylalanine hydroxylase (PheOH) deficiency is inherited as an

autosomal recessive trait. The associated hyperphenylalaninaemia phenotype is highly variable, primary due to allelic heterogeneity in the *PAH* locus. The aim of this study was to investigate the relationship between individual *PAH* mutations and biochemical and metabolic phenotypes in patients with phenylketonuria (PKU) and mild hyperphenylalaninaemia (MHP).
In this study, a total of 184 independent *PAH* chromosomes (92 unrelated patients with PKU and MHP residing in Lithuania) were investigated.
All 13 exons of the *PAH* gene of all PKU probands tested were scanned for DNA sequence alterations by denaturing gradient gel electrophoresis (DGGE), mutations were identified by direct fluorescent automated sequencing or by restriction enzyme digestion analysis of a relevant exons. *PAH* genotype-based prediction of metabolic PhOH deficiency phenotype in PKU/MHP patients form Lithuania was estimated by methods of assigned value (AV) and functional hemizygosity.
Our data provide evidence that a simple genotype-phenotype correlation does exist in most patients with PheOH deficiency: we observed a perfect match between the expected and observed phenotypes in 96% of the cases investigated.
The results obtained confirm that methods of functional hemizygosity and AV sum are applicable for the estimation of the genotype-phenotype correlation in the investigated group of PKU/MHP patients.

P508. Genotype-phenotype correlation in phenylketonuria patients from Ukraine

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In our study we have investigated genotype-phenotype relationships in a group of patients with PKU from the Ukraine. The mutation analysis was performed in101 unrelated patients. 144 mutant alleles were characterised. There were 11 different mutations and 15 different genotypes. In order to establish genotype-phenotype relationship, we have collected clinical data for 18 patients with two identified mutations. According to the established classification, based on the two most important biochemical parameters (pretreatment serum phenylalanine concentration and phenylalanine tolerance), 15 patients have “classical” PKU, 2 have “moderate” PKU and 1 has “mild” PKU. The major group of patients with “classical” PKU comprised homozygotes for the R408W mutation (7) and compound heterozygotes of various genotypes (R408W/R158Q, R408W/R261Q, R408W/Y414C, R408W/Ivs10nt546, R408W/Ivs12nt1, S273F/R413P). Among patients with “moderate” PKU we have identified two different genotypes: R408W/P281L and R408W/R252W. It is interesting to note that these two patients with late diagnosed PKU have “moderate” PKU, yet their genotypes consist of three PKU mutant alleles with zero residual *in vitro* enzymatic activity. The patient with “mild” PKU has genotype R408W/Y414C. It is important to note that the same genotype was identified for a patient with severe “classic” PKU. On the basis of this genotype–phenotype correlation data, we propose the existence of a non-*PAH* modifier gene involved in PKU pathogenesis.

P509. Phenylketonuria mutations at the phenylalanine hydroxylase gene in population of Moldova.

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Introduction. Phenylketonuria (PKU) is an autosomal recessive disease that results from a reduction in phenylalanine hydroxylase (PAH) activity. The *PAH* gene has been cloned and mapped to chromosome 12q22-24.1 and it spans approximately 90kb. The cDNA is 2.5kb long and contains 13 exons that vary in length from 150 to 350bp.
Methods. In this study we examined 48 Moldavian families with classical PKU. Genomic DNA from patients and parents was extracted from whole blood using standard procedures. PCR amplification was performed for various exons of the *PAH* gene as described previously. A diagnostic test for the presence of *PAH* mutations was performed by digesting the PCR amplified DNA with restriction enzymes StyI, DdeI, MspI, AvaI, HinfI and RsaI. The fragments were separated by 7.5% polyacrylamide gel electrophoresis and stained by ethidium bromide.
Results. A total of 100 mutant PKU alleles were analysed for 8 prevalent Caucasian mutations in the *PAH* gene. The most common molecular defect was R408W (51%), with a very high degree of homozygosity (34.2%). The P281L mutation accounted 6 per cent of all mutant alleles, R158Q and R252W for 3% each. Rarer mutations included IVS10nt546, R261Q and IVS12nt1, at 2% each. The I65T mutation, that has been predominantly described for moderate PKU, was not detected in our population. **Conclusion.** Mutation analysis in the population of Moldova identifies 71% of PKU alleles, and is suitable for carrier detection and direct prenatal diagnosis.

P510. The molecular basis of phenylketonuria in Latvia

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Phenylketonuria is an autosomal recessive disease caused by deficiency of hepatic phenylalanine hydroxylase (PAH), a non-heme iron mono-oxygenase which catalyzes the conversion of phenylalanine to tyrosine. It is characterized by hyperphenylalaninemia leading to impaired cognitive development and function.
Characterization of the molecular basis of PKU in Latvia has been accomplished through analysis of 96 unrelated chromosomes from 50 Latvian PKU patients. *PAH* gene mutations have been analyzed through a two-step approach. First, *PAH* exons 5, 7, 11 and 12 were tested for the R158Q, R252W, R261Q, G272X, IVS10nt-11g>a and R408W mutations by PCR followed where necessary by digestion with the appropriate diagnostic enzyme. Subsequently ‘broad range’ denaturing gradient gel electrophoresis analysis of the 13 *PAH* gene exons was used to study uncharacterized PKU chromosomes. A mutation detection rate of 98% was achieved. 12 different mutations were found, with the most frequent mutation, R408W, accounting for 76 % of Latvian PKU alleles. Six mutations (R408W, E280K, R158Q, A104D, R261Q and P281L) represent 92 % of PKU chromosomes. 28 (58 %) of 48 completely characterized PKU patients are homozygous for R408W, the remaining 20 patients are compound heterozygotes. This is consistent with the fact that most Latvian PKU patients have the classical form of the disease. These results show a high degree of homogeneity in the molecular basis of PKU in Latvia. *PAH* VNTR and STR alleles have also been identified and minihaplotype associations with PKU mutations were determined.

P511. Identification of novel mutations in the *PAH* gene in phenylketonuria patients in India

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Phenylketonuria (PKU) is an autosomal recessive disorder caused due to mutations in the phenylalanine hydroxylase (*PAH*) gene. The mutations result in deficiency in the enzyme activity leading to toxic accumulation of phenylalanine in the body resulting in mental retardation and organ damage. More than 400 mutations in the *PAH* gene have been identified so far in various populations. We have begun the first molecular genetic analysis of PKU in India. Three patients (including two siblings) were diagnosed with PKU based on the presence of highly elevated levels of phenylalanine in the urine and blood samples (by HPLC analysis). All the 13 exons of the *PAH* gene were PCR-amplified by using primers listed in http://www.geneticahumana.lt/MOLGENT_pages/PAH-protocols.htm. DNA sequencing was carried out for both strands of the 13 exons for all three patients. We report here two novel mutations that result in PKU. One mutation is present in the 3’ splice site of the second intron (c.168-2A->G) in the two siblings (the parents were heterozygous for the same mutation) and the second mutation is present in the first intron (c.60+5G->A) of the *PAH* gene. This is the first report of a *PAH* mutation occurring in the 3’ splice site of the second intron. The c.60+5G->T mutation in the first intron has been reported previously but this is the first report of the G->A mutation for this position. This

genetic analysis will be useful for prenatal diagnosis and genetic counseling and for the identification of mutations in different ethnic populations.

P512. Analysis of new PAH gene mutation IVS12+del4 in phenilketonuria patients from Russian regions.

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Phenylketonuria is an autosomal recessive disease caused by mutations in the phenylalanine hydroxylase gene (*PAH*). More than 400 mutations have so far been described. This disease is associated with severe mental retardation.
We have studied the most frequent mutations in exons 3, 5, 7, 11 and 12 of the *PAH* gene by ACRS PCR analysis in patients from different Russian regions.
As well as known mutations, we have identified a new mutation IVS12+del4 in exon 12 of *PAH*. This mutation is a 4 bp deletion which results in absence of the first site splice nucleotides, but the following four nucleotides are the same. According to the splice site prediction program this mutation should make a weak splice site, but we have no RNA samples to confirm this. We have studied 116 PKU patients from the Samara, Voronez, Ekaterinburg and Moscow regions and 81 healthy controls from the same regions. We have found Ithe VS12+del4 mutation in 6 independent chromosomes (2.6%) among the affected people, and in 1 chromosome in the control group that may be considered as a heterozygous carrier.

P513. Mutation and haplotype analyses on 21 Spanish patients with GM1 gangliosidosis: 13 novel mutations

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GM1 gangliosidosis and Morquio B disease are distinct disorders both clinically and biochemically, although they arise from the same hereditary deficiency of lysosomal acid beta-galactosidase. Three major clinical phenotypes are distinguished in GM1 gangliosidosis according to age of onset and severity of symptoms: infantile, juvenile and adult. GM1 gangliosidosis is a neurosomatic disease noted by visceromegaly and neurologic symptoms, whereas Morquio B is a mucopolysaccharidosis free of neurological symptoms with skeletal involvement.
We performed mutation analysis on 21 Spanish GM1 patients by sequencing the 16 exons of the GLB1 gene and all the 42 mutant alleles were characterized. We found 18 different mutations, 13 of which are novel. All the mutations reported here were confirmed by restriction enzyme analysis. None of the novel mutations were found in 100 control chromosomes. Besides, four Morquio B patients are currently being analysed.
We also analysed 4 previously described polymorphisms (L10P, L12L, R521C, S532G) as well as a new one (IVS12+8) and used them to perform a haplotype analysis on the GM1 gangliosidosis patients. This haplotype analysis showed that 5 patients of gypsy origin who shared the same mutation (R59H) also shared the same haplotype, indicating a possible common origin of the mutation in this ethnic group.

P514. Lysinuric protein intolerance: functional studies of SLC7A7 mutations.

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Lysinuric protein intolerance (LPI, MIM 222700) is an autosomal recessive defect of cationic amino acid (CAA) transport at the basolateral membrane of epithelial cells in the intestine and kidney, caused by mutations of the *SLC7A7* gene. CAA transport is mediated by y+L system which is represented by a heterodimer consisting of 4F2hc and SLC7A7. This activity is also exerted by also another heterodimeric complex composed by 4F2hc and SLC7A6. The co-expression of 4F2hc with SLC7A7 or SLC7A6 induces the system y+L activity. Two basic molecular aspects of LPI are still unclear: the extreme variability of the LPI phenotype along with a multisystem involvement, and the possible capacity of SLC7A6 to compensate for CAA transport when SLC7A7 is defective. By direct sequencing of *SLC7A7* gene (all exons and exon-flanking intronic sequences), we identified 17 causative mutations in LPI patients, originating from Italy, Tunisia, Greek, Algeria, Pakistan and Japan. We expressed nine of these mutations in *X. laevis* oocytes and mammalian cells and we explored the role of a possible interaction between SLC7A7 and SLC7A6. We found that all mutations abolish arginine transport as compared with the 'wild-type' control except for the F152L mutation that reduces arginine transport by about 50%. In addition, we identified a mutation, E36del, with a dominant-negative effect. Our results indicated a heterotetrameric structure of the CAA transporter and a possible interference between SLC7A7 and SLC7A6. This view will improve understanding of the molecular mechanisms underlying the multisystem involvement of this severe disease.

P515. Analysis of the *CYP21* gene by *Taq* I digestion in a PCR product 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. For the identification of the *CYP21* gene deletion, it is necessary to digest genomic DNA with *Taq* I endonuclease followed by Southern analysis using radio-labeled probe to examine the existence of the 3.2/3.7-kb fragment. This procedure is time-consuming and involves the use of radioisotopes. Here, we present a direct PCR-based amplification procedure for direct analysis of deletion in the *CYP21* gene using a locus-specific sense primer located in the *tenascin* B (*TNXB*) gene deleting 120-bp in *tenascin* A (*TNXA*) and an antisense primer located at the 5' terminus of the *CYP21P* and *CYP21* genes. Since a primer is lacking in the *TNXA* gene for the amplification of the *CYP21P* gene, a 6.2-kb fragment of the *CYP21* gene including the neighboring *TNXB* gene was derived. The PCR product was then directly subjected to *Taq* I digestion and analysis on agarose gel. Our result indicates that the PCR amplification is specific for the *CYP21* gene and the PCR product generated is used not only for the identification of the *CYP21* gene but also for the examination of the deletion status of *CYP21* gene.

P516. Phenotype and gene changes in congenital adrenal hyperplasia (CAH) due to 21 hydroxylase deficiency in the Republic of Macedonia

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Congenital adrenal hyperplasia is a recessively inherited disease caused by mutations and gene conversion in the *CYP21* gene. Various clinical forms have been described and documented, from severe salt losing syndrome to late onset forms that appear only in adolescence.
Our study aimed to explore genotypes in patients with CAH and different clinical expression.
29 patients with CAH were completely genotyped. Most of them had the salt losing form (N=16), 5 had the simple virilizing form, and 8 had the late onset form of the disease. Genotype analysis was performed using SSCP and PCR with sequence specific primers.
The most common mutation (39.4 % of explored chromosomes) was I2 splicing mutation, followed by the V281L (21.2 %), del + 8bp del (15.2 %), and G318 (12.1 %). In general, genotypes corresponded to the phenotype as described in the literature. Thus, I2 mutation was most common in salt losing forms, whereas V281L appeared frequently in late onset forms of the disease. In simple virilizing forms, different combinations of gene conversions were seen. Parents of

the children were also genotyped, and interestingly, two fathers were homozygous for I2 mutation without any clinical expression. It seems that other mechanisms than genotype only might contribute to the final expression of the disease.
In conclusion, genotype/phenotype correlation in children with CAH in Macedonia does not differ significantly from other reported studies in Europe. Late onset forms are more common as described in Mediterranean countries.

P517. C/EBPbeta-dependent activation of the TNFalpha-inducible expression of MEFV, the gene involved in FMF

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Familial Mediterranean fever (FMF) is a recessively inherited inflammatory disorder, characterized by recurrent attacks of fever and serositis and due to mutations in MEFV, a gene encoding a protein named marenostrin or pyrin (M/P). Although the function of M/P remains unknown, several lines of evidence --including those deduced from the disease phenotype of FMF patients, the tissue-specific expression of MEFV, as well as the TNFalpha-inducibility of MEFV gene expression -- strongly suggest that this protein is implicated in the regulation of inflammatory processes.
The purpose of this study was to determine the mechanisms by which TNF-alpha regulates MEFV gene expression. To test this hypothesis, HeLa cells were transfected with a 1kb-fragment of the 5'-flanking region of the human MEFV gene linked to a luciferase coding sequence, and grown in the presence or in the absence of TNF-alpha. We showed that MEFV promoter activity was increased by TNF-alpha (5 to 10-fold).
By performing deletion and mutation analyses of the MEFV promoter together with electrophoretic mobility shift assays, we have demonstrated that TNFalpha-induced expression of MEFV is dependent on both NFkB p65 and C/EBPbeta. These two transcription factors, however, act differently on the TNFalpha-dependent transcription of MEFV: C/EBPbeta represents the key regulatory factor that is required to confer cell responsiveness to TNF-alpha, whereas NFkB p65 increases this response by means of a cooperative interaction with C/EBPbeta, thereby providing an unusual example of a cross-talk between C/EBP and NFkB pathways in TNF-alpha signaling.

P518. Expression and subcellular localisation of pyrin proteins carrying the most common mutations involved in familial mediterranean fever

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Mutations in MEFV, a gene encoding the pyrin, are associated with familial Mediterranean fever, a genetic condition characterised by febrile episodes of serosal inflammation. The function of pyrin is still unclear, although recently, the ASC (Apoptotis Speck protein containing a CARD) protein has been shown to interact with the pyrin domain of pyrin, both proteins being colocalized in specks, suggesting that pyrin may play a role in regulation of apoptosis.To study the influence of MEFV mutations on the subcellular localisation of pyrin, we transiently expressed in HeLa cells wild type pyrin-GFP (pyrin fused to GFP protein) or pyrin-GFP harbouring either the M694V, M694I, V726A, M680I or E148Q mutation; we also co-expressed the different forms of pyrin-GFP (wild type and mutated forms) together with ASC (fused to a V5 tag and revealed by a Cy3-conjugate secondary antibody). In cells transfected with pyrin, we found that all the mutated pyrin-GFP proteins localised exclusively in the cytoplasm, with a pattern similar to the one observed with the wild type pyrin-GFP. In cells co-expressing ASC and pyrin-GFP, the green fluorescence due to pyrin-GFP and the red fluorescence corresponding to ASC were concentrated in specks, whatever the pyrin-GFP construct (wild type or mutated forms). These results suggest that these MEFV mutations do not affect the subcellular localisation of pyrin. In addition, whatever the consequences of the mutant pyrin protein in the regulation of apoptosis, it is tempting to speculate that these consequences do not result from an absence of ASC/pyrin colocalisation.

P519. MEFV gene analysis in patients with familial Mediterranean fever from Karabakh

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Familial Mediterranean fever (FMF) is an autosomal recessive disorder common in populations of Armenian, Turkish, Arab or Sephardic Jewish ancestry. To delineate the mutation spectrum in another population of Mediterranean extraction, we investigated 50 patients living in Karabakh, the population of which has been isolated from the actual frontier of Armenia since about 100 years.
Screening for mutations of all MEFV coding exons and intronic boundaries by DGGE revealed a mutation spectrum significantly different from that reported in Armenia. The frequency of non-identified (NI) MEFV alleles is much higher among patients from Karabakh than among those from Armenia (26% vs 7%, P=5.10-5). As no particular MEFV haplotype was over represented among the NI alleles, we excluded the existence of a unique and rare MEFV mutation resulting from a founder effect that could have escaped our mutation screening procedure. The spectrum of identified mutations among Karabakhtsis and Armenian patients is also different: the M694V and R761H mutations were more frequent among Karabakhtsis patients (P=0.01 and 0.04, respectively) and, conversely, the V726A mutation represented only 5% of identified alleles among Karabakhtsis patients, whereas it accounted for 26% of characterized FMF alleles in Armenian patients (P=0.0003); the M680I mutation was found at a similar frequency in the two populations. No new MEFV mutation was identified among the Karabakhtsis patients. Altogether, these results show that the mutation spectrum differs significantly between the two populations, an observation which may result from the relative independent evolution of each population since the beginning of the XXth century.

P520. Familial Mediterranean Fever in the Hellenic population of Greece and Cyprus.

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Familial Mediterranean Fever (FMF) is a hereditary inflammatory disease with autosomal recessive transmission. Typically it presents as acute episodes of periodic fever accompanied by abdominal pain, chest pain, or joint pain. Appearance of renal amyloidosis indicates severe prognosis. About 40 mutations have been identified so far, some of them being very frequent. 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 136 Cypriot MEFV chromosomes analysed, the results are: V726A 27.2%; F479L 21.3%; M694V 21.3%; E148Q 5.9%; M694I 2.2%; R761H 3.7%; M680I 1.5%; unknown 18.4%. Mutation F479L is rather rare in other populations. Preliminary evidence suggests that this frequent, in the Cypriot population, 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 8 of 78 patients carried it, supporting its mild nature. In a number of Hellenic samples from Greece tested, the F479L mutation was not present, whereas in a cohort of patients with childhood onset of FMF, F479L was found in only one patient in heterozygosity. In this same cohort the severe mutations M694I and M694V were highly represented, in accordance with the childhood onset of disease. Funded by the Cyprus Research Promotion Foundation.

P521. Clinical diagnosis of familial Mediterranean fever versus genotyping of *MEFV*

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Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by recurrent attacks of fever and serositis, common in populations of Sephardic Jewish, Armenian, Arab and Turkish origin. Early diagnosis is crucial to start colchicine therapy that prevents the occurrence of attacks and amyloidosis. It is based on two sets of established clinical criteria (from Livneh et al.; Tel Hashomer) that are widely used in clinical practice. More recently, identification of *MEFV* mutations has provided an objective diagnostic criterion for FMF. To assess both the sensitivity and the specificity of those accepted clinical criteria, we screened *MEFV* for mutations in a large sample of patients (n=417) from at-risk populations. Mutation analysis included the screening of at least exons 10, 5, 3 and 2.
The table shows of the genotypes of patients according to the clinical probability of FMF as indicated by the criteria from Livneh et al. or Tel Hashomer.

Diagnosis of FMF		Two mutations	One mutation	
Livneh et al.	Yes	142	93	
	No	17	6	15
Tel Hashomer	Definite	72	22	
	Possible	42	25	26
	Unlikely	45	52	116

The sensitivity and specificity of the criteria from Livneh et al. are 89% and 8%, respectively, whereas, regarding Tel Hashomer criteria, sensitivity is 72% and specificity is 65%. Those results, which should be taken into account for management of this disease, leave open the possible existence of so far unknown *MEFV* mutations and/or genetic heterogeneity.

P522. Molecular genetic diagnosis of Familial Mediterranean Fever by PCR and reverse-hybridization

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Familial Mediterranean fever (FMF) is an autosomal-recessive inherited inflammatory disorder that is characterized by recurrent, short, self-limiting bouts of fever, accompanied by pain in the abdomen, chest or joints, and sometimes associated with erysipelas-like erythema. The most severe complication is progressive amyloidosis, ultimately leading to renal failure. FMF predominantly affects Turks, Arabs, Armenians and Sephardic Jews (carrier rates up to 1:5), but has been observed in lower frequencies throughout the Mediterranean area. It is caused by mutations within the marenostrin/pyrin gene (MEFV) on chromosome 16p13.3, which differently affect the severity of the disease phenotype and the risk to develop renal amyloidosis. Owing to the rather nonspecific clinical symptoms, molecular genetic analysis significantly improves early and correct diagnosis of FMF, and allows to commence lifelong prophylactic treatment of affected individuals with colchicine.
We have developed a reverse-hybridization assay (FMF StripAssay) for the rapid and simultaneous detection of the following 12 MEFV mutations: E148Q, P369S, F479L, M680I (G/C), M680I (G/A), I692del, M694V, M694I, K695R, V726A, A744S, R761H. The test is based on multiplex DNA amplification of exons 2, 3, 5 and 10, and hybridization to a teststrip presenting a parallel array of allele-specific oligonucleotide probes for each mutation. The entire procedure from blood sampling to the identification of mutations requires less than 6 hours, and hybridization/detection may be carried out manually or essentially automated using existing instrumentation (e.g. TECAN profiBlot). The test is simple and convenient, requires very small amounts of samples, and can easily be modified to include additional mutations.
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P523. Novel Intron Alterations - Molecular Findings in Familial Mediterranean Fever

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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 represents the most severe complication of the disease which primarily affects populations bordering the Mediterranean Sea, but due to migrations FMF becomes a more common medical problem.
In 1997, the gene *MEFV* responsible for FMF was identified. The gene product consists of 781 amino acids encoded by 10 exons. The specific protein function remains unclear but its expression in polynuclear leukocytes suggests an essential role in inflammation processes.
Two cases, with intronic alterations will be reported. Both patients were 6-year-old boys presenting with recurrent fever attacks in short intervals associated with episodes of severe pain. Their inflammatory attacks lasted for years being resistant to therapy. The molecular analysis of the *MEFV* gene in the first case revealed a deletion of the nucleotide G in position +148 and a transversion C>A in position +146 in intron 8 (IVS8+146C>A/IVS8+148delG). The second case was a carrier of the well characterized amino acid substitution E148Q in exon 2. In addition, a novel transversion A to C in nucleotide position +47 in intron 6 (IVS6+47A>C) was detected. The described alterations in the introns were not found in 200 chromosomes of a control group. The putative splice aberrations will be discussed together with the clinical phenotypes as they could be associated with the disease or represent rare polymorphisms.

P524. Long term stabilization after bone marrow transplantation in an adolescent case of metachromatic leukodystrophy

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Metachromatic leukodystrophy is a neurolipidosis due to a deficiency of arylsulfatase A (ASA) which gives rise to an excess of undegraded sulfogalactosylceramides (sulfatides). We have treated a case of a 19 year old man by bone marrow transplantation. He started, at 18 years-old, spino cerebellar manifestations with difficulties in swallowing. MRI evidenced a leukodystrophy. His nerve conduction velocity was increased. His verbal and performance IQs were 80. The nerve biopsy evidenced metachromatic deposits. ASA in leukocytes was 5 Units (normal 50-80 Units). He received an allograft after intense intense immunosuppressive therapy, total body irradiation and anti-lymphocyte serum. The graft was well tolerated and there were no signs of rejection. The hematopoietic system was reconstituted 75 days after the transplantation. After 5 years evolution, he was stabilized neurologically and his IQ was stable. ASA was also stable at 19 units. MRI was unchanged and sulfitiduria persisted. Except for ASA, the biological parameters were not modified although there was no clinical deterioration. Bone marrow transplantation is a treatment option in MLD with slow evolution as observed in this young adult.

P525. Initial experience with enzyme replacement therapy (ERT) in children and adolescents with Fabry disease.

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Fabry disease (FD) is an X-linked lysosomal storage disorder caused by the deficiency of α-galactosidase A. Clinical onset of the disease typically occurs during childhood. Whereas adults with FD are treated with enzyme replacement since several years, experience in patients of younger age is lacking. Therefore a clinical study with affected boys and girls was initiated.

We included 6 children (2 boys and 4 girls) into the open label safety study. In addition to general clinical assessments, ophthalmological examination, 24h-urine test, ultrasound of the kidney, ECHO, ECG, vestibular function test, audiology, standardized cold exposure testing, Gb3 measurements in blood and urine and pharmakokinetic evaluation studies were carried out at the beginning and at the end of the study. All children received 0.2mg/kg agalsidase alfa (Replagal®) intravenously every two weeks over 40 min.
Until now 6 children, age 6 ½ -17 years, with FD participated in this study. The MSSI (Mainz Severity Score Index) ranged from 13-21, none was asymptomatic. Baseline revealed 2/6 with chronic pain, 4/6 had fever pain crisis, 3/6 pathological cold exposure test, 6/6 cornea verticillata, 3/6 proteinuria, 1/6 autoimmune glomerulonephritis, 1/6 peripheral vestibulopathy, 0/6 hearing impairement, 3/6 gastrointestinal signs, 1/6 partial AV-channel, 1/6 ECG changes. Algasidase alfa was very well tolerated. Pharmakokinetic data were quite similar to those seen in adult patients.
This study demonstrated beneficial and safety effects of ERT in a number of children and adolescents. Longterm data will be followed in the FOS program (international Fabry database).

P526. *In vitro* gene targeting of *Cftr* in mouse embryonic stem cells

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Cystic Fibrosis (CF) is a lethal genetic disease resulting in a reduced Cl^- permeability caused by mutations in CFTR gene. An innovative gene modification approach, based on Small Fragment Homologous Replacement (SFHR), allows for the *in situ* modification of specific genes and was used to delete 3-bp in exon 10 of *Cftr* locus. This study is based on the introduction of small DNA fragments (SDF ~700 bp) into cells. The SDF pairs with its genomic homologous and replaces the endogenous sequence with the exogenous introduced fragment sequence.
Mouse D3 embryonic stem (ES) cells were transfected with an SDF homologous to *Cftr* exon 10 sequence, except for the ΔF508 mutation. Fragments were previously complexed with cationic liposome (Gene Porter, GTS) at different charge ratio (+/-). Confocal analysis confirmed the presence of Cy5 fluorochrome labelled DNA fragments in the nucleus of transfected cells. Modification at the appropriate genomic locus by DNA fragment introduction and expression of the modified *Cftr* mRNA was determined by allele-specific PCR analysis. RT-PCR products obtained from transfected cells were cloned and sequenced. Quantitative PCR (Applied Biosystem 7000) and Western Blot analysis are in progress to estimate the percentage of gene modification and the defective protein respectively.
These results are encouraging as a demonstration that SFHR may be effective for the development of cell gene therapies for genetic diseases by *ex-vivo* gene modification of pluripotent stem cells.
Work supported by Italian Ministry of Health, Italian Ministry of Education, University and Research, by CF Foundation and Pennsylvania CF Research, Inc.

P527. Correction of fatty acid oxidation by fibrates in carnitine palmitoyl transferase 2 deficient fibroblasts

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Carnitine Palmitoyl Transferase 2 (CPT2) deficiency, one of the most common inborn mitochondrial fatty acid oxidation (FAO) defects, has several clinical presentations. In the mild adult form, characterized by myalgy and episodes of rhabdomyolysis, residual FAO flux and CPT2 enzyme activity measured in patient fibroblasts are significantly higher than in the severe neonatal form of CPT2 deficiency. Our objective was therefore to test whether fibrates, which possibly stimulate ß-oxidation, could have beneficial effects on FAO in CPT2-deficient fibroblasts.
Bezafibrate, fenofibrate, ciprofibrate, or gemfibrozil were added for 3 days in the culture medium of mild-type CPT2-deficient fibroblasts, at concentration ranging from 50 to 800 µM, and ^{3}H-Palmitate (^{3}H-Pal) oxidation was measured according to standard methods. Bezafibrate induced a dose-dependant increase in ^{3}H-Pal oxidation, maximal at 800 µM (3.3±0.3 nmol ^{3}H-Pal oxidized /h/mg prot versus 1.8±0.2 in vehicle-treated cells; $p<0.001$), which lead to restore normal values. Other fibrates tested had less or no effects. Kinetic studies (2 to 72h; 800 µM bezafibrate) demonstrated increases in ^{3}H-Pal oxidation starting from 6h, with maximal effects at 24h. Bezafibrate treatment (800µM, 3days) also resulted in a 48% increase in residual CPT2 enzyme activity, and in a 50-60% increase in CPT2 gene transcripts determined by quantitative RT-PCR in deficient fibroblasts. In contrast, bezafibrate failed to increase ^{3}H-Pal oxidation in fibroblasts from patients with severe CPT2 deficiency. These are the first data suggesting that fibrates could correct mild CPT2 deficiency, and this approach could be tested in other ß-oxidation defects.

P528. Enzyme replacement therapy in the tolerant mouse model of Pompe disease.

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Deficiency of acid alpha-glucosidase (GAA) results in generalized deposition of lysosomal glycogen manifesting as myopathy and cardiomyopathy. Although many lysosomal disorders are corrected by enzyme replacement therapy (ERT) that provides 1-5% of normal cellular activity, the reversal of pathology in skeletal muscle in murine Pompe disease requires much higher GAA activity, as shown by somatic induction of transgenic GAA expression in GAA-/- mice. Furthermore, skeletal muscle is not easily accessed by the exogenous enzyme. We have demonstrated in a study with rhGAA (20mg/kg/weekly for up to 5 months) in tolerant GAA-/- mice that skeletal muscle cells take up little enzyme compared to liver and heart. Glycogen reduction was less than 50%, and some fibers showed little or no glycogen clearance. A 100mg/kg dose of rhGAA resulted in higher activity and up to ~75% glycogen clearance. The enzyme reduced heart muscle glycogen to undetectable levels at either dose. Skeletal muscle fibers with glycogen showed immunoreactivity for LAMP-1/LAMP-2, indicating that undigested glycogen remained in proliferating lysosomes. Differential transport of enzyme into lysosomes may explain the strikingly uneven pattern of glycogen removal. In the murine model, glycogen clearance was more pronounced in type 1 muscle fibers, and histochemical analysis suggested an increased mannose-6-phosphate receptor immunoreactivity in these fibers. Importantly, a modest glycogen reduction resulted in improved muscle strength in some experimental groups. These studies suggest that ERT, although at much higher doses than in other lysosomal diseases, has the potential to reverse cardiac pathology and to reduce the glycogen level in skeletal muscle.

P529. Acquired β-Thalassemia intermedia phenotype in a patient affected with juvenile myelomonocytic leukemia

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Juvenile myelomonocytic leukemia (JMML) is characterised by elevated postnatal fetal hemoglobin (HbF) expression up to 30% in association with monosomy 7 and up to 70% in cases with a normal karyotype. We describe a case of JMML with normal male karyotype, and hematologically normal parents, presenting with 70% HbF, 30% HbA and no detectable HbA_2. The synthetic ratio of the globin chains was compatible with a β-thalassemia intermedia phenotype. No molecular defects justifying this phenotype were found on the β-globin gene clusters of propositus or parents, indicating that reactivation of foetal or deactivation of postnatal erythropoietic expression was not caused by alterations on the β-globin genes

cluster. Constant reactivation of foetal gene expression in postnatal life is a long searched but never achieved therapeutic approach that could cure many patients affected with sickle cell disease and β-thalassemia major. The foetal erythropoiesis observed in this patient could be explained by a modification in the chromatin structure of the β-globin gene cluster in the early precursors of an invading malignant clone. Alternatively, by blocking or reactivation of an unknown foetal specific erythropoietic factor, coded elsewhere on the genome, either by chromosomal rearrangements or chromatin structure changes. Using COBRA technology on EBV transformed original lymphoid cell lines no evidences for major chromosomal rearrangements were found. As a higher resolution method we are considering array comparative genomic hybridisation (CGH) at this moment.

P530. Quality of life in patients with Fabry disease

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BACKGROUND: Fabry disease is an X-linked metabolic disorder caused by deficiency of a-galactosidase A. This leads to accumulation of globotriaosylceramide in different organs (e.g. kidney, gastrointestinal tract, heart and nervous system). Enzyme replacement therapy (ERT) has been available since 2001, and positive clinical effects have been reported. However, there are no published data on the effects of ERT on health-related quality of life (HRQOL).
METHODS: HRQOL was therefore assessed in 145 male and female patients (mean age, 38 years) with Fabry disease treated with agalsidase alfa and enrolled in FOS - the Fabry Outcome Survey. HRQOL was measured using the validated EQ-5D questionnaire.
RESULTS: Before treatment, patients with disease manifestations in the following organ systems had a significant reduction in HRQOL: kidney ($p<0.008$), musculo-skeletal system ($p<0.02$), gastrointestinal tract ($p<0.001$) and nervous system ($p<0.001$). EQ-5D scores were significantly ($p<0.001$) improved after ERT. In addition, there were significant improvements in 8 of 11 subscales of the Brief Pain Inventory after ERT. We also investigated the influence of ERT on HRQOL based on quality-adjusted life years (QALYs). After ERT for a mean of 10 (range, 3-21) months with agalsidase alfa, 191.4 QALYs were generated. HRQOL at baseline was the only factor correlating with increased QALYs after treatment. This is not surprising, as it is easier to improve HRQOL in patients with lower EQ-5D scores at baseline than in those with higher scores.
CONCLUSION: In conclusion, ERT with agalsidase alfa significantly improves HRQOL in patients with Fabry disease.

P531. Desmin supplementation as a possible gene therapy in epidermolysis bullosa simplex (EBS)

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Epidermolysis bullosa simplex (EBS) is an autosomal dominant skin disorder, characterised by blister formation on mild trauma. According to the severity of the phenotype, EBS can be classified as Dowling-Meara, Kobner or Weber-Cockayne. All three forms are caused by mutations in either keratin K5 or K14 and the severity of the phenotype can often be correlated to the position of the mutation. The keratin networks of EBS cells tend to collapse and aggregate under various stress conditions, and this is the probable underlying cause of the observed skin fragility.
Five keratinocyte cell lines, expressing EBS-associated keratin mutations of differing severity, were subjected to a range of physical stresses. Monitoring their early stress responses to osmotic shock, we found a direct correlation between the severity of the keratin mutation carried and the speed of JNK (a stress-activated protein kinase) activation.
Considering that EBS cells are only disrupted when they are subjected to some sort of trauma, we hypothesised that a small amount of structural reinforcement of their network could allow them to behave as normal. We therefore transfected all EBS cell lines with human desmin, a muscle specific type III intermediate filament protein, and subjected them to stress assays. Our results suggest that desmin is indeed capable of giving structural reinforcement to the EBS cells, which, once transfected, appear to be more resistant to stresses such as osmotic shock, cell stretching, wound healing and heat shock, than the untrasfected cells.
These observations may have value in designing therapeutic strategies for EBS.

P532. Enzyme Replacement Therapy for Gaucher Disease in Taiwan

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The treatment of Gaucher disease(GD) has been dramatically improved after the development of enzyme replacement therapy (ERT) with intravenous administration of macrophage-targeted glucocerebrosidase. ERT with imiglucerase was introduced to Taiwan in October, 1998. Up to now, there are 14 patients regularly receiving the ERT which's fully subsidized by the government (National Health Insurance). Twelve patients have type 1 and 2 have type 3 GD. Sex ratio is M:F=3:11. Seven are children and 7 are adults with mean age of 20.8 years. Five patients have undergone splenectomy before the ERT. All receive intravenous injection of imiglucerase 60U/Kg q2wk. All except type 3 patients respond well with improvement of hemoglobin concentration (4.1gm% increase) and platelet count (212,000/uL increase), and marked decreases in biochemical markers. All of them show prominent decrease in liver size. The 7 young patients enjoy remarkable linear growth. Quality of life improves mainly due to significant reduction of bone pain/crisis rate. The type 3 patients show moderate degree of psychomotor retardation, but slow catching-up of the developmental milestones is observed. There are no serious adverse effects documented. Only 2 patients experienced self-limited skin eruption, and one developed tremor of the limbs which resolved later. Conclusion: ERT has reversed the visceromegaly and hematological/biochemical abnomalities of our GD patients, and alleviated bone pain/crises. Quality of life of the patients is dramatically improved by the therapy. Still, ERT for type 3 patients remains to be further evaluated.

P533. Effects of Enzyme Replacement Therapy on Renal Function in Fabry Disease.

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Fabry disease (FD, alpha-galactosidase A deficiency) is an X-linked hereditary disorder leading to pathologic accumulation of glycosphingolipids in lysosomes. Accumulations in the vascular endothelium of the kidney, heart, and brain lead to many of the clinical manifestations of disease. A phase 3 double-blind, placebo-controlled clinical trial with recombinant human alpha-galactosidase (r-haGAL, Fabrazyme, Genzyme) in 58 patients with FD showed safe and effective clearance of GL-3 from the endothelial cells of the kidney, heart, and skin. All 58 patients entered and have subsequently been treated with r-haGAL during an ongoing open-label extension study for up to an additional 24 months. The mean estimated glomerular filtration rate (GFR) and serum creatinine for the study population were normal at baseline. After 20 weeks in the pivotal trial, placebo patients had a mean change in GFR of 0 and r-haGAL patients had a mean decrease of 3.7. There was no change in serum creatinine. Once these patients entered the extension

trial, both GFR and serum creatinine values remained stable after 24 additional months of r-haGAL therapy. The median urinary protein/creatinine ratio has remained stable after 24 months into the extension study, indicating stabilization of proteinuria (n=30). A subset of 10 patients had low GFR values at the start of treatment. After 24 to 30 months of therapy, 8 of these 10 patients exhibited either stabilization (no change) or improvement in renal function based on GFR values. These results suggest that early treatment with r-haGAL may slow the progression or prevent renal disease in FD.

P534. Targeted gene repair of *hprt* mutations by 45 base single stranded oligonucleotides

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Targeted repair of a single base in a gene of an eucaryotic cell by specific oligonucleotides is currently a controversial technique. Here, we introduce the repair of point mutations in the hypoxanthine-guanine-phosphoribosyl-transferase (HPRT) gene as an additional model system to test targeted gene repair. In human, *Hprt* mutations cause Lesch-Nyhan syndrome. Using hamster V79 cells, we generated three cell lines with one *hprt* point mutation each. These cell lines were treated with specific single stranded 45 base phosphothioate modified oligonucleotides and selected by HAT medium. The surviving clones were investigated for the repair of the respective *hprt* mutation. Treatment with the oligonucleotides was successful in repairing all three *hprt* mutations (*hprt* cDNA position 74, C→T; position 151, C→T; position 400, G→A). The repair rate was very low but reproducible. We suggest that this system allows to investigate targeted gene repair in dependence of the target sequence and the oligonucleotides used.

P535. Use of chimeraplasts on cells from Gaucher disease patients

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Current gene therapy approaches for the treatment of genetic diseases are based on the addition of an intact copy of a gene to restore the function that is lacking as a consequence of a mutated gene. However, several drawbacks arose when clinical trials begun. To overcome some of these limitations, novel strategies have recently emerged. One of them is chimeraplasty, a new methodology that uses chimeric RNA/DNA oligonucleotides (chimeraplasts), designed to contain the normal sequence of the region to be corrected. Once inside the nucleus, the chimeraplast pairs with homologous genomic DNA and generates a mismatch which is supposed to be corrected by the DNA repair mechanisms of the cell. This strategy has been applied to correct different genetic defects and some very promising results were reported.
We have tried to use a chimeraplast to repair Gaucher disease mutations in cultured fibroblasts from Gaucher disease patients. First of all, we showed that the chimeraplasts reached the fibroblast nucleus. We then assayed the level of in vitro correction by a mammalian cell-free extract using E. coli cells. The system consisted on the correction of a mutant antibiotic resistance gene in a bacterial plasmid with a chimeraplast. The correction frequency was much lower than previously described. Furthermore, preliminary results on fibroblasts from the patients were negative.
Recently, a strong criticism has been raised against this technique and many of the first successful results are now questioned. Our negative results support this criticism.

P536. Fabry Disease: Enzyme Replacement Therapy (ERT) Improves Gastrointestinal Symptoms

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In Fabry disease, an X-linked lysosomal storage disease due to α-galactosidase A (α-Gal A) deficiency, gastrointestinal symptoms including chronic severe abdominal cramps, diarrhea, nausea, vomiting, and food intolerance markedly impact quality of life. These symptoms result from lysosomal globotriaosylceramide (GL-3) deposition in vascular endothelial and perithelial cells, small unmyelinated neurons, and perineurial cells of the gastrointestinal system (Seth et al, Am. J. Gastrol. 1981). Conventional treatment including Lomotil provides limited benefit.
Clinical trials of α-Gal A replacement in Fabry disease (1mg/kg q2wk) have shown clearance of the accumulated GL-3 from plasma, kidney, liver, heart, and skin (*Eng et al, Am. J. Hum. Genet. 2001; N. Eng.l J. Med. 2001*). In four classically affected males (aged 20-40 years) with severe gastrointestinal symptoms participating in the Fabrazyme Phase I/II or III trials and extension studies (Genzyme Corporation), ERT (1mg/kg q2wk) markedly improved or reversed the gastrointestinal symptoms. Prior to ERT, these patients had abdominal pain and severe diarrhea with 7-10 bowel movements (BM) 2-5 times/wk to 6-9 BM/day. Vomiting, food intolerance, and poor weight gain were associated symptoms. After 6 months of ERT, all four patients reported “no or only occasional” abdominal pain or diarrhea, discontinued Lomotil, and gained 3-8 kg. Quality of life and food tolerance were markedly improved. Thus, ERT with Fabrazyme markedly improves gastrointestinal symptoms and quality of life in patients suffering from the gastrointestinal manifestations of Fabry disease.

P537. Ribozyme Gene Therapy Approaches for Keratin Disorders

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There are now 18 keratin genes known to be involved in dominantly inherited epithelial disorders. Due to the dominant negative effect of the mutant keratins, conventional gene replacement therapy is not appropriate.
Ribozymes are small catalytic RNA molecules that can be designed to cleave mRNA molecules at specific sites. Among the several potential ribozyme sites in K14 mRNA, five were selected based on the best RNA folding predictions. Ribozymes were protected from in vivo RNAse degradation by addition of hairpin sequences derived from U1 snRNA, and constructed as mini-genes driven by the U1 snRNA promoter. Three ribozymes were capable to cleave K14 mRNA in vitro, and two of them shown rapid cleavage following transfection into cultured keratinocytes. These experiments demonstrate the capability of ribozymes to specifically degrade abundant mRNA species such as keratins, paving the way for gene therapy approaches were all endogenous keratin might be ablated and replaced by a modified ‘ribozyme-immune’ keratin gene.
Ribozymes might also be designed to specifically target the mutant mRNA in cases where the mutation creates a suitable cleavage site. By modification of the ribozyme conserved catalytic subunit, we were able to develop a novel ribozyme that specifically cleaves K14-R125C, the most common mutation in DM-EBS severe form. This is an interesting approach for keratin diseases and other dominant-negative disorders in which increasing expression of the wild-type/mutant allele could be sufficient to correct the phenotype.
Currently, we are developing transgenic models in order to assess ribozyme therapy efficacy and safety in vivo.

P538. How many loci for the His475Tyr polymorphism of the glutamate carboxypeptidase II gene?

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The glutamate carboxypeptidase II (GCPII) gene (GenBank accession no. AF007544), assigned to chromosome 11p11.2, encodes for the folylpoly-glutamate carboxypeptidase (FGCP) enzyme which digests polyglutamylated folates into monoglutamyl folate. Three different studies evaluated the role of the His475Tyr (C1561T) polymorphism of the GCPII gene in the determination of plasma folate or total homocysteine levels in relation to human diseases by using two different sets of primers. Another polymorphic sequence, a (TAT)2/3 repeat, was also identified in intron 13.
Using the GCPII cDNA sequence (FGCP, GenBank accession no. AF176574) as a query we found that another sequence, located on chromosome 2p24.3, is present in the H. sapiens genomic contig sequence database. When the AF007544 sequence is used as a query for a BLAST search in the same database, two nearly identical contigs are found on chromosome 11 (GenBank accession no. NT_033232.2) and chromosome 2 (GenBank accession no.

NT_005334.10). Both sequences include 19 putative exons and a potential (TAT)2/3 repeat. Either pair of primers used for the His475Tyr genotyping, may amplify a DNA fragment with the same size and oligonucleotide sequence by annealing to both sequences. Taking into account the evidence of two copies of a GCPII-like gene, we are concerned for the reliability of results of genetic studies carried out so far. Our data call for further molecular studies on the GCP II gene before further epidemiological evaluation of the His475Tyr and (TAT)2/3 polymorphisms is carried out.

P539. The molecular mechanisms of interferon signalling in lymphoid cells under the action of ionizing irradiation

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Interferons are the family of multifunctional cytokines that are encoded by intronless genes and play key roles in cell metabolism. The activity of interferons is based on the expression of interferon-inducible protective genes (e.g. encoding 2',5'-oligoadenylate-synthetase, double-stranded RNA-activated protein kinase, etc.). Uncovering the pathways that mediate interferon-inducible gene expression under ithe nfluence of various factors has become very urgent in recent years. We report a study of interferon signalling under the action of ionizing irradiation which is known to cause breakdown of gene expression and originate heritable diseases.
The key interferon-induced enzymes were studied in two experimental models: *in vitro* (in human lymphoblastoid Namalwa cells) and *in vivo* (in animal lymphoid cells, isolated from spleen and thymus). The activities of 2',5'-oligoadenylate-synthetase and dsRNA-dependent protein kinase were shown to increase in both cell systems after irradiation at 0.5 Gy dose. The *in vitro* treatment of cells with different interferon inducers (e.g. poly(I)×poly(C), tilorone, cycloferone) caused an intensification of the radiation-induced amplification of the activity of both enzymes in irradiated cells.
The post-radiation increase of 2',5'-oligoadenylate-synthetase and dsRNA-dependent protein kinase activity suggests an enhancement of the expression of interferon-dependent proteins. As the interferon inducers amplify the activity of the investigated enzymes, it makes possible to predict the stimulation of interferon gene expression under the action of irradiation.
The investigation of interferon signalling in conditions of radiative influence will provide a useful tool to further understanding the molecular mechanisms of the development of radiation-induced genetic pathologies.

P540. Mutation Of Serotonin 5-ht2b Receptor In Fenfluramine-associated Primary Pulmonary Hypertension

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Background - Appetite-suppressant drug fenfluramine is implicated in primary pulmonary hypertension (PPH) but the molecular pathways that mediate this effect are unknown. A mouse model incriminates a vasopressor effect mediated by the serotonin 5-HT2B receptor, but this receptor has also been shown in other models to mediate pulmonary arterial vasodilation via nitric oxide production.
Methods and Results - We analyzed the 5-HT2B gene in 5 patients with appetite-suppressant drug-associated PPH. We found a mutation causing premature truncation of the protein product in one. The mutation was not found in 80 control subjects and no 5-HT2B mutation was found in 18 PPH patients not associated with appetite-suppressant drug intake. Functional analysis of the transfected receptor expressed either transiently in COS cells or stably in CHO cells demonstrated that the mutated receptor fails to activate the second messenger inositol-phosphates cascade and subsequent intracellular calcium release, in spite of normal expression at the cell membrane. The mutated receptor has no constitutive activity, and produces no dominant negative effect on the wild-type receptor.
Conclusion - The 5-HT2B mutation found in a fenfluramine-associated PPH patient results in loss of receptor function, consistent with haploinsufficiency of a nitric oxide-dependent vasodilator or antiproliferative effect.

P541. The effect of Maras Powder on DNA methylation and micronucleus in blood lymphocyte and buccal tissue

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In the South East region of Turkey, especially around Kahramanmaras and Gaziantep, there is a habitual smokeless tobacco called Maras Powder (MP). In this study we investigated the effect of MP on DNA methylation and micronucleus formation in habitual users. Buccal and peripheral blood DNA from 80 subjects (40MP users, 20 smokers and 20 nonusers/nonsmokers) were isolated and digested with Msp1 (methylation insensitive) and Hpa II (methylation sensitive). The software programme used to interpreted the results reported the data as pixels (pc). Average HpaII fragmentation was 0.34pc in MP users, 0.26pc in smokers and 0.22 pc in non-users/non-smokers. The MspI fragmentation averaged 0.59pc in users, 0.40pc in smokers and 0.34pc in non-users/non-smokers in peripheral blood DNA.
MspI digestion was different in all three groups. While MspI fragmentation was 0.70pc in users, it was 0.55pc in nonsmokers/nonusers and 0.63pc in smokers. HpaII fragmentation was 0.32pc in users, and 0.27pc in both smokers and nonsmokers/nonusers. This result indicates that MP causes more hypomethylation (on epithelial DNA) comapred to levels in smokers and non-smokers.
Mean MN cell percentage was 1.93 ±1.24 in MP users, 0.95±0.85 in nonsmokers/nonusers, 1.82±0.98 in smokers. Average MN is significantly higher in smokeless tobacco users and smokers than nonsmokers/nonusers ($p<0.05$). There was no statistical difference between smokers and MN users ($p>0.05$).
In conclusion, our study suggests that Maras Powder causes DNA hypomethylation and increases micronucleus formation.

P542. Regional and cellular brain distribution of proteins supporting copper balance in mammalia

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In eukaryotes, the safe transport of copper ions, which are essential for the activities of several crucial enzymes, is realized by coordinated interaction of cell- and tissue-specific autonomous metabolic copper systems (MCSs). Inherited and ecologic-related disorders of copper balance cause a number of neurodegenerative diseases such as Menkes', Wilson's, Alzheimer's, Parkinson's, prion diseases etc. Many features of function of the brain MCS as well as the structure and subcellular localization of its components remain unknown.
We investigated the expression of two copper-transporting P-type ATPases (ATP7B and ATP7A) and ceruloplasmin (CP), the main intercellular copper transporter, in cerebral cortex, neuroglia, cerebellum, pituitary gland and choroid plexus. Using specific antibodies, the immunoreactive polypeptides of these proteins should be detectable in the cellular secretory pathway membranes by Western-blot analysis. ATP7A was found in the plasma membranes from cerebral cortex, neuroglia, cerebellum, pituitary gland but not choroid plexus. ATP7B is expressed only on the plasma membranes from choroid plexus and pituitary gland.
The glycosylphosphatidylinositol (GPI)-anchored form of CP was localized to the plasma membranes of all brain regions, except choroid plexus. The expression of GPI-CP was verified by RT-PCR analysis. The transcripts of soluble CP were revealed in RNA isolated from all brain branches. GPI-CP mRNA was not only found in choroid plexus.
Our data suggest that the copper balance in brain is supported by complex MCS, consisting of semiautonomous copper systems of various brain departments. *Supported by RFBR (01-04-49597, 02-04-07611), "Integration" (I0064)*

P543. Identification of post-transcriptional regulatory elements of the RET gene through comparative sequence analysis.

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Comparative sequence analysis is a simple way to analyse the vast amount of data obtained by the Genome Project of different organisms. This analysis allows us to identify conserved genomic elements playing a functional role in fundamental biological process, including the control of gene expression. The RET proto-oncogene is characterized by a complex pattern of post-transcriptional regulation, such as alternative splicing and differential polyadenylation. In order to clarify this control mechanism, we compared the human RET 3'UTR (4kb) with the RET sequences of 13 vertebrate species (chimpazee, baboon, swine, cow, cat, dog, mouse, rat, chicken, frog, zebrafish, pufferfish and torafugu), We found a high degree of conservation in the last alternative spliced exons as well as in the donor and acceptor splice sites (>80%). When restricted to mammals, our analysis showed conserved sequences also in non-coding regions, suggesting the existence of further regulatory elements. One of these is 91 bp long and is localized in the intronic region immediately upstream the last polyadenylation signal. This sequence contains an AUUUA motif, which is known to destabilize mammalian mRNA. We constructed a hybrid reporter gene, characterized by this RET conserved fragment cloned downstream the luciferase sequence. After transfection in SK-N-BE, a neuroblastoma cell line, we observed a significative decrease of luciferase activity in the hybrid construct compared to the control vector, suggestive of change in gene expression. Additional characterization of this and other RET conserved sequences, which may play an important role in mRNA stability and alternative splicing, is currently under investigations.

P544. Direct haplotype detection in the upstream region of the dopamine D4 receptor (DRD4) gene

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The dopamine D4 receptor (DRD4) gene is one of the most polymorphic genes among the neurotransmitter systems in the brain. This special feature and the presumptive role of the dopaminergic system in the development of neuro-psychiatric disorders have drawn the attention to the DRD4 gene in psychogenetic association studies. 10 SNPs and a 120-basepair duplication polymorphism have been described in the 5' untranslated region of the gene. Most of these polymorphisms are assumed to influence the transcription activity of the gene. Haplotype structure of these sites, however, has not yet been investigated. In order to identify specific effects of certain haplotypes on psychiatric phenotypes, several direct haplotype detection systems have been worked out in our laboratory.
Here we present data on the analysis of the 120 bp duplication together with one of the SNPs by a special allele-specific PCR employing an allele-specific primer for the appropriate SNP and including the region of the repeat polymorphism. The haplotype of various SNPs is examined by bidirectional allele-specific amplification, and alternatively by RFLP applying several restriction endonucleases simultaneously.
Additionally, it is also important to carefully design the genotyping methods when analyzing a highly polymorphic region, because the SNPs in close proximity can disturb each other's reliable genotyping. This problem is also demonstrated by the -615AG and the -603Tdel SNPs.
The genotyping and haplotyping methodologies demonstrated here have a widespread application in genetic association studies of polygenic diseases such as psychiatric disorders.

P545. Functional characterisation of the HOX11L1 5'-flanking region to identify genes involved in enteric nervous system development.

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The transcription factor HOX11L1 is specifically expressed in tissues derived from neural crests cells. It plays a crucial role in the development of the enteric nervous system, as confirmed by knock-out mice exhibiting megacolon and myenteric neuronal hyperplasia. To identify other genes involved in human congenital disorders characterised by defect of intestinal innervation, we have focused on HOX11L1 regulation.
HOX11L1 promoter presents three GC-rich regions and lacks conventional TATA and CCAAT boxes. After determining, by 5'-RACE, the transcriptional initiation site at 338 nucleotides upstream the ATG start codon (+1), we cloned 2 kb of the 5'-flanking region fused to the luciferase gene and performed transient tranfection assays in different cell lines. By deletion analysis, we identified a minimal sequence (-524/-338) sufficient to the constitutive expression of the gene in all the studied cells. This region contains putative binding sites to Sp1 (-411/-398) and AP1 (-361/-350). Moreover, we associated an enhancer activity of the HOX11L1 promoter in neuroblastoma cells to the region between -1711 and -1396, characterised by consensus sequences for different transcription factors. A further deletion analysis of this specific region identified a Sp1 site as responsible for the enhancer activity. To confirm the role of the above factors in the minimal sequence and in the distal region, we performed transient transfections with promoter-reporter constructs carrying mutations abolishing the binding of the specific factors. Our results suggest a synergism between distal and proximal Sp1, likely achieved by direct interaction, acting as the main mechanism of promoter regulation .

P546. Protamine gene expression and marked differences in protamine content and P1/P2 ratios in sperm cells from Percoll fractions between patients and controls.

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Marked differences in protamine gene expression have been previously reported in infertile patients when analysing the overall sperm cells present in semen. However, it is known that different sperm cells types are present in a simple ejaculate, which differ in their motility and morphology. In this work we have analysed the protamine expression and P1/P2 ratio of subpopulations of human spermatozoa at different stages of maturation, isolated by density gradient centrifugation of ejaculated spermatozoa obtained from a group men of proven fertility, and groups of oligozoospermic or asthenozoospermic individuals attending to our Assisted Reproduction Unit. A karyotype and a Y chromosome microdeletion analysis was performed to all participants to rule out known genetic causes of infertility. Four different fractions were collected from each semen sample. Differences in the motion and morphology were found between the fractions in each of the groups studied. However, no significant differences in the P1/P2 ratio were found between fractions within the same group of samples indicating that the P1/P2 ratio and the expression of protamines is to some extend independent of the morphology and motility of sperm cells. In contrast, statistically significant differences were found in the P1/P2 ratio and in the relative expression of protamines between groups pointing out to independent origins of infertility. Funded by a grant from Fondo de Investigaciones Sanitarias (FIS 99/0422 to RO).

P547. RNA editing affects mRNA splicing of the 5HT2C receptor gene.

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In the precursor RNA of the 5HT2C receptor gene (HTR2C), sequence containing the 3' end of exon 5 and the 5' end of intron 5 is predicted to form a stable stem-loop structure. This stem-loop is flanked by two alternative splice sites (GU1 and GU3) and contains the preferred donor splice site (GU2) and, in the stem, five exonic adenosines which are susceptible to editing. We have additionally identified two novel intronic editing sites (F and G) in the stem of the predicted stem-loop. We examined the distribution of editing isoforms in brain regions from control individuals and observed significant differences in editing frequencies between GU2-spliced, GU3-spliced and unspliced RNA. Splicing at GU1 removes all editing sites. In order to investigate the relationship between RNA editing, secondary structure and splicing, we transfected HTR2C minigene constructs in different cell lines using A/G mutations to model A/I editing. These experiments have shown that editing at most sites favours splicing at GU2 over GU1, and that these effects appear to be unrelated to secondary structure. When combined, these editing mutations often greatly enhance splicing at GU2 at the expense of GU1, particularly

with constructs to model the common editing isoforms seen in the brain. These generate increased splicing at GU2 and, for some editing isoforms, also at GU3. These splicing patterns are consistent with our observations in brain and validate this model system. These results strongly suggest that A/I RNA editing has a considerable effect on splice site selection.

P548. Modulation of SOX2 and SOX3 gene expression during differentiation of human neuronal precursor cell line NTera2

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The SOX genes comprise a family of transcriptional regulators implicated in the control of nervous system development. The developing brain is the major site of expression of many Sox genes. Sox2 and Sox3 genes are predominantly expressed in the immature undifferentiated cells of the neural epithelium throughout the entire CNS.
NTERA2 is a human embryonal carcinoma cell line that phenotypically represents undifferentiated, pluripotent embryonic stem cells. In the presence of retinoic acid, cells differentiate into mature neurons providing an in vitro model for studying human genes that promote and regulate neural differentiation.
In this study it is shown for the first time that the retinoic acid-induced neuronal differentiation of NTERA2 cells is accompanied by down-regulation of SOX2 and up-regulation of SOX3 gene during early phases of induction. These data suggest that the effects of retinoic acid on neural differentiation of NTERA2 EC cells might be mediated by modulation of SOX2 and SOX3 gene expression.

P549. Characterization of the human *Iroquois* (*IRX*) homeobox genes: proposal for a systematic nomenclature.

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The group of Iroquois (*IRX*) genes is a small family of homeobox genes of the TALE group encoding DNA binding proteins and regulatory transcription factors which are important for pattern formation in all higher organisms including vertebrates, insects and nematodes. They contain a homeodomain, an IRO domain and frequently a poly amino acid domain. The evolutionary conservation of *IRX* genes is reflected in their conserved genomic organization of three clustered genes, one cluster is present in *Drosophila* and two clusters in vertebrates with the six genes named *IRX1-IRX*6 or *IRO1-IRO6*, depending on the organism. Cluster A is located on chromosome 5p15.33 and cluster B on chromosome 16q12.2 in *Homo sapiens*. Two of the human genes are believed to be fully characterized and we have characterized the cluster organization, gene structure and expression pattern of the remaining four human *Iroquois* homeobox. Comparative analysis of the *Iroquois* genes in ten different organisms (human, mouse, rat, zebrafish, fugu, *Ciona intestinalis*, chicken, *Xenopus*, *Drosophila, C.elegans*) demonstrate conservation of both genes and clusters, with intra-gene and inter-gene structural conserved features that may have importance for the function and regulation of the Iroquois genes. Due to the inconsistencies of the present nomenclature, we support a systematic nomenclature in analogy with what is used for the *HOX* genes, where cluster A is composed of *IRXA1*, *IRXA2* and *IRXA3,* and cluster B of *IRXB1*, *IRXB2*, and *IRXB3*, as proposed by Peters et al. (2000).

P550. New data on novel human gene MOB deduced from brain-specific cDNA clone Hmob33

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The novel human gene *MOB* localized on human chromosome 10 came from the Hmob33 clone which was derived from a human medulla oblongata cDNA library. The *MOB* gene was demonstrated to span over 320 kb and to consist of 11 exons and 10 introns. The results of 5'-directed primer extension reaction confirmed that the 5'-end of exon I represents the 5'-end of the transcribed region of the *MOB* gene. The presence of a promoter region just upstream of the 5'-end of exon I was revealed by computer analysis. *MOB* transcripts were detected in human cerebellum, hippocampus, forebrain cortex and lung. The 5'-end of the coding region lies within exon VII and the 3'-end within exon XI. The 5'-untranslated region of *MOB* mRNA includes exons I-VI and part of exon VII. Exon I is rich in G and C and seems to be highly structured; three short ORFs are localized within exons I, V and VI. Five transmembrane domains and one SAM-domain were predicted by computer analysis within the Mob protein. A number of homologues for this protein were found in *Mus musculus*, *Drosophila melanogaster* and *Caenorhabditis elegans* databases. The genomic structure of the corresponding mouse genes appeared to be similar to that of the human *MOB* gene. *In silico* comparison of the corresponding mouse mRNAs with human genomic sequences indicates that human chromosomes 4 and 10 might bear two new genes related to *MOB*. We assume the existence of a novel gene family encoding transmembrane proteins.

P551. Analysis of human SOX14 promoter region

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The *SOX* genes comprise a family of genes related to the mammalian sex-determining gene *SRY* which play important roles during animal development. SOX proteins display properties of both transcription factors and architectural components of chromatin.
The *SOX14* gene is a member of the B subfamily of *SOX* genes that has been mapped to human chromosome 3q22-23. Expression analysis of *SOX14* gene has shown that this gene is active in developing brain, spinal cord of chick and mouse, as well as in the human HepG2 hepatocellular carcinoma cell line.
Although many *SOX* genes have been characterized in detail, little is known about the transcriptional regulation of the *SOX* genes themselves. Computer analysis of 5 kb of the *SOX14* 5' flanking region showed that it lacks a TATA box, but it contains a typical CCAAT box located 586 bp upstream of the ATG codon. Sequence analysis using MatInspector program revealed potential cis-acting elements for multiple transcription regulators including Sp1, NF1, ZBP89, Myf5, TBX5 etc.
A 1.3 kb fragment of the *SOX14* 5' noncoding sequence showed promoter activity in a CAT reporter gene assay in transiently transfected HepG2 cells.
In order to analyse proteins which bind to the *SOX14* promoter we have analysed a 688 bp DNA fragment of the 5' *SOX14* region using gel shift assays with HepG2 nuclear extracts. These experiments revealed 14 complexes within this region with highly specific DNA binding properties.

P552. Determining the role of transcription factor BTEB3 in the control of smooth muscle-specific gene expression in vivo

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Control of gene expression in vascular smooth muscle cells (VSMCs) is important in development and disease. During normal development smooth muscle α-actin (SM α-actin) is expressed prior to SM22α with smooth muscle myosin heavy chain (SM-MHC) expressed last. Dedifferentiation of these cells is a major pathological change seen in a number of conditions including atherosclerosis and restenosis after angioplasty. Recent evidence has implicated the Krüppel like family of transcription factors (KLF family) in the control of SM-specific gene expression.
BTEB3, a KLF identified in our laboratory, is widely expressed in both adult and embryonic mouse tissues. Our studies have implicated BTEB3 as a selective regulator of the smooth muscle-specific gene, SM22α, in VSMCs but not of either SM-MHC or SM α-actin.
To investigate the role of BTEB3 further we use two different methods to modify its expression in mice, standard knock out and siRNA transgenic mice. To make siRNA transgenic mice first two siRNA vectors were developed and showed that they knock down > 85% of BTEB3 expression in vitro. The siRNA construct was used to make transgenic mice and phenotype was analysed at different embryonic stages. Preliminary data showed that BTEB3 siRNA transgenic mice are not viable from ED 12 although the phenotype does not show full

penetration. The effect of knock down of BTEB3 will be compared with the effect of knock out of BTEB3.

P553. Desired: Single (living) cells, single chromosomes, polar bodies, blastomeres or related. Answer: LMPC

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Many fields in the area of human genetics need the use of very clean samples as a prerequisite for reliable results. Whether in preimplantation- or prefertilization diagnosis, prenatal diagnosis, cancer genetics, tumor biology and so on, pure samples are indispensable.
The LMPC technology (this stands for laser microdissection and pressure catapulting) allows microdissection of single cells, cell clusters, chromosomes etc. and their transfer directly into or onto a collection device. These collected cells or chromosomes can be investigated as singles or pooled for molecular or proteomic analyses.
Any kind of tissue from various sources (also archival histological samples) and even subcellular structures can be captured using this laser method.
Even living cells can be collected in this way and processed further. This new approach can be used to establish a homogeneous cell population out of a heterogeneous cell population (e.g. after transfection experiments). It is possible to obtain 100% homologous cell populations for expression studies. In addition this protocol serves as a basis to develop the procedure to isolate homologous living cells from biopsies for real in vivo studies.
The key technology of LMPC can also be used for facilitating in vitro fertilization and polar body biopsy.
A special software program (Metafer P from MetaSystems) combined with LMPC allows the automated detection and collection of rare events on the object slide (e.g. cells or metaphases) and saves a lot of time in finding e.g. FISH labelled cells or fetal cells in maternal blood.

P554. New Advances in DNA Sequencing Chemistry

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Fluorescent dideoxy sequencing has advanced greatly since the early days of automated DNA sequencing about 15 years ago. Cycle sequencing using thermally stable DNA polymerase enzymes provided a robust method of sequencing plasmids over a much wider and lower range of concentrations than was previously possible. Fluorescent dideoxy terminators reduced the number of steps, equipment and supplies required to perform sequencing reactions, and BigDye®Terminators provided several fold additional sensitivity. These advances greatly facilitated the rapid sequencing of the human genome. Researchers today are challenged to finish the difficult-to-sequence areas of the human genome and other organisms, and to obtain highly accurate sequences of individuals in studying polymorphisms. We have recently released two new sequencing kits that will help to address these challenges. Here we present BigDyeTerminators v3.1 and 1.1 Cycle Sequencing kits. We will illustrate improvements with both our work as well as the results of our confirmation test sites.

P555. Alternatively included exons show higher conservation of surrounding intron sequences than constitutive exons in *NF1*, *CFTR*, *PER3*, *CARS* and *SYT7*

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It is still not fully understood to what extent intronic sequences aside from exonic sequences contribute to the regulation of the different forms of alternative splicing as alternative cassette exons events, exon isoform events and intron retention. We investigated cassette exon events by comparative genomic analysis of human and mouse in five well-characterized genes, *NF1*, *CFTR*, *PER3*, *CARS* and *SYT7*. *NF1* comprises 61 coding exons. High intron identity around the 52 constitutive and four alternatively skipped *NF1* exons is restricted to the close vicinity of the exons. In contrast, we found on average high conservation of intron sequences over three hundred base pairs up- and downstream of the five alternatively included *NF1* exons. The investigation of *CFTR, PER3*, *CARS* and *SYT7* supported the high intron identity around alternatively included exons. In *CFTR* the mean intron identities around the eight skipped exons do not differ considerably from those around the constitutive exons. In addition, in *NF1* and *CFTR*, we detected three highly conserved small intronic elements, but we found no correlation of the conserved regions to several intronic elements known to be involved in the regulation of splicing. In the investigated genes, the difference in intron conservation could point to a difference between the regulation of alternative exon inclusion and the regulation of alternative exon skipping and constitutive exon splicing. The availability of both mouse and human genomes will be helpful for additional genome wide investigations to elucidate to what extent our finding can be generalized.

P556. Insights as to the function of Dymeclin, the protein product of the Dyggve Melchior Clausen syndrome (DMC) gene

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DMC is an autosomal recessive skeletal dysplasia with mental retardation. The causative gene has recently been identified on chromosome 18. The predicted protein product of the DMC transcript appears novel with no known homology to any known protein family. Electron microscopy of skin in DMC reveals dilated rough endoplasmic reticulum and enlarged and aberrant vacuoles, leading to the hypothesis that the progressive clinical features of DMC result from abnormal handling of an unidentified compound.
To commence investigation of the function of the DMC gene we have performed RT-PCR using RNA extracted from a broad range of tissues and shown the DMC transcript to be widely expressed in contrast to the apparent bone and brain specific clinical phenotype. We next performed subcellular localisation of the DMC gene product (Dymeclin) using a myc tagged DMC construct transfected into Hela cells. Dymeclin appears to localise to the endoplasmic reticulum. Bioinformatic analysis of the predicted protein suggest the presence of myristoylation and dileucine motifs known to be involved in sorting and targeting of proteins. Taken together these findings suggest that the DMC protein is involved in the trafficking of intracellular compounds.
Furthermore, in a collaborative study, 6 of 7 mutant alleles predict premature truncation and consequent loss of function. Studies to identify Dymeclin interacting proteins are now underway.

P557. MBD4: more complex than previously thought?

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MBD4 (alternatively known as MED1) is a mismatch-specific G/T or G/U DNA glycosylase capable of binding the mismatch repair protein MLH1. It can also process DNA containing 5-methylcytosine. Mutations in MBD4 have been reported to occur in over 40% of microsatellite unstable sporadic colon cancers, and defective MBD4 can promote gastrointestinal tumour formation in mice carrying an APC germ line mutation. The study of MBD4 is thus relevant to an understanding of the maintenance of genomic stability and thus, potentially cancer development in man. To facilitate the investigation of MBD4 function, we developed a novel polyclonal antibody to the amino-terminal region of MBD4. Unexpectedly, in immunofluoresecence analyses in HeLa cells, the novel antibody and a commercially available anti-MBD4 antibody consistently indicated a sub-nuclear localisation of the antigen, consistent with a nucleolar localization. In addition, we report the identification of a potentially novel, truncated MBD4 product In HeLa cells. Intriguingly, truncated

MBD4 retains the glycosylase domain but the methyl-binding domain is lost. These unexpected observations suggest that the biology of MBD4 may be more complex than previously indicated.

P558. Factors affecting lymphocyte EBV transformation success rates

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ECACC has provided an Epstein Barr Virus (EBV) transformation service for human genetic research for over 15 years. Following a thorough process review and a study based on normal healthy volunteers, ECACC has optimised its EBV transformation process. As a direct result ECACC is now able to transform more than 600 samples per month with an average transformation success rate(TSR) of 95%.
In the study Peripheral Blood Lymphocytes (PBLs) were separated using accuspin tubes and then cryopreserved. EBV transformation was achieved by addition of a cocktail of EBV and phytohaemaglutinin (PHA) in culture medium. After a minimum 14-day incubation with medium changes every 2-3 days the successful cell lines were subcultured and then cryopreserved.
Several factors, thought to influence TSR were investigated. These included; initial PBL numbers, two different cell culture matrices (tissue culture tubes and 4 well plates), effectiveness of PHA from different suppliers and at different concentrations, and virus efficacy, including batch comparison against a reference standard. The Chemometic Nucleocounter was also evaluated as a rapid method of cell counting.
Other factors investigated were the effect of liquid nitrogen storage on PBL viability; the incubation of transforming cells in a 5% CO2 atmosphere compared to the addition of HEPES buffer, and increased cell line recovery from frozen ampoules.
Results of these investigations will be presented and discussed.

P559. Co-incubation provided no evidence that FGF23 is a substrate for PHEX

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X-linked hypophosphatemia (XLH) and autosomal dominant hypophosphatemic rickets (ADHR) are characterized by renal phosphate wasting, rickets and ostemalacia. XLH is caused by mutations in the PHEX gene, which encodes a type II membrane protein belonging to the family of zinc metalloendopeptidases. ADHR is caused by gain of function mutations in the fibroblast growth factor 23 (FGF23). FGF23 is a secreted factor that is cleaved between aa179 and aa180. Administration of the intact form to mice causes phosphate wasting, whereas the cleavage products show no activity. It is tempting to speculate that PHEX cleaves FGF23. But so far, ambiguous results have been reported in this respect. In order to investigate whether FGF23 is a substrate of the endopeptidase PHEX, we prepared constructs of recombinant FGF23. We also generated a secreted, soluble form of PHEX (secPHEX) by site-directed mutagenesis (1). The recombinant proteins were stably expressed in HEK293 cells and harvested from conditioned media. secPHEX and FGF23 were co-incubated up to 4 hours at 37°C. Reactions were stopped with sample buffer and peptides were separated on SDS-PAGE. Immunoblot analysis was performed with specific PHEX and FGF23 polyclonal antibodies. secPHEX activity was confirmed by degradation of PHTrp107-139 and inhibition with EDTA. Despite the efficient activity of secPHEX, our results provided no evidence that FGF23 was proteolytically processed in the presence of secPHEX. These experiments cannot rule out the possibility that FGF23 may require a specific cofactor for processing.
1) Boileau G et al. Biochem J 2001,355:707-13.

P560. The provision of a high throughput service for EBV transformation of peripheral blood lymphocytes (PBLs)

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The Human Genetic Cell Bank (HGCB) at ECACC, was created to secure a renewable and expandable source of human genomic DNA, and has provided support to major human genetic projects since 1989. Approximately 40,000 subjects, and 700 clinical genetic disorders are represented in the collection, and funding sources include MRC, Wellcome Trust, Diabetes UK and other charitable associations and commercial organisations.
The operation comprises two main processes. Firstly; the preparation and cryopreservation of PBLs from blood samples, and secondly, the manufacture of a lymphoblastoid cell bank using Epstein Barr virus transformation.
A significant increase in the demand for this service in the last two years raised a series of challenges. In order to meet these challenges, a batch system has been introduced. By processing PBLs in groups of 50 from a single project, we are able to effectively track samples and monitor transformation success rates of both individual projects, and overall. This system, combined with the introduction an in-house bar-code-driven information management system, has meant that we are able to respond more quickly to customer requests, and can provide more detailed information.
As a direct result of the introduction of these, and other measures we are currently able to process and successfully track in excess of 1000 blood samples and 600 transformations per month, while maintaining average success rates of approximately 95%.
The HCB response to the increased demand for its service, and the effect of the measures introduced as part of our continuous improvement policy will be presented.

P561. Gene expression analysis using Pyrosequencing™ technology

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The Pyrosequencing technology is based on sequencing-by-synthesis, where the sequential DNA polymerase incorporation of nucleotide tri-phosphates is detected by the release of pyrophosphate (PPi) in a real-time enzyme cascade detection system. The amount of PPi released is directly related to the number of DNA molecules present in the reaction. Therefore, quantitative data is obtained, as previously has been shown in allele frequency determination analyses and for quantification of HIV molecules.
We investigated the Pyrosequencing technology for quantification of specific nucleic acids, such as cDNA. Oligonucleotides were made with DNA sequences identical to the target sequences except for a few bases that functioned as references in the quantification. As the PCR primer binding sites were the same for target and reference, the two templates were competitively amplified in a single tube. After PCR amplification including a known amount of reference oligonucleotides, the target and reference sequences were simultaneously analysed in a Pyrosequencing reaction. The peak heights specific for the target and for the reference were compared, and the difference reflected the pre-PCR amount of target molecules. Initial experiments indicate that small differences in cDNA expression levels (two- to fivefold) can be accurately detected. The method generates sequence data acting as an internal specificity control of the polymerase priming, and several of the peaks can be used for the quantification analysis. A main advantage is that the analysis is performed on a Pyrosequencing instrument possible to use in a multitude of applications for genetic analyses.

P562. Detection of Tsga10 transcripts in mouse and rat testis

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Spermatogenesis converts a stem cell population of spermatogonia into a highly differentiated population of sperm in the testis. In the course of this pathway cells pass from a maintenance state through mitotic divisions, meiosis and finally terminal differentiation. Surprisingly little information is available about any of the genes which have a role in this pathway. We have previously shown by Differential Display (DD) RT-PCR that a novel gene, TSGA10 is expressed in human testis but not in a variety of non-spermatogenic tissues. Hence, TSGA10 is a testis-specific gene, which maps to 2q11.2. In this project we have used RT-PCR and *in situ* hybrization to investigate the expression of Tsga10 in adult rat and mouse testis and in mice embryos. RT-PCR shows that Tsga10 is expressed

in rat testis once spermatocytes have progressed to the late pachytene stage. It was present in mouse whole embryo (day 7, E7) and in mouse embryonic brain, but was not detected in earlier mouse embryos (E2-E4). Also it shows that Tsga10 has possibly an alternative splicing in mice testes and embryos but not in mice brain. *In situ* experiments were used to locate Tsga10 expression in cryostat section of mature mouse testis. Tsga10 transcripts show a pronounced expression from secondary spermatogonia to later spermatogenic cells including those closer to the lumen of the seminiferous tubules. Our results are the first to demonstrate that the transcripts for Tsga10 are present in spermatogenic cells and that they mostly exist in meiotic and post meiotic cells.

P563. Positional clustering of differentially expressed genes on human chromosomes 20, 21 and 22.

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Background: Clusters of genes co-expressed are known in prokaryotes (operon) and were recently found in several eukaryote organisms, including Human. According to some studies, these clusters consist of housekeeping genes, whereas other studies suggest that these clustered genes exhibit similar tissue specificity. Here we further explore the relationship between co-expression and chromosomal co-localization in the human genome, by analyzing the expression status of the genes along the best-annotated chromosomes 20, 21 and 22.
Methods: Gene expression levels were estimated according to their publicly available ESTs and gene differential expressions were assessed using a previously published statistical test. Gene sequences for chromosomes 20, 21 and 22 were taken from the Ensembl annotation.
Results: We identified clusters of genes specifically expressed in similar tissues along chromosomes 20, 21 and 22. These co-expression clusters occurred more frequently than expected by chance and may thus be biologically significant.
Conclusion: The expression of colocalized genes might be due to higher chromatin structures influencing the gene availability for transcription.

P564. Gene expression in a partial trisomy 16 animal model of Down syndrome

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Down syndrome (DS), caused by trisomy for chromosome 21 (T21), is the most common genetic cause of mental retardation. In addition to the mental retardation and facial characteristics, there are many other phenotypes, including congenital heart disease, early onset Alzheimer's disease and an increased risk of leukemia. The presence of an extra copy of certain but not all of the approximately 225 HC21 genes are predicted to contribute to aspects of the complex DS phenotype. It is often assumed that all genes from the extra chromsome 21 will be expressed at a level 1.5x relative to diploids. However, since regulation of gene expression is complex, involving regulatory loops, many genes may not conform to this prediction. Identifying which genes are overexpressed, and to what levels, is obviously important to understand genotype-phenotype correlations in DS. The development of microarray technology has allowed the simultaneous examination of expression levels of many genes to be compared across tissues. However, there are certain limitations to microarray experiments: sensitivity and dynamic range of detection, large RNA quantities required. We have used a high-throughput real-time quantitative PCR (qPCR) assay to examine the expression level of 115 genes (including 82 HC21 orthologues) in the Ts65Dn mouse model of DS. Tissues examined so far include brain, heart, liver and muscle. The qPCR assay has enabled us to detect genes which are overexpressed, and those which are not, in Ts65Dn thus helping to identify those genes contributing to the DS phenotypes.

P565. The PAX6 and PAX2 allelic variant databases.

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PAX6 and PAX2 are members of the paired box-containing PAX family of transcriptional regulators. Mutations in both genes are associated with congenital malformations of the eye; PAX6 mutations cause aniridia (OMIM 106210) and related phenotypes, while PAX2 mutations underlie renal-coloboma syndrome (OMIM 120330, also called papillorenal syndrome). Disease-associated and neutral allelic variants of PAX6 and PAX2 are archived on the web using MuStaR software at http://pax6.hgu.mrc.ac.uk/ and http://pax2.hgu.mrc.ac.uk/ respectively. These databases provide a valuable resource for those working on PAX6 and PAX2 as well as those who are interested more generally in mutation spectra. The PAX6 database currently contains 227 records and the PAX2 database (in the process of being updated) contains 35. While PAX6 has mutations of all types scattered throughout the length of the open reading frame, pathological PAX2 mutations are confined to just three exons (2, 3 and 5). Around half of all PAX2 mutations involve expansion of an oligo-G tract in exon 2.

P566. Half Way Up The Ladder

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The HUGO Gene Nomenclature Committee (HGNC) has to date provided unique gene symbols for over half of the estimated 30,000 human genes. As we progress through the genome assigning new symbols to the annotated sequence, we are also identifying sequence for a number of genes where previously only the chromosomal location was known. To help us with this challenge we have set up a „**Virtual Gene Nomenclature Workshop**", with a webpage for each chromosome based at URL: http://www.gene.ucl.ac.uk/nomenclature/workshop/virtual.html
We hope that by using both the knowledge of the scientific community and the processing power of the genome browsers, we will be able to add significantly to the number of genes identified in the human sequence. Genes currently without sequence include at least:

- 459 pseudogenes, e.g., ACTGP4, actin, gamma pseudogene 4
- 234 genes identified by in situ hybridisation, e.g., HSPCAL1, heat shock 90kDa protein 1, alpha-like 1
- 165 enzymes, e.g., HSD17B5, hydroxysteroid (17-beta) dehydrogenase 5
- 96 zinc finger genes, e.g., ZNF57, zinc finger protein 57
- 72 antigens identified by monoclonal antibodies, e.g., MIC5, antigen identified by monoclonal antibody R1
- 43 2D gel spots, e.g., PNI2, protein spot in 2-D gels 33kDa

We continue to assign gene symbols requested by individual scientists, databases, and journals. Recently, this has included approving a number of new symbols for the chromosome projects' forthcoming genome publication coinciding with the 50th anniversary of the double helix.
Please see our website at http://www.gene.ucl.ac.uk/nomenclature/ and contact us via email at nome@galton.ucl.ac.uk for further information, or to offer your help.

P567. Testing of an automated PCR parameter design program for use with OptimaseTM Polymerase.

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We have designed a web-based tool for the generation of PCR Protocols for Optimase™ Polymerase. This tool will produce PCR protocols for use with the high-fidelity PCR enzyme Optimase™ Polymerase. To generate a PCR protocol, primer sequences and anticipated PCR product length are entered, followed by selection of the desired PCR protocol type (either simple 3 step or touchdown PCR). The software will then create a PCR protocol appropriate for that fragment that will give high-quality amplification and low misincorporation when using Optimase™ Polymerase.
We demonstrate here the simplicity and accuracy of the software and validate its predictions for several primer sets demonstrating that the conditions generated lead to a high yield and clean product produced

by Optimase™ Polymerase. This minimises the need for optimising PCR reactions using a gradient block thermocycler to determine the best annealing temperature or PCR conditions to use.
This tool is accessed through Mutationdiscovery.com™, a website supported by Transgenomic, that enables customers to share information about the diseases, genes and methods. This site includes entries for thousands of genes, and each entry presents known variations in the context of the genomic sequence for the gene. The site also includes PCR and DHPLC protocols for the detection of both new and previously observed genetic variations.

P568. Evaluation of fidelity of data generated from linearly amplified RNA in the Affymetrix platform

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Analysis of transcript levels using DNA microarrays representation requires 1-10 micrograms of total RNA. However the amount of RNA that can be practically isolated from laser microscope captured cells is much less than the microgram range. Although linear isothermal RNA amplification methods are used to generate analyzable amounts of RNA, the fidelity of array results using amplified material has not been fully validated in the Affymetrix platform. The expression profiles of MCF10A and HTB-19 cells (a normal epithelial and breast cancer cell lines respectively) were compared in two separate experiments using Affymetrix HG-U95Av2 chips. In the first experiment 10µg total RNA isolated from these cells were labeled and hybridized according to the standard protocols by Affymetrix. In the second experiment 100ng total RNA was amplified then labeled and hybridized according to Affymetrix GeneChip Eukaryotic Small Sample Target Labelling Technical Note (Version 1). The correlation of relative gene expression changes (n=12500) between results of the two experiments is weak (r=0.482) if no data filtering is applied. However if “absent”, and “marginal” calls are filtered out and only “present” calls are included in the analysis (n=3918), the correlation is substantial (r=0.803). These results indicate data filtering is important for the accurate reporting of gene expression changes using amplified RNA

P569. An effective and quick magnetic particle transfer based system for genomics and proteomics sample preparation.

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The identification and characterization of genes and their protein products are essential issues in genomics and proteomics for understanding how cell growth and differentiation are regulated. In order to study the complex network of cell functions at reasonable speed and convenience, the methods for isolating and purifying DNA, mRNA and protein should be easy, rapid and reliable. Until now the main drawback of the techniques is that the time required for isolating nucleic acids and proteins with conventional methods may take hours or even a few days, can be labor-intensive, and may require use of toxic chemicals.
Here we describe the PickPen™ magnetic particle transfer technology and its use for the sample preparation in genomics and proteomics using magnetic particles coated with an appropriate chemical surface. For example, for the purification of mRNA we use long oligo-(dT)30 to increase the selectivity or for the purification of His-tagged proteins we have optimized the IMAC coordination chemistry for different downstream applications (e.g. pull-down analysis). The use of the new PickPen™ technology together with appropriate magnetic particles shortens the conventional protocols to 10–15 minutes. Moreover, the technology allows the purification of a variety of different molecules from the same sample. With the novel 8-magnet PickPen™ eight samples can be processed in parallel at the same time. The isolated biomolecules are of the highest quality and can be used for various downstream applications.

P570. The Third Party Annotation Dataset in EMBL-Bank: Call for Submissions

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The EMBL nucleotide sequence database now accepts third party annotation (TPA) entries of existing sequence data.
Until 2002, in its role as the principal European nucleotide sequence collection, EMBL-Bank has collected and distributed primary nucleotide sequence and annotation data. Primary data is defined as annotated sequence that has been determined by a submitter and their team. Primary database entries remain in the ownership of the original submitter and the co-authors of the submission publication.
We have experienced increasing demand from our users for opportunities to re-evaluate primary data by way of TPA. Indeed, with the availability of ever increasing quantities of primary data and the development of novel computational and experimental techniques to analyse sequence data, we believe that the TPA dataset will heighten the quality of information provided to database users as a whole.
Types of entry in the TPA dataset include reannotation of existing primary entries, combinations of novel sequence and existing primary data and annotation of trace archive and whole genome shotgun data.
Entries form a subset of EMBL-Bank and are identifiable as TPA. The TPA dataset is available from all existing EMBL-Bank sources.
Modifications to Webin, our custom web tool for EMBL-Bank submissions, have recently been completed to include TPA-specific fields. Submitters are required to provide accession and sequence version numbers as well as nucleotide locations for all primary entries to which their TPA submission relates. Submitters are encouraged to maintain their TPA entries using the EMBL update web form.

P571. Tools for the design of genetic studies

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Tools for the Design of Genetic Studies
Genetic studies of groups of individuals are frequently conducted in order to assist in the identification of genes associated with complex diseases. These studies require the locating and genotyping of possibly large numbers of individuals and can be expensive and time consuming. Moreover, the optimum parameters for the study are often difficult to estimate.
We introduce a set of simulation techniques which can assist in making such estimates. These techniques are based around a sophisticated tool for simulating the time-development of a human population. Parameters such as recombination fractions, reproduction-rate profiles and population admixture and stratification can be specified as required.
We identify statistical measures to determine signal-to-noise ratios in samples selected from these simulated populations. We also present results demonstrating the sensitivity of the signal to parameters such as sample size, recombination fractions and the nature and location of trait and marker loci.
It is anticipated that these techniques will assist those conducting genetic studies to make informed choices in the design of their study and hence to minimise the cost and maximise the effectiveness of such studies.

P572. Combining genetic linkage analysis with microarray gene expression profiling in multifactorial diseases.

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Identification of genes involved in diseases with complex genetic backgrounds remains challenging. Most recent genome-wide screens have failed to identify genes, due to the significant amount of genes within susceptibility loci.
In order to bring down the number of genes of interest within these loci we have developed a method, capable of combining genetic linkage analysis and microarray gene expression profiling. By converting the genetic map in use with genetic linkage analysis to a physical one and

by physically mapping the cDNAs or oligonucleotides in use on the microarrays, we were able to overlay genetic linkage graphs with gene expression profiles.
A Java based computer program was developed, that acts as a central repository for the combined analysis of genetic linkage and gene expression data. It includes a viewer, capable of showing karyobands, genes, markers, linkage graphs, gene expression levels, SNPs and additional annotation data simultaneously. Additionally data management and filtering functionality was implemented and the databases Ensembl, LocusLink, Gene Ontology and Unigene were integrated.
We tested the program's functionality in celiac disease, a multifactorial gluten-sensitive enteropathy, with the combined analysis of a genome-wide screen in 82 Dutch families with affected siblings and microarray gene expression profiles of 18118 cDNAs in 29 biopsy experiments.

P573. Isocode® ID, an Archiving Device Designed for the Storage of cDNA Libraries.

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Genetic studies often utilize cDNA libraries to search for „genes of interest". Library storage can be very expensive and laborious. We present an alternaitve to freezer storage of clones by using IsoCode ID archiving matrix. IsoCode® ID is based upon the well-established IsoCode technology that protects DNA contained in biological samples from degradation and microbial contamination. When a sample is applied to IsoCode, the chemistry lyses the cells, dissociates proteins from nucleic acids and destroys nucleolytic enzymes. DNA, suitable for PCR amplification, transformation and genetic typing, is eluted from the matrix in water by a simple heat step. IsoCode ID incorporates a color indicator into the IsoCode chemistry that clearly distinguishes where the sample was placed. We show that a variety of vectors and cDNA clones can be safely stored at room temperature and subsequently recovered from IsoCode ID. Recovery of clones utilized electroporation procedures to transform *E.coli* HB101 cells. In addition, cDNA clones recovered from the matrix were amenable to PCR amplification; the amplicons generated were consistent with control amplicons for each clone tested. We show that the DNA of a 650 base pair sequence isolated from a cDNA clone is unchanged while stored on IsoCode ID at room temperature. IsoCode ID was shown to be compatible with several types of DNA polymerases including Taq, TaqGold™, FastStart Taq, *Pfu*Turbo® and YieldAce™. In conclusion, IsoCode ID serves as an excellent matrix for room temperature storage and retrieval of cDNA clones from libraries typically stored at –80°C as glycerol stocks.

P574. Generation of new genes in the human trypsinogen family

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A primary driving force in genome evolution is duplication. Provided that one copy of a gene remains functional, the second copy is redundant and thus is free to accumulate mutations without deleterious effects on an organism's phenotype. This plays an important role in the generation of novel genes.
The human trypsinogen family was inadvertently sequenced due to its intercalation within the T cell receptor (TCR) beta locus, which was among the earliest targets of the human genome project (Rowen et al. Science 1996). Nine trypsinogen genes are separated physically into two groups. Group I (T4~T8) is located towards the 3' end of the TCR beta locus and consists of five tandem 10kb repeats in 70kb between V beta 4S1 and D beta 1. A sixth member (T9) has been translocated from chromosome 7q35 to chromosome 9p13. Group II (T1~T3) is located towards the 5' end of the TCR beta locus. Among these genes, T4, T8, and T9 have been assigned to the three well characterised forms of trypsinogen (cationic, anionic, and meso-trypsinogens). The other genes represent relic genes, pseudogenes or expressed pseudogenes.
Having integrated the current available genomic, transcriptomic and proteomic data, we provided evidence that T1 is a likely novel gene generated from the highly diverged group II trypsinogen genes, and T9 is gradually losing its function as a trypsinogen but is gaining a novel function in the form of a chimaeric protein.

P575. Comparative mapping of a part of human chromosome 1 studied by genetic linkage analysis in cattle

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Human chromosome 1 contains 8.4% of the total human genome. Two orthologous genes were chosen from human chromosome 1. One of these loci, was Fc fragment of the IgG receptor (CD64 or FCGR1B) mapped to human chromosome (HSA 1p12) is expected to map to cattle chromosome (BTA 3) and other Na+/K+ transporting alpha 1 (ATP1A1) mapped to HSA 1p13 was close to an evolutionary breakpoint. Two sets of PCR oligonucleotide primer pairs for ATP1A1 were designed to amplify 3'UTR and intron 2 in cattle. The primers to amplify FCGR1B were obtained from Bishop and associate (1994) where it is listed as FCGR2. This primer amplify a DNA segment contains microsatellite with 12 TG repeats. SSCP analysis were performed to develop polymorphism for amplified fragment from ATP1A1 and denaturing PAGE gel were used to separate FCGR1B polymorphic microsatellite product. Twenty three full-sib families of International Bovine Reference Panel (IBRP) were genotyped for each polymorphism. The genotypic data for each locus was submitted to the Cattle Genotypic Database (CGD) and analyzed using CRI-MAP v2.4 (SunOS). Then lodscores and maximum likelihood estimates of recombination were calculated between these loci and all other loci in the cattle genotypic database using the TWOPOINT option of CRI-MAP. The multipoint map for chromosome 3 was built up by successive refinement of the position of the loci. Both FCGR1B and ATP1A1 were linked to at least 10 previously mapped markers on BTA 3 and extend the comparative mapping of the short arm of human chromosome 1.

P576. Comprehensive annotated database of K channel mutations

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An extensive number of disease causing mutations in human K+ channels have been identified. We have constructed a web-based database aiming to assemble all available molecular and clinical information regarding K+ channel mutations. The database will automatically collect and categorize public information from the Internet though new data will be reviewed manually for quality and consistency. The database system has been built as a customizable general purpose scientific database system where emphasis has been put on annotation forums of all data units, change logging, source reference and cross-linkage, easy collaboration with external databases and containment of experimental data. http://www.multiplex-network.de

P577. A human DNA resource for use in association studies and the quality control of high throughput SNP studies

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Gene variants contribute to virtually every human disease, conferring susceptibility or protection directly or indirectly by influencing interactions with environmental factors. SNPs are the most widespread type of DNA variant in the human genome and are increasingly commonly used in association studies.
To facilitate these - and similar - studies, a high quality, standardised, authenticated control DNA resource has been generated. 480 EBV immortalised cell lines were generated from patrial UK blood donors

in order to provide a reproducible and renewable source of control DNA. Aliquots were organised into 5 panels of 96 (HRC-1 to 5) with the first two panels also available in a PCR-ready 8x12 array format. We provide evidence that use of this DNA results in a tight clusters of co-ordinates in the scatter plot generated by the ABI 5' nuclease SNP assay. The clusters correspond to the heterozygous and homozygous states. The tightness of the clusters facilitates scoring. This suggests that validation of a SNP assay is best achieved using high quality reference DNA, such as the ECACC HRC resource. Such validations are of special importance in a high throughput setting such as that provided by MRC geneservice.

P578. A Novel Method for 'Centrifuge Free' Plasmid DNA Purification from *E. coli* Broth without the Use of Organic Solvents

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Nucleic acid purification has traditionally required centrifugation and precipitation steps, and modern methods typically involve the use of chaotropic agents and organic solvents. These introduce restrictions in scaling to high throughput, and more elegant technologies are needed to drive the genomics industry further and faster. We present a new technology, which completely removes the need for these chemicals, and uses protocols that can eliminate precipitation, centrifugation or dehydration of DNA, therefore reducing the risk of damage to the final product. The technology is based on an immobilized switchable positive charge, which switches to neutral at pH > 8.0. The switchable positive charge can be derivatised on almost any surface, creating the opportunity for a vast number of purification formats. This method utilising derivatised magnetic beads for plasmid purification is reliable, efficient and easy to use. This technology allows plasmid purification from bacterial broth making plasmid purification easier and vastly improves the handling on automated workstations, especially in high through-put laboratories. We have shown that this immobilised charged technology can applied to many formats including magnetic beads, cartridges and plastics.

P579. Reliable genotyping of Thiopurine Methyltransferase polymorphisms by MALDI-TOF MS

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The correlation of genetic polymorphisms, in particular SNPs, with pharmacologic effects found in pharmacogenetic studies may revolutionize future medicine. The possibility to avoid severe side effects of drugs and to adapt therapy considering genetic predispositions will be one major benefit of the elucidation of the human genome and its diversity. On the other hand, to make use of this potential reliable and cost-effective technologies are necessary. We investigated 475 human samples for three polymorphisms in the thiopurine methyltransferase gene which are known to affect S-methylation of commonly used anticancer drugs like azathioprine, thioguanine or 6-mercaptopurine using the novel *GENOLINK MT* system and *genoSNIP* assay. After PCR amplification, amplicons containing the polymorphic sites were used as templates in a triplex allele-specific primer extension reaction with oligonucleotides containing an internal photocleavable building block. The extended biotinylated primers were bound to streptavidin-coated microtiterplate cavities and washed. Subsequently, the 3' part containing the genotype information was cleaved off by UV-light and transferred to a MALDI sample plate. Measurement of the exact molecular masses of the fragments allowed determination of the respective genotypes. Pipetting steps, measurements and allele calling were performed automatically. In this blind study, all genotypes could be determined unambiguously and were consistent with results previously obtained using DHLPC and DNA sequencing. Because of its reliability, throughput and cost reduction capabilities, the *GENOLINK MT* system is highly suitable for pharmacogenomic studies and diagnostic purposes.

P580. Haplotyping genotypes: some problems

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Several projects plan to infer haplotypes from genotypes observed in populations or sub-populations differing in health or ancestry. Here I explore some simple problems in the diallelic case and compare the few deduced haplotypes with the many inferred haplotypes provided by programmes in common use.
It is obvious that haplotypes cannot be deduced from genotypes with more than one heterozygote. I code haplotypes by compact allelic sequences such as
‚Haagg..', ‚Haaag..' and genotypes by ‚GaaHg.. ,
where the heterozygous allele is coded ‚H': this also doubles as a parameter with a prior value of a half defining the probability of a specified allele being in the inferred haplotype.
An simple example based on known imaginary short haplotypes is shown below.
Known Inferred (unordered) Chosen
Haaagga {iaaagga,iaaaggg} Iaaaggg
Haagggg {iaagggg,iaagaga} Iaaggga
GaaHggH GaaHggH
Haaaggg {iaaagga,iaaaggg} Iaaaggg
Hagagga {iagaggg,iagagga} iagagga
GaHaggH GaHaggH
Only three of the four known haplotypes are inferred, the fourth being lost.
Iterative algorithms that optimise values of H at each locus using the small haplotypes deduced from homozygous segments are liable to reach ‚cusps' ending with values of 1 or zero, leading to fewer inferred haplotypes and multiple representation of others. There are problems when with EM algorithms applied to parameters whose most likely values reach fixation.
In random pairs of most mammals the expectation of less than four substantial haplotypes is low. Inferred haplotypes should be termed ‚inferred haplotypes'.
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P581. Mammalian RNA Profiling on Bead-based Microarrays

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We have adapted Illumina's bead-based array technology for studying gene expression. The array matrix format, described below, currently allows gene expression profiles to be obtained for ~ 700 genes from 96 samples at a time, enabling thousands of samples to be processed a day. The array matrix comprises 96 individual arrays arranged in an 8-by-12 matrix that matches the well spacing of a standard microtiter plate. Each individual array in the matrix holds ~ 1,500 different oligonucleotide probes. The probes are attached to 3 micron beads, which are assembled into wells at the end of an optical fiber bundle to make an array. Since there are many more wells than probe sequences, multiple copies of each bead are present in the array. This built-in redundancy improves robustness and measurement precision. Through a Latin Square experiment using 79 spiked yeast transcripts spiked into mouse RNA, we have demonstrated detection of transcripts present at ~ 1:100,000 in mouse cellular mRNA. Measurement of gene expression is linear over a 2.3-2.8 log range, and precision is sufficient to detect 2-fold differences in expression between samples. By measuring cell-specific gene expression in samples of mouse B and T cell RNA mixed at various ratios, we show that this high level of performance extends to endogenous mammalian genes. The combination of hundreds of genes per array and the sample throughput of the matrix format should enable "many genes, many samples" applications that are not adequately addressed by current gene expression technologies.

P582. Bioinformatical recognition of Potassium-channel sequences

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Potassium (K-)channels comprise a large and diverse class of membrane proteins involved in a great variety of cellular functions. K-channel α-subunits consist of at least two transmembrane regions that frame the ion-conducting pore-domain, the only conserved region among all K-channels. At the extracellular opening of the pore a „selectivity-filter" provides the K^+-specificity.
K-channel gene identification with conventional methods like pattern recognition and even emotif (Nevill-Manning et al., JMB 1997) produce a large number of false positives caused by the close relationship among all ion-channel pores and therefore have limited use for K-channel-gene screening.
Here we introduce a method that is based on a highly specific signature and a new algorithm analysis not the amino acid sequences themselves but physico-chemical properties of their residues.
Focusing on only the conserved pore region and the selectivity-filter, the motif comprises 25 residues, carrying sufficient specific information to be used within this algorithm.
The algorithm converts the signature into a „consensus-feature-string" describing biochemical and steric features of the signature. Therefore, a potential hit represents a match to a stringent order of properties and not a amino acid sequence.
The method was tested with a set of 1419 sequences, consisting of 462 K-channel pore sequences, 178 pore-domain related sequences, 188 K-channel β-subunits and 591 random sequences.
Using conventional pattern recognition to recover 90% of all K-channels, leads to a false positive rate of 30%. Even when recovering 99% of the K-channel,.t he false positive rate of our method is smaller by a factor of 10.

P583. Integrated sequence-based genotyping of viral populations using the ABI PRISM® SeqScape® Software v2.0 and 3100 Genetic Analyzer system from Applied Biosystems

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Sequence-based mutation detection and verification methods are successfully employed in various applications where it is critical to know both the exact location and identity of a genetic change, including the identification of mutations resulting in drug susceptibility or resistance in microorganisms, human identification via analysis of mitochondrial DNA sequence patterns, species-specific identification of microorganisms and phylogenetic analyses. The ABI PRISM® SeqScape® Software version 2.0 from Applied Biosystems is a dedicated sequence comparison tool containing new features and algorithms which facilitate and accelerate the analysis of DNA sequencing data for genotyping. The expanded Reference Data Group allows discontinuous numbering of reference sequence components, differentiation between sequence regions such as introns and exons, and customized data display. The quality value-based Consensus Caller™ algorithm in combination with the integrated library search functionality allows for accurate, easy and rapid genotyping and sequence variant identification.
Until recently, viral populations have been classified according to their antigenic characteristics, but the advent of rapid and affordable protocols and instrumentation has made automated nucleic acid sequence analysis the most reliable and accurate method for evaluation of viral genome variation. Studies using sequencing-based genotyping of Hepatitis C virus (HCV) and Human Papilloma virus (HPV) populations have resulted in the development of a classification system for these genomes. Here, the new functionalities of SeqScape® Software version 2.0 are used to genotype to the subtype level various HCV and HPV isolates resolved using an ABI PRISM® 3100 Genetic Analyzer, and the results compared to that obtained with classical genotyping methods.

P584. Large-scale homology modelling of non-synonymous single nucleotide polymorphisms

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Single nucleotide polymorphisms (SNPs) represent about 90% of human DNA sequence variation. Non-synonymous SNPs (nsSNPs) are those that cause amino acid sequence changes. They are therefore more likely to affect protein function and to be related to disease. A large-scale systematic study of the effects of nsSNPs on protein structure and disease relationship will help derive predictive rules to identify deleterious amino acid variants and thus aid in the assessment of disease susceptibility.
In this study, human protein variants present in the Swiss-Prot database were classified into two datasets: disease-related and non-disease-related. Automatic modelling using the Swiss-Model server was performed if suitable structural templates (> 70% identity to the sequence) could be identified from the Protein Data Bank.
In total, 3500 models (2800 disease-related and 700 non-disease related), each containing one nsSNP, were created. Preliminary analysis regarding to the distribution of secondary structure, residue accessibility and size showed that accessibility and the secondary structure of the mutation site have a better discriminative power than change in residue type and size. In particular, it was found that about 36% of the disease-related nsSNPs are located in regions with low accessibility, while only 19% of non-disease-related nsSNPs are in these areas.
While the detailed analysis of the structural features of nsSNP is still underway, new Swiss-Prot variant webpages are being implemented through the ExPASy server to provide public access to the essential information of each protein variant, as well as their homology models (when available).

P585. The Human Proteomics Initiative

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SWISS-PROT is a protein sequence and knowledge database characterized by high quality manual annotation, minimal redundancy and high level of integration with other databases.
In this context, the goal of the HPI project is to annotate all known human protein sequences and their mammalian orthologs, according to SWISS-PROT high quality standards.
For each known protein, a wealth of information is provided that includes the description of its function, domain structure, subcellular location, similarities to other proteins, etc. A special emphasis is laid on post-translational modifications (PTM), isoforms produced by alternative splicing, polymorphisms and disease-linked variants.
While all human proteins are, in the context of this project, equally important, a special effort is being made in 2002 to annotate proteins encoded on chromosomes 20, 21 and 22, which were the first chromosomes to be fully sequenced and partially annotated. Currently, SWISS-PROT is nearly synchronised with the current state of knowledge of proteins encoded on these chromosomes. In 2003, we will focus on chromosome 14, while maintaining chromosomes 20, 21 and 22 up-to-date.
Release 40.40 (January 17th 20032) contained **9'001** annotated human sequences. These entries were associated with about **23'200** literature references; **22'600** experimental or predicted PTMs, **2'800** splice variants and **15'100** polymorphisms (the majority of which are linked with disease states). Up-to-date statistics are available at http://www.expasy.org/sprot/hpi/hpi_stat.html.

P586. Analysis of Gene Expression in Mice Hemizygous for the DiGeorge Syndrome Region using Affymetrix Microarrays.

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DiGeorge Syndrome(DGS) is usually associated with a 1.5-3Mb heterozygous deletion on chromosome 22q11.2 in human patients. The main features of the phenotype are congenital heart disease, parathyroid and thymic hypoplasia, velopharyngeal insufficiency, cleft palate and learning difficulties. Chromosome engineering methods have produced a mouse model of DGS carrying a 1.2Mb (18 gene) deletion on proximal chromosome 16 which is syntenic to the human

deleted region. Heterozygously deleted mice (termed Df1+/-) have cardiovascular defects of the same type as those in DGS, aswell as thymic, parathyroid defects and learning and memory impairment. To understand the genetic aetiology of the defects seen in the Df1 mouse, RNA was extracted from the branchial arches of 3 e10.5 embryos and hybridised to Affymetrix microarray chips (Df1+/- n=8; wild type n=7). Genes in the Df1 deleted region served as internal controls as the gene dosage is 50% of wild type levels. Several genes involved in angiogenesis and cardiac development have been identified as possible targets of Df1 deleted genes, which are now being followed up.

P587. Exact molecular quantification by the refined quantitative fluorescent PCR and molecular-cytogenetic analysis of Y sequences from gonads and peripheral blood in a Turner syndrome patient.

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The objective of this work was to determine and compare representation of cell lines with Y sequences in gonads and peripheral blood in a Turner syndrome patient.
Methods. The refined technique of the quantitative fluorescent polymerase chain reaction (RQF PCR) with utilization of AMELX/Y locus was applied for molecular quantification. Fluorescent in situ hybridization technique (FISH) was used for detection of gonozomes on tissue print preparations and on slices from frozen tissue.
Results. On the basis of a formulated calibration curve, the following Y mosaics were deducted: right gonad 13,5 %; left gonad 23,4 %; peripheral blood 31 %. Universally applicable model of mosaic determination on the molecular level was formulated. Y sequences detected by FISH method were found preferentially in certain cell types. **Conclusion.** Sensitivity of RQF PCR together with the FISH technique made possible a significant progress in the analysis of gonozomal mosaics. The finding of an uneven representation of Y positive cell lines and their nonrandom distribution is considerable too.

P588. Molecular profiling of embryonic versus adult neural stem cells: a transcriptome analysis

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Neural stem cells (NSC) are used to replace lost populations in neurodegenerative disease animal models where they integrate and functionally recover the damage. The therapeutic use of adult neural stem cells is still limited by the scarce knowledge of their real potential in comparison with fetal NSC. Embryonic and adult neural stem cells are both able to proliferate and to differentiate in vitro and in vivo, but extensive molecular and cellular comparative data are still missing.
In order to address this issue we performed an expression profiling study of neural stem cells derived from murine brains during primary neurogenesis (embryonic day 12) and adult life. The high density oligonucleotide Genechips (Affymetrix) were used.
Our results show that temporal changes do exist in NSC gene expression. Interestingly, besides significantly modulated genes, several transcripts appear to be specifically expressed only in embryonic or in adult NSC, consistent with the hypothesis that temporally different regulatory mechanisms may play a role in neural stem population specification during neurogenesis. Transcription factors such as Zic1, Zic3 and engrailed 2 show a marked expression in embryonic NSC. A Real Time PCR assay was used to confirm data. A validation was achieved also on different biological replicates thus giving significance to the obtained data.
A map of the differentially regulated pathways in embryonic and adult NSC will be useful to integrate expression profile data with NSC cellular features. These results will also help understanding the obscure mechanisms underlying the transdifferentiation process of adult stem cells.

P589. Down regulation of the Sonic Hedgehog signalling pathway during neuronal differentiation of human teratocarcinoma (NT2) cells

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Neural stem cells (NSCs) in the fetal and adult central nervous system has the capacity of selfrenewal and differentiation into neurons. These cells are of interest for understanding of the basic developmental biology of the brain as well as the development of brain tumours. Furthermore, it is believed that NSCs may serve as potential therapeutic reagents for neurodegenerative disorders. However, little is known about the biology of NSCs, thus investigations of e.g. the signalling pathways regulating the survival and differentiation of these cells are needed.
Human teratocarcinoma NT2/D1 cells can be induced by retinoic acid (RA) to differentiate into postmitotic central nervous system neurons. We have investigated the Sonic hedgehog (SHH) signalling pathway during neuronal differentiation of NT2 cells using real-time RT-PCR based expression analysis of mediators and targets of SHH signalling.
Our preliminary results suggest that NT2/D1 cells may serve as a human model for investigation of signalling transduction in NSCs. The data show that SHH signalling is down regulated during neuronal differentiation of these cells. The data also indicate that the NT2 precursor cells can compensate for the direct inhibitory effect from RA and suggest that RA inhibition of SHH signalling may involve other yet unknown signalling pathways in these cells.

P590. A possible role of Bcl-2 and Bcl-X in the aberrant programmed cell death seen in type I spinal muscular atrophy

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Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by mutations in the SMN1 gene. The degeneration and loss of the anterior horn cells constitute the major neuropathological finding in SMA and the mechanism and timing of this abnormal motor neuron death is under study. It has recently been reported that the fetal SMA spinal cord shows a significant increase in cells with DNA fragmentation, suggesting that the programmed cell death is aberrantly increased in type I SMA during development (Soler et al, Brain 2002, 125:1624-1634; Fidzianska and Rafalowska, Acta Neuropathol 2002, 104:363-368). We analyzed the expression of two antiapoptotic proteins, Bcl-2 and Bcl-X, in control and SMA fetal spinal cords. In control fetuses, Bcl-2 expression is predominant in motor neurons at 12 weeks, but reduced at 15 weeks. On the other hand, no Bcl-X expression was detected in motor neurons at 12 weeks, but it is present at 15 weeks. This period coincides with an increase in physiological motor neuron death and with the establishment of functional neuromuscular synapses. Both proteins may complement each other for the survival of early postmitotic neurons before appropriate synaptic connections are established. At 15 weeks, SMA spinal cord showed a marked decrease in the levels of Bcl-2 and a delay in the expression of Bcl-X in comparison with controls. The difference in the pattern and degree of expression is consistent with a role for both proteins in the aberrant programmed cell death observed in type I SMA (Supported by FIS 02-1275)

P591. Spectrum of mutations of the LDL-R gene in Greek patients with familial hypercholysterolemia.

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Familial hypercholisterolemia (FH) is an autosomal codominant disease, caused by mutations in the low density lipoprotein receptor (LDLR) gene. In order to identify the spectrum and the distribution of the mutations in the LDLR gene in the Greek population we investigated 132 FH patients of greek origin. The patients all fulfilled the clinical criteria for FH and were recruited from the Hippocratio hospital. We used a mutation screening assay based on the highly sensitive denaturing gradient gel electrophoresis (DGGE) technique combined with DNA sequence analysis. In total, mutations regarded as cause of the disease were identified in 44 patients (33%) representing 13 different mutations. We did not identify any patients with the common mutation (apo B 3500) causing familial ligand defective apoB-100. The most frequent mutation according to our results was found to be Africaner 2 (1285 G>A) on exon 9 of the LDLR gene. We further identified 9 point mutations which were not considered to affect the function of the gene, and thus were regarded as polymorphic changes. Future studies should aim at identifying the importance of these mutations in offering differentiated treatment in patients with FH and unravelling the involvement of other genes in this disease, hence to provide a more differentiated prognostic and therapeutic evaluation in FH.

P592. The analysis of mutations NADH-cytochrome b5 reductase gene for hereditary methemoglobinemia in Yakut population

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Hereditary methemoglobinemia is an autosomal recessive disorder characterized by NADH-cytochrome b5 reductase deficiency.
Two forms of this enzyme are known, a membrane-bound form in somatic cells, and a soluble form in erythrocytes. There are three types of hereditary methemoglobinemia: an erythrocyte type (type I)- is deficient only in the erythrocytes of patients manifesting mild cyanosis, a generalized type (type II)-in which deficiency of the enzyme in all tissues of patients manifesting cyanosis, mental retardation and a blood cell type (type III)- the enzyme defect is observe in all blood cells. The different types of methemoglobinemia are the result of mutation in the DIA1 gene which is localized at chromosome 22q13 and contains 9 exons. At present 15 different mutations of this gene have been described at type I b5R deficiency. Yakutya is the most large endemic hearth of hereditary methemoglobinemia. Since Yakutya is situated near to China and Japan we decided to investigate 3 point mutations of the DIA1 gene (Leu72Pro, Val105Met, Arg57Gln) which were previously described in the Chinese and Japanese populations. Using restriction analyses of PCR-amplified fragments of exons 3 and 4 of the DIA1 gene we did not find any of these point mutations in 19 children of Yakutya with hereditary methemoglobinemia type I, indicating that in Yakutya there is a specific mutation of the studied gene for this population.

P593. A novel transgenic mouse model with the R192Q Familial Hemiplegic Migraine mutation: generation and preliminary (electrophysiological) characterization.

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Mutations in the CACNA1A gene which encodes the alpha1A ($Ca_v2.1$) subunit of neuronal P/Q type Ca^{2+} channels cause a wide spectrum of diseases, including Familial Hemiplegic Migraine (FHM), Episodic Ataxia type 2 (EA-2), and Spinocerebellar Ataxia type 6 (SCA6).
We generated a transgenic FHM mouse model by introducing the R192Q FHM mutation in the orthologous mouse gene. Heterozygous and homozygous mice are viable and do not show an overt clinical phenotype.
P/Q-type Ca^{2+} channels are expressed at presynaptic active zones of central synapses and the peripheral neuromuscular junction (NMJ), and mediate neurotransmitter release. Earlier studies revealed synaptic defects at diaphragm NMJs of the natural $Ca_v2.1$ mouse mutant *Tottering*. Using *in vitro* micro-electrode methods we observed an increase in spontaneous quantal ACh release and an increase in rundown of evoked release at high rate nerve stimulation frequency (40 Hz) (Plomp et al., *Brain* 2000;123:463-471).
Measurements at the NMJ of homozygous transgenic R192Q mutant mice showed an increased spontaneous ACh release (by ~140%), compared to wild-type control. Upon slight depolarization of presynaptic terminals by 10 mM K^+ the difference in spontaneous release between R192Q and wild-type NMJs became more pronounced, i.e., it was ~380% higher in the mutant. ACh release at R192Q NMJs was sensitive to the P-type channel blocker ω-Agatoxin-IVA, confirming involvement of this type of calcium channels.Our results suggest that synaptic dysfunction is likely to contribute to FHM symptoms. The novel R192Q FHM transgenic mouse model might prove to be highly valuable to unravel migraine related pathophysiological mechanisms.

P594. Re-evaluation of genetic heterogeneity in multiple exostoses.

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Multiple exostoses (EXT) is a bone disorder characterized by bony outgrowths, mainly located in the juxtaepyphyseal parts of the long bones. EXT is a genetic heterogeneous disorder with three presumed loci: EXT1 on 8q24, EXT2 on 11p11-p12 and EXT3 on 19p. Both EXT1 and EXT2 genes have been isolated and encode glycosyltransferases involved in heparan sulfate biosynthesis.
In the past mutation analysis, mostly performed by SSCP, resulted in the identification of an EXT1 or EXT2 mutation in approximately 70-80% of the cases. We have now performed direct sequencing of all EXT1 and EXT2 coding exons in several patients, including some which failed to show a mutation in previous SSCP studies.
Several new mutations were identified in our set of patients with most mutations being inactivating private mutations. The fact that in several patients which were negative in a previous SSCP based screening now a mutation was identified, illustrates the importance of the choice for a more sensitive detection technique in diagnostic settings.
At present the few mutation negative patients are analyzed with intragenic EXT1 and EXT2 markers and FISH to screen for deletions within these genes. Additionally RNA analysis should reveal smaller deletions and splicing defects caused by intronic mutations. It can be expected that after this extensive mutation analysis an EXT1 or EXT2 mutation can be identified in close to 100% if not all of the patients, raising serious questions about the existence or at least importance of the EXT3 locus at chromosome 19p.

P595. Mutation screening of Endoglin and ALK1 in French patients with Hereditary Hemorragic Telangiectasia

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Hereditary Hemorragic Telangiectasia (HHT) is an autosomal dominant vascular disorder. The main lesions, arteriovenous malformation, can cause frequent and abundant epistaxis, pulmonary, intestinal or brain hemorrhage and cardiopathy due to a shunt effect. Most HHT are due to mutations in endoglin (HHT1) or ALK1 (HHT2) genes, which products are involved in the TGFbeta signaling pathway. Genetic screening of patients is important for both diagnostic confirmation and to focus the clinical survey on asymptomatic carriers.
97 French unrelated probands with confirmed (81%) or suggested (19%) HHT diagnosis were screened by heteroduplex for both genes. A germline mutation was found in 62% of the patients (n=60), 19 of them in endoglin, including 9 small insertions/deletions, 6 missense and 4 intronic mutations. Each patient had a different mutation. Forty-one mutations were found in ALK1, including 2 nonsense, 18

missense, 20 small insertions/deletions and 1 intronic mutations. Only two of the missense mutations observed have been previously reported. None of the mutations was found in 130 French unaffected individuals. Three ALK1 mutations were of particular interest: 1112_1113insG was present in 10 individuals sharing a common haplotype, supporting the hypothesis of a founder effect. Two missense involved the same codon: R411W and R411P, respectively observed in 5 and 2 unrelated patients. Haplotype analysis was in favor of a mutation hot-spot.
Our series show that germline mutations in HHT patients are found in both endoglin and ALK1 genes and affect most of the coding sequence.

P596. Characterization of Hereditary Haemorrhagic Telangiectasia (HHT) in the Italian population

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Hereditary Hemorrhagic Telangiectasia (HHT) o Rendu-Osler-Weber Syndrome (OMIM#187300) is an autosomal dominant disorder, characterized by aberrant vascular development.
Mutations in at least two genes have been shown to be associated with HHT: endoglin (*ENG*) on chromosome 9 and *ALK-1* on chromosome 12; the proteins encoded by the two genes are expressed predominantly on endothelian cells and are involved in TGF-β signaling.
We examinated 65 patients, 53 belonging to 30 unrelated families and 12 sporadic cases.
Genomic DNA isolated from peripheral blood was used to amplify the 15 exons of ENG and the 9 exons of the coding region of ALK-1.
We performed SSCP and HA analysis to search for mutations in the two genes.
The amplified DNA samples showing variant electrophoretic pattern were sequenced.
We identifed 5 novel mutations in the *ENG* gene: 3 deletions, 1 insertion and 1 nonsense substitution. Another nonsense mutation, already described, and 8 polymorphisms were found. In three instances we are dealing with a de novo mutation.
In the *ALK-1* gene we found nine missense mutations, 3 of which never described previously, in 12 unrelated families.
In the affected members of one family a transversion in a consensus sequence of a splicing acceptor site was observed. Splicing assays are in progress in order to evaluate the effects of this mutation on gene expression.
In the families who share the same *ALK-1*mutation, characterization of tightly linked microsatellite markers to this locus is in progress to verify the possible existence of a founder effect.

P597. Functional impact of mutations underlying pulmonary hypertension in hereditary haemorrhagic telangiectasia

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Mutations in the genes *ALK-1* and *ENG* encoding type I and accessory receptor members of the TGF-beta cell-signalling pathway, underlie the vascular dysplasia hereditary haemorrhagic telangiectasia (HHT). Primary pulmonary hypertension has been associated with mutations in *BMPR2*, a type II receptor member of the TGF-beta family.
We have recently described several patients with pulmonary hypertension (PH) and HHT with mutations in *ALK-1*. In the present study we have investigated a cohort of a further nine families with both PH and HHT.
We performed sequence analysis of *ALK-1*, *ENG* and *BMPR2*.
Five *ALK-1* mutations were identified in six families, with one mutation seen in two apparently unrelated patients in our cohort. All were missense mutations within the kinase domain of *ALK*-1. Two mutations are novel and are conserved across evolution and different TGF-β family receptors. Three have previously been described in patients with HHT alone.
To investigate the impact of the mutations identified we transiently transfected HeLa cells with GFP-tagged mutant constructs to determine the cellular localisation of mutant protein. The majority of identified *ALK-1* mutations in HHT and PH prevent the localisation of transiently transfected ALK-1 to the cell surface in HeLa cells, with ALK-1 being retained intracellularly within the endoplasmic reticulum. Plexigenic pulmonary hypertension appears to be more common in HHT subjects harbouring *ALK-1* mutations generating haploinsufficiency. The molecular mechanisms underlying this life threatening complication of HHT warrant further investigation.

P598. Congenital afibrinogenemia: expression and analysis of novel mutations affecting fibrinogen assembly or secretion.

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Congenital afibrinogenemia (MIM #202400) is a rare, autosomal recessive disorder characterized by the complete absence of circulating fibrinogen. Since our identification of the first genetic defect for this disease, a deletion of 11 kb eliminating nearly the entire FGA gene, numerous causative mutations have been described. Apart from three missense mutations in FGB, all mutations described so far are null mutations, predicted to cause total lack of the corresponding fibrinogen chain. During the course of a prenatal diagnosis for a Palestinian family with two affected daughters, we identified a novel nonsense mutation in the FGB gene, W467X (exon 8) which was predicted to lead to the production of a truncated protein missing only 25 amino acids from the C-terminus. The identification of this relatively mild afibrinogenemia mutation in homozygosity in the two affected patients prompted us to study the W467X mutation further. Expression of the W467X mutant FGB cDNA in combination with wild-type FGA and FGG cDNAs showed that the W467X mutant protein was stably expressed. However, fibrinogen molecules containing the mutant beta-chain were not secreted into the media, although hexamer assembly appeared unimpaired, confirming the necessity of intact C-terminal portions of the fibrinogen beta-chain for the secretion of functional fibrinogen hexamers. Two other missense mutations identified in afibrinogenemia patients: FGA R178P, in the coiled-coil region of the fibrinogen alpha chain, and FGB G444S in the C-terminal portion of the beta chain, are currently under analysis in order to investigate their putative effect on fibrinogen chain assembly and secretion.

P599. Nonsense mutation in exon 3 of the growth hormone receptor (GHR) in severe GH insensitivity (Laron syndrome) and the issue of the origin and function of the GHRd3 isoform

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Mutations in the growth hormone receptor gene (GHR) cause congenital GH insensitivity (GHI), a genetic disorder characterized by severe growth retardation associated with high serum concentration of GH and low serum levels of IGF-I. Molecular defects have been identified in all GHR coding exons, except exon 3, a sequence that encodes part of the extracellular domain of the receptor. In humans, GHR transcripts exist in two isoforms differing by the retention (GHRfl) or the exclusion (GHRd3) of this particular exon. As shown recently, such a dimorphic expression pattern -of unknown significance- could result from a retrovirus-mediated deletion event

involving exon 3. This model for the generation of those two isoforms, however, leaves open the possibility that GHRd3 transcripts also arise from GHRfl alleles through alternative splicing. Here, we report the identification of the first mutation in exon 3 of the GHR (W16X) in a patient with GHI and who also carries another nonsense mutation in exon 4. Intrafamilial correlation analyses of genotypes (presence of normal or mutant GHRfl and/or GHRd3 alleles), GHR expression patterns, and phenotypes provided direct evidence against an alternative splicing of exon 3: in particular, this exon was retained into transcripts originating from the GHRfl-W16X allele in both the patient and his mother. These observations, given the normal phenotype of the heterozygous parents, revealed also that a single copy of either GHRfl or GHRd3 is sufficient for normal growth.

P600. Molecular investigation of eight Iranian achondroplasia patients

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Achondroplasia (ACH) is the most common genetic form of dwarfism, inhereited as an autosomal dominant trait with 100% penetrance. The estimated frequency of ACH is one in 26,000 with at least 80% of the cases being sporadic. A gene for ACH was recently localized to 4p16.3 by linkage analysis. The ACH candidate region includes the gene encoding fibroblast growth factor receptor 3 (*FGFR3*). DNA studies revealed a point mutation in the *FGFR3* gene in ACH patients. Most of them have a G→A transition at nucleotide 1138 of the cDNA. A G→C transversion at the same position has been reported in a few patients. Both mutations result in substitution of arginine for glycine at position 380 in the transmembrane domain of the mature FGFR3 protein. For eight clinically determined cases of achondroplasia, we used FGF-DT primers to amplify a 164 bp product from genomic DNA that includes the complete membrane spaning domain of FGFR3. Sequencing the amplified region revealed a G→A transition in five subjects.

P601. MURR1: a new copper transport protein?

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Copper is an essential trace element for the survival of all organisms, although it is highly toxic above a certain threshold. To ensure proper copper homeostasis, copper import, distribution and export are well-regulated processes. Two homologous copper transport proteins have been identified, which, when dysfunctional, cause either copper deficiency (Menkes disease) or copper accumulation (Wilson disease) in man. Non-Wilsonian hepatic copper toxicosis in man has been described to be phenotypically very similar to copper toxicosis (CT) in Bedlington terriers, which is a frequent genetic disease unique to this breed. We recently established that canine CT is due to a large deletion encompassing exon 2 of MURR1. We also performed MURR1 mutation analysis in 23 patients with non-Wilsonian hepatic copper toxicosis. Although no mutation was found the MURR1 gene remains to be a candidate gene for other patients with non-Wilsonian hepatic copper toxicosis. The function of the MURR1 gene is still unknown but we hypothesize that the MURR1 protein is involved in regulation of biliary copper excretion . Based on the amino acid sequence, MURR1 is predicted to be a cytoplasmic protein. To begin to address the function of MURR1, we developed specific polyclonal antibodies and performed a yeast-two hybrid screen to identify interacting proteins. Further characterization of MURR1, and its pathway, may lead to disentangling the complexities of copper metabolism in mammals and to the identification of new genes involved in copper homeostasis, which will be candidates for non-Wilsonian hepatic copper toxicosis.

P602. Phosphorylated histone H2AX foci and acetylated H3 distribution in bleomycin-treated T cells of Ataxia Telangiectasia and Nijmegen Breakage Syndrome patients

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Cell exposure to X-rays or radiomimetic drugs induces DNA double-strand-breaks in a dose-dependent way and the recruitment of repair factors into distinct nuclear foci. H2AX, a variant form of the H2A histone, is known to be phosphorylated (gamma-H2AX) at break sites by ATM, the protein mutated in AT, after a few minutes of treatment. In T cell-lines from patients affected with AT and NBS we analysed gamma-H2AX foci after treatment with bleomycin. Cells from two normal controls showed high levels of gamma-H2AX-positive cells after 5-30' treatment, followed by a decrease ascribable to successful repair after 45' in drug-free medium. In one patient with classical AT, homozygous for the truncating mutation 3802delG, positive cells did not change during treatment, nor following the recovery period, in agreement with the lack of ATM activity. Another patient with a milder phenotype, homozygous for the splice-site mutation 3576G>A known to preserve partial ATM activity, showed a delayed response, indicative of slower H2AX phosphorylation.
Cells from two patients with NBS were capable of H2AX phosphorylation, as evidenced by increased positive cells after treatment. This, however, persisted during the recovery period due to unrepaired breaks, in accordance with the fundamental role of the NBS1-MRE11-RAD51 complex in repair downstream of H2AX phosphorylation. Double staining of acetylated H3 histone-gamma-H2AX was also performed in order to investigate possible relationships between double-strand-break repair and chromatin accessibility. Our preliminary finding of redistribution of H3 acetylation in gamma-H2AX-positive cells may suggest functional coordination of early events as H2AX phosphorylation with widespread chromatin remodelling.

P603. Novel SBDS mutations and gene conversions associated with Shwachman-Diamond Syndrome

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Shwachman-Diamond syndrome (SDS [OMIM 260400]) is an autosomal recessive disorder with clinical features including exocrine pancreatic insufficiency, variable skeletal defects, and haematological dysfunction. Patients are at risk of bone marrow failure and malignant transformation to acute myelogenous leukemia. The gene responsible for SDS, named SBDS, maps to the interval at 7q11 previously defined by linkage analysis and family studies. Although highly conserved orthologues of SBDS exist in archaea and eukaryota, the predicted protein has no homology to any characterised functional domain. Several indirect lines of evidence suggest that it may be involved in some aspect of RNA metabolism. A pseudogene copy of SBDS, with 97% nucleotide sequence identity, resides in a distally duplicated segment of 305 kb. Recurring exon 2 gene conversion mutations, the results of recombination between the paralogous duplicons, account for 75% of SDS-associated alleles. We report 13 novel SDS-associated mutations distributed across SDBS, including 7 missense mutations, 2 splice site mutations, a frameshift mutation, and an in-frame deletion of 2 amino acids. Two additional gene conversion mutations have also been identified, including a 4 bp deletion in exon 3, and two coincident nucleotide substitutions in exon 4. Each of these mutations occurred only once or twice in our international collection of over 200 families, and always occurred with

a common conversion mutation on the second allele. The frequency and distribution of disease-causing gene conversion events in SBDS suggest that some regions of the gene are more prone to aberrant recombination with the pseudogene.

P604. Development of uniplex and multiplex assays for determination of single-base changes in CARD15 gene by use of microelectronic array technology

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Microelectronic DNA chip devices represent an emerging technology for genotyping. We developed a protocol for rapid detection of the three most common clinically relevant single-base changes in CARD15 gene. Mutations in CARD15 gene have recently been identified in patients with Crohn's disease, a chronic inflammatory bowel disease, which is one of the most frequent causes of gastrointestinal morbidity in Western Europe and Northern America. Primer pairs, with one containing a 5'-biotin group, were used to PCR-amplify the region encompassing each single-base change to be interrogated. Double stranded PCR products were electronically targeted to discrete sites on streptavidin-coated gel pads surfaces by use of a NanoChip® Molecular Biology Workstation. After denaturation, the biotinylated strand immobilized on the test site was interrogated using allele-specific dye-labeled oligonucleotide reporters for detection of wild-type and variant sequences. Protocols were validated by genotyping 100 samples previously typed by DNA sequence analysis and/or Pyrosequencing™ analysis. Results were totally concordant with results obtained previously by other genotyping methods. The analysis of amplified DNAs required 4–6 h for typing each mutation in 100 samples. A protocol for simultaneous testing of the three single-base changes on a single test site was also optimised and validated. This multiplex format allowed us to immobilize the three PCR products, containing the mutation sites, on the same microelectrode of the NanoChip, thus reducing cost and time for the single analysis to one third. The NanoChip microarray technology is then confirmed as an accurate and convenient method for rapid screening of clinically relevant single-base changes.

P605. Clinical and Genetic Heterogeneity in Desbuquois Dysplasia.

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Desbuquois dysplasia is a rare autosomal recessive chondrodysplasia characterized by short stature, joint laxity, facial dysmorphism, a 'Swedish key' appearance of the proximal femur, advanced carpal and tarsal bone age, and hand anomalies consisting of phalangeal dislocations and an extra ossification centre distal to the second metacarpal. However, the latter changes are not consistently observed in all Desbuquois patients, defining two distinct groups, based on the presence or absence of hand anomalies. Here, we first report on a genome-wide search in four inbred Desbuquois families originating from France, Sri-Lanka, United Arab Emirates and Morocco, selected for the presence of typical hand anomalies in order to guarantee homogeneity of the samples. The disease locus was assigned to chromosome 17q25.3 (Zmax = 4.61 at θ =0 at locus *D17S1806*) in the 9.5 cM interval defined by loci *D17S802* and *D17S1822*. In order to test the hypothesis of genetic heterogeneity of Desbuquois dysplasia depending on X-ray features of the hands, linkage of the 17q25.3 region was subsequently tested in three inbred Desbuquois families without typical hand abnormalities originating from Asia, Morocco and Turkey. Heterozygosity for six polymorphic microsatellite DNA markers of the 17q25.3 candidate region in all affected members permitted us to exclude this locus in this clinical subtype. The present study supports the genetic heterogeneity related to radiographic heterogeneity and will hopefully help in identifying the Desbuquois dysplasia genes.

P606. A survey of FVIII in a Belgian population of severe and mild Haemophilia A patients.

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Haemophilia A is an X-linked bleeding disorder caused by reduced or absent FVIII protein due to mutations in the *FVIII* gene. Nearly 45% of the patients with a severe presentation have an inversion of the *FVIII* gene. Investigation of the mutations in the other patients is hampered by the size of the gene - 26 exons spread over 186 kb - and the "private" character of the mutations.
We report on a survey of 112 Flemish families with severe (97), mild (13) or moderate (2) Haemophilia. Patients were first analysed by Southern blot. The common inversion was identified in 54/97 (severe) cases (56 %). Subsequently, the complete gene was screened by Chemical Cleavage of Mismatches (CCM) (Freson *et al*, 1998, *Hum Mut* **11**:470-9) or Denaturing High Performance Liquid Chromatography (DHPLC). In total, 20 different missense mutations, three nonsense, 15 small deletions or insertions, one splice mutation, 2 larger deletions, and one insdel were found. The latter one results in the duplication of 8 amino acids from codon 2303 to 2310. The majority (23) of these mutations have not been described by other groups. Two missense mutations, V234F and L625V, probably have a common Belgian founder: they have been found in respectively 6 severe and 7 mild to moderate Belgian patients which belong both to 3 main families.

P607. A spectrum of mutations of the Prop1 gene in 48 Greek patients with Multiple Pituitary Hormone Deficiency (MPHD)

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Prop1 gene defects constitute the most frequent cause of genetically determined MPHD. Prop1 gene mutations lead to deficient synthesis of GH, Prl, TSH and gonadotrophins in variable severity and timing of onset.
Molecular genetic analysis was carried out by PCR and direct sequencing in 48 Greek patients exhibiting deficiency of at least two pituitary hormones. Prop1 gene mutations were detected in 17 patients (35%). Observed genotypes: seven GA296del (homozygotes), five A150del (homozygotes), one R73C (homozygote), two GA296del/A149del (compound heterozygotes), one GA296del/R73H (compound heterozygote) and one GA296del/Q83X (compound heterozygote). Hence, in our population, the mutation GA296del was detected in 14/34 chromosomes and the A150del in 12/34 chromosomes. The mutation Q83X is a novel mutation and it is a C to T transition at nucleotide 247 resulting in a premature stop (CAG to TAG). The resulting protein is truncated with only 82 out of 226 aminoacids of the normal peptide. The Q83X mutation was found in an 11 weeks old infant, examined for prolonged jaundice caused by low TSH hypothyroidism.
In conclusion, the distribution of mutations in our group is analogous to those observed in other populations, while Q83X has only been detected in a Greek patient. Etiologic classification of pituitary insufficiency not only leads to proper management of the pathologic condition but also to the appropriate genetic counseling and management of prospective pregnancies.

P608. Consequences of a Guanine insertion in myosin binding protein C gene: Two aberrant mRNA species and downregulation of both normal transcripts and protein in a patient suffering from hypertrophic cardiomyopathy

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Insertion of one G in exon 25 of the cardiac myosin binding protein-C gene (MyBP-C; MYBPC3) was previously shown to be associated with hypertrophic cardiomyopathy (family EA; Moolman et al., 2000). The insertion produces a cryptic splice donor site (SDS) causing loss of 40 bp in mRNA (exon 25) and a premature stop codon in exon 26. We have investigated consequences of this mutation by Real-Time Quantitative RT-PCR of RNA from cardiac tissue of an EA patient, and by analysing mRNA from HeLa cells transfected with minigenes comprising exon 25 (+/- G insertion) plus adjacent exons and introns. In cardiac tissue we identified one normal and two aberrant mRNA species, one with loss of 40 bp in exon 25, and one with an intact exon 25, but an unspliced intron 25 in addition. Two closely spaced SDS's (40 bp) may have mutually been inhibitory. Bimodal distribution of mutated transcripts was reproduced in transfected HeLa cells. The competition hypothesis was substantiated by elimination of the native SDS in the minigene resulting in the disappearance of intron 25 sequences in mRNA.
Quantification of mRNA in patient tissue showed a reduction of wildtype mRNA to ~50% of control. A similarly reduced value was found for MyBP-C protein. About 10% of the total mRNA were aberrant, with internally deleted and incompletely spliced mRNA being roughly equal. Since truncated MyBP-C protein was undetectable in affected tissue, prevention of aberrant mRNA translation and/or degradation of protein might together result in haploinsufficiency as basic mode of disease.

P609. Unusual genomic rearrangement in SLC3A1 in cystinuria patients

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Cystinuria is a common inherited disorder of defective cystine and dibasic amino acid transport in the kidney. The two disease causing genes *SLC3A1* and *SLC7A9* encode the renal transport system rBAT/b0,+AT. While point mutations in the two genes are well-known to cause cystinuria, only few studies aimed to identify gross alterations. Therefore we developed real-time PCR assays using TaqMan™ technology and screened our cohort of 49 cystinurics for copy number deviations in the *SLC3A1* gene. RT-PCR and DNA sequencing were used to confirm and characterize the rearrangements.
In seven patients we could detect the same large duplication of more than 30 kb in the *SLC3A1* gene spanning from introns 4 to 9. We analyzed this mutation in detail and identified the transition of the tandem duplication from intron 9 to intron 4. The duplication was accompanied by a small inversion of 25 bp and a 2 bp deletion in intron 9. Alu sequences were localized near the duplicated region but are not directly involved in the rearrangement. We presume that the inversion event in intron 9 provokes a chromatin structure that stimulates the duplication. This mechanism is probably supported by the Alu elements within the affected genomic regions.
This study is the first report on a duplication in *SLC3A1* and demonstrates the utility of real-time PCR in screening for mutations causing cystinuria.

P610. Mutations in *MATN3* cluster in the β-sheets of the A-domain and prevent secretion of matrilin-3 from the rough endoplasmic reticulum

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Mutations in the matrilin-3 gene have recently been shown to underlie a relatively mild variant of multiple epiphyseal dysplasia. This autosomal dominant form of MED is characterised by normal stature, joint pain and stiffness and early onset osteoarthritis. Following a targeted mutation screen of *MATN3* in sixty unrelated individuals with MED we now report the identification of four novel mutations (A219D, I192N, T120M and E134K), one previously published mutation (R121W) and one non-synonymous polymorphism (E252K).
All the mutations are located within the β-sheets of the A-domain of martilin-3, suggesting that they have a deleterious effect on the folding and/or function of this domain. Preliminary expression studies established that these mutations prevented the secretion of matrilin-3 A-domain *in vitro*. Interestingly, the non-synonymous polymorphism, which is located in the α-helical region of the A-domain and may therefore a less deleterious effect on it's structure, does not affect the secretion of matrilin-3 A-domain *in vitro*.
EM analysis of chondrocytes from a patient with the R121W mutation showed the accumulation of granular material within the rER *in vivo*, thereby confirming that the misfolding of the A-domain results in the retention of full-length matrilin-3. These findings suggest that MED resulting from *MATN3* mutations may share common disease mechanisms with MED resulting from *COMP* mutations such as the retention of misfolded mutant protein.
In summary identification of additional *MATN3* mutations and a study of their structural and functional consequences will help our understanding of the molecular cell-pathology of MED and provide the rationale for targeted therapy.

P611. Polymorphisms resulting in defects of premRNA processing as causes and predisposition to diseases

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Pre-mRNA splicing relies on the correct identification of protein coding sequences from non-coding sequences. The spliceosome (a multi-protein complex) is responsible for the production of mature mRNA molecule. The assembly of this complex relies on the presence of a „core" of cis-acting sequences together with additional cis-acting elements, localised either in the coding sequence or introns, which may enhance or antagonise splicing. Such complexity, allows fine-tuning of the splicing process, but carries a high risk of derangement following even minor mutations. In fact 15% of genetic diseases concern dysfunction in pre-mRNA splicing causing the distinction between polymorphisms and splicing pathogenetic mutations to become an increasing challenge.
We focus upon genetic diseases arising from unusual splicing defects, uncovering novel pathways of RNA processing in a variety of genes. In the ATM gene we have identified a novel type of cis-acting sequence, essential for correct intron removal. The importance of exonic sequences in the regulation of splicing is highlighted by our studies on CFTR exon 9 and exon 12 and in NF1 exon 37. We show that missense, nonsense and even silent variations (this last frequently discarded as not pathogenetic in genomic screening) may cause splicing defects.
In addition we show U1snRNP-pre-mRNA interactions to be key factors involved in both the ATM element dysfunction and the NF1 gene exon 3 skipping. Both polymorphims abolish a U1 snRNP-pre-mRNA complex. A modified U1snRNP is capable of restoring correct splicing of both pre-mRNAs. providing a promising future prospect for the development of novel therapeutic strategies.

P612. Two cases of congenital afibrinogenemia caused by potentially elusive mutations.

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Congenital afibrinogenemia is a rare autosomal recessive coagulation disorder characterised by severe fibrinogen deficiency and haemorragic manifestations of variable severity.
A mutational spectrum, comprising one large deletion and 29 point mutations (11 nonsense, 8 insertion/deletion, 8 splicing, and 2 missense mutations), has been so far determined. All causative mutations are located in the fibrinogen genes coding for fibrinogen Aalpha-, Bbeta-, and gamma-chains. In this work, analysis of the fibrinogen cluster in two unrelated afibrinogenemic probands identified two novel mutations (table).
The 15-kb deletion removes the last two exons of the Aalpha-chain gene and almost the entire Aalpha-Bbeta intergenic region. Sequencing of the deletion breakpoints showed the presence of direct and inverted repeats that could mediate an “illegitimate” recombination. This represents the second large deletion identified in the fibrinogen cluster that, if present in the heterozygous state in compound heterozygous afibrinogenemic patients, could escape mutational screening performed by standard PCR.
L172Q missense mutation involves a highly conserved residue located in a coiled-coil region. *In-vitro* expression experiments of the mutant L172Q fibrinogen revealed secretion to normal levels. Inspection of nucleotide sequences around the 5157T>A mutation revealed the possible activation of a cryptic acceptor splice site. Semi-quantitative fluorescent hot-stop RT-PCR analysis, performed on RNA extracted from cells transfected with a minigene Bbeta-construct containing the missense mutation, demonstrated altered mRNA processing (exon skipping) in about 90% of transcripts. These results underline the importance of analysing exonic mutations also at the mRNA level, even when they affect nucleotides far from splicing junctions.

*Numbering according to GenBank: Aalpha, AC107383; Bbeta, M64983; §Numbering omitting signal peptide

Proband’s origin	Gene Exon	Nucleotide* Amino acid§	Mutation type (Genotype)
Thailand	Aalpha 5-6	del47645-62872 del152-end	15-kb deletion (homozygous)
Italy	Bbeta 4	5157T>A L172Q	Missense/splicing (heterozygous)

P613. Identification of mutations that perturb RNA splicing using genomic DNA and a functional minigene assay

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Techniques for the identification of sequence variations in different genes have become faster and cheaper. These technological improvements have made possible the frequent use of DNA diagnostic tests in clinical practice but have also made the distinction between neutral polymorphisms and pathogenic mutations an increasing challenge.
This challenge is illustrated by mutations that cause abnormalities of premRNA splicing. The effect of a given nucleotide variation on splicing can be predicted from genomic DNA sequence analysis only if mutations alter highly conserved canonical splicing signals. However it is extremely difficult to predict the effects of changes in intronic and exonic sequences not obviously involved in the splicing process.
We present here an efficient and simple test that has proved a valuable tool for the identification of those mutations that cause splicing defects. The assay uses genomic DNA making it feasible to carry this out in the molecular diagnostic laboratory as part of the analysis of the DNA sample provided by the referring clinicians. Using this system two mutations in the Nf1 gene were analysed. Firstly a nonsense mutation in exon 37 and secondly an intronic mutation downstream of exon 3. Both of these were found to dramatically affect splicing. The intronic mutation was then rescued by co-transfection in this assay of a U1 snRNP complimentary to the mutation. Further intronic mutations in the CFTR gene were analysed at positions +8 to +11 of exon 9, these variations affected splicing by altering a TIA binding site.

P614. Microphthalmia with Linear Skin Defects (MLS): different approaches to identify the molecular bases of this syndrome

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Microphthalmia with linear skin defects (MLS) is a rare X-linked dominant, male lethal developmental disorder. It is mainly characterized by linear skin defects that disappear with age, and microphtalmia. This condition is associated with monosomy of Xp22.3. The molecular characterization of breakpoints in patients with Xp22.3 rearrangements allowed the definition of the MLS critical region. We are applying different approaches to identify the gene responsible for this disorder.
We have identified seven new cases of MLS patients and we are currently defining their breakpoints by FISH to redefine the critical region. Furthermore we have assembled a complete transcription map of the critical region and we are performing mutation analysis on three exceptional MLS cases without chromosomal rearrangements. The mutation analysis is being carried out on transcripts already known to be associated with the critical region as well as putative novel transcripts we have identified by bioinformatic analysis. So far no abnormalities have been identified.
We are currently testing gene expression levels of transcripts spanning 2.5Mb across the critical region by Real Time PCR analysis of RNA from MLS patients without chromosomal rearrangements. Furthermore we are performing a comparative analysis between the human and murine genomes across the MLS critical interval in order to identify Conserved Sequence Tags potentially involved in the pathogenesis of this disorder.

P615. Mutations In The Col4a4 Gene In Relation To Familial Hematuria

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Mutations in COL4A4 gene have been reported in autosomal recesive and dominant type of Alport syndrome (AS), as well as benign familial hematuria (BHF) or thin basement membrane disease (TBMD). BHF probably affects at least 1% of the population and is characterized by persistent microscopic glomerular hematuria, sometimes with proteinuria or hypertension, thinning of the glomerular basement membrane (GBM) and normal renal function. Considering the similarities in GBM abnormalities, TBMD cannot be clinically differentiated from the initial stages of AS. It has recently been suggested that TBMD represents a carrier state of autosomal recessive or X-linked AS. We analyzed 22 patients with confirmed BHF and 12 AS patients who had tested negative in COL4A5 gene screening also for COL4A4 mutations. Non-isotopic single stranded conformation analysis (SSCA) after amplification of each exon with boundary intronic sequences by the polymerase chain reaction (PCR) was used for all 51 exons of COL4A5 and 47 exons of COL4A4 gene. While no mutation was identified in COL4A5 gene in all 34 patients COL4A4 mutation screening disclosed eleven common polymorphisms that are shared in part with other populations and six novel mutations: G774R (GGT>CGT), G789G (GGA>GGT), 2860+2T>G, R908W (CGG>GGG), D1049H (GAC>CAC)+del 5 bp, 3506-8T>G and 4081-8G>C comprising three missense mutations, one frameshift and two potentially splice site mutations. Furthermore, we found one silent mutation, one rare variant in a non-coding region, and several polymorphism with a very low heterozygosity. This study confirms the importance of the COL4A4 gene in the pathogenesis of benign familial hematuria.

P616. Novel nt1005delG mutation of FOXC2 gene in a family having hereditary lymphedema-distichiasis syndrome with variable expression of renal disease and diabetes mellitus

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Mutations of FOXC2 gene on 16q24.3 were previously implicated in the etiology of Lymphedema-Distichiasis (LD) Syndrome (MIM 153400), which is an autosomal dominant disorder that classically presents as late onset lymphedema of the limbs, and double rows of eyelashes (distichiasis). This disorder has substantial clinical heterogeneity, and additional abnormalities such as cardiac defects, cleft palate, extradural cysts, and various ophthalmologic problems were also observed in some families with this syndrome. In this study, we report a family with LD phenotype in six affected relatives over three generations. In addition to LD, four individuals have renal disease, and three patients have diabetes mellitus (DM). The severity and progression of both diseases show extensive clinical variations. Automated sequence analysis of FOXC2 gene in this family revealed nt1005delG mutation, which results in immediate truncation of the protein without any extra amino acids. The presence of DM and/or renal problems is not a classical feature of LD. We believe that the manifestation of these symptoms might be associated with the novel nt1005delG mutation. High expression of FOXC2 (based on quantifying ESTs from Unigene clusters) in pancreas and kidney tissue, beneficiary role of FOXC2 to overcome insulin resistance (Cederberg et al., 2001. Cell: 106, 563-573.), and highly pleiotrophic nature of FOXC2 mutations (Erickson et al., 2001. J Med Genet 38, 761-766) strongly support our hypothesis.

P617. A novel mutation 11571C>G in *ALMS1* gene in sibs with Alstrom syndrome and different clinical presentations

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Alstrom syndrome (ALMS) is an autosomal recessive disorder whose main features are pigmentary retinal dystrophy, obesity, non-insulin dependent diabetes mellitus and sensorineural hearing loss. Respiratory, renal, hepatic and cardiac involvement have been also described. Recently, the gene mutated in this syndrome (*ALMS1*) has been identified on 2p13 and 13 different mutations have been reported to date.
We observed 2 Italian brothers affected by ALMS, born to non-consanguineous healthy parents. Both sibs presented pigmentary retinopathy and generalized obesity. Neither alterations in glucose metabolism nor genital abnormalities were observed. Mental development was normal. Karyotype was 46,XY.
There were major differences in the clinical course of the 2 patients. The elder brother suffered from bronchial asthma poorly responsive to conventional therapy by the age of 3 months. He further developed sensorineural hearing loss and acanthosis nigricans on his neck. The younger brother had a dilated cardiomyopathy without heart failure. By molecular analysis of the *ALMS1* gene, we identified in both patients a homozygous novel mutation (11571C>G) in exon 16, causing a premature stop codon (Y3820X).
To date, no genotype-phenotype correlation in ALMS has been reported. Indeed, only a few patients have been molecularly characterized so far. The present report of 2 ALMS sibs with different clinical courses shows that the same *ALMS1* mutation can produce differences in the phenotype, even within the same family. Accurate clinical description in combination with molecular characterization of more patients is necessary to define a genotype-phenotype correlation in ALMS.

P618. Two cases of misinterpretation of molecular results in Incontinentia Pigmenti, and a PCR-based method to discriminate NEMO/IKKg gene deletion

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Familial Incontinentia pigmenti (IP, MIM308300) is a rare X-linked dominant disorder which affects ectodermal tissues. Over 90% of IP carrier females have a recurrent genomic deletion of exons 4-10 of the NEMO/IKBKG IKKgamma (MIM 300248) gene, which encodes a regulatory component of the IkB kinase complex, required to activate the NF-kB pathway.
In IP, mutations in NEMO lead to complete loss of NF-kB activation, creating a susceptibility to cellular apoptosis in response to TNF-alpha. This condition is lethal for males during embryogenesis while females, who are mosaic as a result of X-inactivation, can survive. Recently, a second non-functional copy of the gene, DeltaNEMO, was identified, opposite in direction to NEMO in a 35.5 kb duplicated sequence tract. PCR-based detection of the NEMO deletion is diagnostic for IP disease. However, we present instances in which ex 4-10 DeltaNEMO pseudogene deletion occurred in unaffected parents of two females with clinically characteristic IP. These were missed by the currently standard PCR-based method, but can be easily discriminated by a new PCR-based test reported here that permits unambiguous molecular diagnosis and proper familial genetic counseling for IP.

P619. Spectrum of mutations of the most common genetic disorders in Bulgaria

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Clarification of the molecular characteristics of a given monogenic disorder is a preliminary step for each national diagnostic centre aiming to develop an effective prophylactic (post- and prenatal diagnosis) programme. Since 2000 the „National Program for Diagnostics and Prevention of the Inherited Disorders, Predispositions and Inborn Anomalies in Bulgaria, 2000-2005" has been introduced.
The present data concerns the molecular characteristics of some of the most common inherited disorders in Bulgaria: CF (32 mutations/ 257 patients), β-thalassemia (17 mutations/ 89), haemophilia A (11 inversions/ 31), DMD/BMD (116 deletions/ 179), SMA (87 deletions/ 156), LGMD2C (1 mutation/ 28), CMT (20 duplications/ 66), HMSNL (2 mutations/ 19), congenital myasthenic syndrome type Ia (1 mutation/ 24) and EDMD-AD (1 mutation/ 1). For some inherited metabolic disorders direct mutation analysis was applied: phenylketonuria (16 mutations/ 37), galactokinase deficiency (1 mutation/ 6), MCAD deficiency and Wilson disease (4 mutations/ 80). The results presented confirm our preliminary expectations of great genetic heterogeneity of all autosomal recessive disorders among Bulgarians. The same is true for Bulgarian Turks. Genetic differences were found among the three major ethnic groups in Bulgaria: Bulgarians, Bulgarian Turks and Gypsies.
We found high carrier frequencies among Gypsies for all but one (phenylketonuria) autosomal recessive disorders, and mutation homogeneity in Gypsy patients was detected. The present results make the Gypsy population suitable for mass screening programmes for some recessive disorders, for example to detect hetero- and homozygotes in galaktokinase and MCAD deficiency, and heterozygote carriers of CF and LGMD2C.

P620. Sequence analysis of cosmid clones mapping to the rhesus major histocompatibility complex (MHC)

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The major histocompatibility complex (MHC) plays a major role in graft rejection and controlls susceptibility to many diseases mostly

of autoimmune or infectious nature. Among the 120 expressed genes of the 3.8 Mb encompassing human MHC, the HLA complex, the most characteristic ones are the highly polymorphic class I and class II genes, whose gene products control specific immune responsiveness.
The rhesus macaque (Macaca mulatta) serves as an animal model for several human infectious diseases, e. g. AIDS and susceptibility to viral infection is mainly controlled by class I molecules. However, knowledge of rhesus macaque class I genes is based on cDNA sequence information and the genomic structure of the rhesus MHC is largely unknown. The sequences of three cosmids have been completely determined, two of them are overlapping cosmids that map to the so called extended MHC class II region and contain the SACM2L and KE4 genes as well as a third cosmid that contains the gene coding for myelin oligodendrocyte glycoprotein (MOG), the major autoantigen involved in multiple sclerosis. Evolutionary analyses will be presented.

P621. Heterogeneous duplications of Xq22 and the *PLP1* gene in Pelizaeus-Merzbacher disease

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Genomic duplications of Xq22 including the whole *PLP1* gene are the most common cause of Pelizaeus-Merzbacher disease (PMD), an X-linked dysmyelinating disorder of the central nervous system. *PLP1* is a dosage sensitive gene, and copy number imbalance causes neurological disease in both humans and animal models.We show that the size of the genomic duplications involving *PLP1* varies greatly between individuals, from less than 300kb to over 4Mb. There is no simple correlation between duplication size and disease severity. Most of the *PLP1* duplications are in a tandem head to tail orientation, but in some cases the extra copy has been inserted elsewhere in the genome. We have mapped the location of the duplication end points in 17 affected individuals, using a combination of interphase fluorescence in-situ hybridisation (FISH), fibre FISH, quantitative PCR and long range PCR. We show that both proximal and distal breakpoints are highly variable between families in contrast to several other genomic disorders. Analysis of DNA sequence in the vicinity of the breakpoints does not suggest the rearrangements are mediated by the common mechanism of non-allelic homologous recombination between flanking low copy number repeats. It suggests a more complex mechanism is involved leading to genomic instability of Xq22. Analysis of the chromosomal breakpoints is elucidating a greater understanding of the mechanisms involved in these unusual genomic rearrangements and transposition events including the *PLP1* gene.

P622. Deletion of the MTMR1, MTM1 and F18 genes in a contiguous gene syndrome with myotubular myopathy and intersexual genitalia

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X-linked myotubular myopathy (XLMTM) is a severe congenital disorder that affects skeletal muscle due to mutations in the MTM1 gene. This gene codes for a lipid phosphatase that acts on the second messenger phosphatidylinositol 3-monophosphate (PI(3)P). The MTM1 gene belongs to a highly conserved multigenic family, with 14 members in humans, that include its two closest homologues, MTMR1 and MTMR2. The latter was recently found mutated in Charcot-Marie-Tooth type 4B neuropathy. Although mutations in MTMR1 have not been described so far, muscle-specific alternative splicing of this gene is altered in muscle cells derived from patients with congenital myotonic dystrophy. We show the characterization of a small Xq28 deletion in a boy affected by a contiguous gene syndrome with myotubular myopathy and hypospadias. The entire MTM1 gene was found deleted in the patient, as well as its centromeric neighbour (F18 gene). Comparison with similar cases previously published confirms the existence of a gene centromeric to MTM1 necessary for genital development. Genomic PCR and Southern blot analysis revealed that the MTMR1 gene, distal to MTM1, was also absent in the proband. The patient did not show other clinical manifestations apart from those corresponding to the described syndrome, indicating that MTMR1 is not essential for human development. Alternatively, its function may be masked by the severe neonatal XLMTM phenotype, and a physiological role manifesting at a later age cannot thus be ruled out.

P623. Molecular diagnostics of hereditary angioneurotic edema (HAE) in Hungary

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Hereditary angioneurotic edema (HAE) is an autosomal dominant disorder characterised by episodic local subcutaneous and submucosal edema affecting the upper respiratory and gastrointestinal tracts. It is caused by deficiency of activated C1 esterase inhibitor protein (C1-INH, type I: reduced serum level, type II: reduced activity) whose function is preventing unnecessary activation of the complement system. The aim of the present study was to determine the disease-causing mutations among Hungarian HAE-patients. The estimated number of affected HAE-families in Hungary is approximately 40-50, out of which 26 families (type I:23, type II:3) managed in a single centre (Budapest) were enrolled in the current study. To detect large deletions/insertions, we used Southern-blotting analysis with BclI and BglII digestions. In the absence of large structural changes, we employed direct capillary sequencing covering the whole coding region and splicing sites of the C1-INH gene. Large deletions were detected in 4/23 (17.4%) type I families. We found the Arg444Cys mutation (reactive site) in each of the 3 type II families. In the remaining type I families, 11 novel mutations (IVS2SD+1G-A, Gln10Stop, 2535-2536delCT, 2580-2621del42bp, 2697-2698insT, Cys108Tyr, IVS3SD+1G-A, Gln201Stop, Asp386Val, 14225delA, 16725ins26bp,) were detected in 14 families affecting primarily exon 3 (7/11) of the C1INH gene. In 5 cases, known mutations were identified affecting primarily exon 8 (5/6). The high rate of new mutations in our patient population supports the concept of genetic instability of the C1INH gene. Our program provides definite molecular diagnosis and allows the widening of known mutation database by examining a distinct population.

P624. Segregation of aldolase B gene mutated allelles in Turkish Families

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Hereditary Fructose Intolerance (HFI) is characterised by vomiting, abdominal pain after taking foods containing fructose and cognate sugars, which induce hypoglycemia, hypophosphatemia, acidosis, fructosuria. HFI is a recessively transmitted disease caused by deficiency of aldolase B in the liver, intestine and kidney. Several mutations in the aldolase B gene were shown to be responsible for this enzyme defect. We present aldolase B mutation analysis in three HFI families from Central Anatolia. We ascertained these families through an affected adult male referred to our department for genetic counseling. Despite absence of consanguinity, pedigree analysis revealed the presence of affected persons in the family of the proband's partner. Since one of the affected persons in the latter family was the offspring of a non-consanguineous marriage, pedigree analysis suggested the presence of a third family also having individuals with aldolase B gene mutations. In the first step of the genetic study, aldolase B mutations were investigated in three affected and three unaffected individuals from two families. A174D was found to be segregating in one family and A149P in the other. Further analysis confirmed the genotypes of individuals and the presence of an additional unrelated family having individuals with the A174D mutation. Since the families investigated in this study are

unrelated, in other words, each mutated allele was inherited from different ancestors for each family, it may be suggested that aldolase B gene mutations have a high frequency at least for the Central Anatolian Region.

P625. Pyruvate Kinase Deficiency (PKD): discovery of a new deletion

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Pyruvate Kinase deficiency (PKD) is a disease characterized by chronic haemolytic anaemia of varying severity from silent carriers to severe a-thalassemia like hydrops fetalis. Molecular mechanisms of this disease are point mutations, with more than 80% private, requiring screening of the 12 exons for molecular diagnostic. Up to now, only three deletions were described.

We studied a Vietnamese family whose son was affected by a severe form of PKD. DHPLC, sequencing and restriction assays of parents and the affected child showed a unique point mutation in exon 7 (N316K) which was homozygous in our proband, heterozygous in his father, and absent in the mother.

No filiation error was found leading to the hypothesis that child had inherited N316K from his father and a deletion including PKLR exon 7 from his mother. To demonstrate this hypothesis, we set up quantitative real-time PCR test with Sybrgreen, normalized using another gene, which confirmed the presence of a deletion of PKLR including exon 7 in the child, the mother and throughout the pedigree. Almost all methods used in laboratories for mutation detection are based upon PCR, and are unable to diagnose deletions. In our family, evidence for a deletion as the second molecular event was found just because the deletion involved the exon bearing the father‘s mutation. This observation highlights the underestimation of deletions in molecular pathology and the need for strategies such as real-time quantitative PCR with Sybrgreen to detect such events.

Pedigree	PK Gene (Raw Data) QDNA(ng)	Relative quantification PK/bGlobin	PK gene/ controls
Father	19.65	0.75	0.97
Mother	6.9	0.46	0.40
Probant	8.18	3.37	0.45

Extended Pedigree	PK Gene (Raw data) QDNA (ng)	RATIO PK/bGLOBIN Relative quantification	PK Gene/ Controls
Grand-mother	12.39	0.51	0.59
Grand-father	21.91	0.78	1.08
Controls (n=18)	20.68	0.76	1

P626. Different variations to fluorescent ARMS assays for achondroplasia, familial dysautonomia and spinal muscular atrophy

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Amplification refractory mutation system (ARMS) is a simple, quick assay that can be applied to several diagnostic applications. We demonstrate three variations of this assay, utilizing fluorescent detection for maximum versatility. Detection on a capillary fluorescent analyser allows detection of fragments that differ in size by as little as 1bp. Therefore wild type and mutant alleles are distinguished by size differences created by the addition of a small number of nucleotides to the end of one of the allele specific primers.

The first assay involves standard ARMS primers to detect the FGFR3 achondroplasia mutations, 1138G>A and 1138G>C. DNA is amplified using three forward primers with their 3‘ nucleotides specific for either the wild type (G) or the mutant (A or C) nucleotides in a single tube with one common fluorescently labeled reverse primer.

The second assay is used for carrier or diagnostic testing for familial dysautonomia, a condition only observed in the Ashkenazi Jewish population and caused by two mutations in the IKBAP gene (R696P and IVS20+6T>C). For this assay the allele specific primers incorporate locked nucleic acids (LNA™), a DNA analog with improved hybridization characteristics, at the allele specific sites, which facilitated the optimization of this assay.

In the final assay we have further demonstrated the versatility of the ARMS approach by developing a quantitative assay for testing carriers of spinal muscular atrophy. Two multiplex ARMS specifically amplify exons 7 and 8 of the SMN1 or SMN2 gene along with a control locus (MPZ) used for quantification.

P627. Achondroplasia In Turkey Is Defined By Recurrent G380r Mutation Of The Fgfr3 Gene

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Achondroplasia, the most common form of skeletal dysplasia in man, is inherited autosomal dominant and causes severe dwarfism. More than 90 % of patients with achondroplasia have a G to A transition or G to C transversion at position 1138 of the fibroblast growth factor receptor (*FGFR3*) gene resulting in substitution of arginine for glycine at position 380 (G380R) of the FGFR3 protein.

In this study, 16 unrelated Turkish patients with achondroplasia were evaluated for the G to A and G to C mutations at position 1138 of the fibroblast growth factor receptor (*FGFR3*) gene. Fifteen out of 16 patients studied carried the G to A mutation heterozygously. None of the patients had the G to C mutation at the same position.

In conclusion, the vast majority of Turkish achondroplasia patients have the same frequent mutation that was defined in patients with achondroplasia from other countries. Our results also support the argument that the G380R mutation of *FGFR3* is the most common mutation causing achondroplasia in different populations.

P628. Identification of Four Novel Mutations in the C1NH Gene Associated with Hereditary Angioedema

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Hereditary Angioedema (HAE, OMIM #106100) is an autosomal dominant disorder characterized by episodic local subcutaneous oedema and submucosal oedema involving the upper respiratory and gastrointestinal tracts. The disease is caused by mutations in the *C1NH* gene which encodes C1 inhibitor protein. Mutations in *C1NH* can lead either to deficient serum level of C1 inhibitor (C1NH) (type I HAE) or non functional C1NH protein (type II HAE).

We screened the *C1NH* gene using the High Performance Liquid Chromatography (DHPLC) analysis in 12 Italian families with multiple affected individuals. Anomalous DHPLC fragments were sequenced and confirmed by segregation and enzyme restriction analysis. All examined patients were characterized genotypically. We identified four novel mutations (c.1214T>C, c.delG1412, c365C>A, c.1376T>C) including missense, nonsense, and splicing types respectively in exons 3, 7, and 8, and confirmed previously described mutations. Laboratory examination showed reduced levels of CH50 and C4, and normal C3 levels. Our results suggest that DHPLC provides an accurate method for the rapid identification of C1NH mutations.

This work was supported by a grant from the Italian Ministry of Health.

P629. Novel missense mutation within exon 5 of the TRPS1 gene in a Mexican patient with severe form of tricho-rhino-phalangeal syndrome

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Tricho-rhino-phalangeal syndrome (TRPS) type I is characterized by sparce hair; bullbous nose; mild skeletal dysplasia with cone-shaped epiphyses, short stature and shortening of the metacarpals and metatarsals. TRPS III is considered a more severe form of the disease. Both entities are due to molecular defects in the TRPS1 gene located on chromosome 8q24.2. TRPS I is due to several mutations that span through the TRPS 1 gene while TRPS type III is due to the specific class of mutation within exon 6 of the gene. In the present study we analyzed the TRPS1 gene in a Mexican patient

with severe form of TRPS that resembles type III. Molecular studies included PCR and DNA sequence analysis of the entire coding regions of the gene. We identified a novel missense mutation within exon 5 (C instead of T) that results in the substitution of cysteine by arginine. In conclusion, we report a novel missense mutation within exon 5 of the TRPS1 in a severe form of TRPS, this data suggests that mutations in exon 6 are not the only allelic variant of the severe form of the disease.

P630. Genetic Analysis Of Normotensive Hypokalemic Salt-losing Tubulopathies In The Italian Population.

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Bartter Syndromes are inherited renal disorders characterised by normotensive hypokalemic salt-losing tubulopathies. Four genes have been identified where loss of function mutations underlie the mechanisms responsible for altered electrolyte reabsorption in four renal membrane proteins: *SLC12A3*, *SLC12A1*, *KCNJI* and *CLCNKB* coding respectively for the sodium-chloride cotransporter NCCT (Gitelman Syndrome), the sodium-potassium-chloride cotransporter NKCC2 (Bartter I), the inwardly rectifying potassium channel ROMK (Bartter II), and the basolateral chloride channel ClC-Kb (Bartter III). The aim of the study was to characterise the molecular defect of a cohort of 74 patients, clinically defined as Gitelman / Bartter Syndromes. Twenty four patients (33%) showed two mutations (13 homozygous and 11 compound heterozygous mutations): based on our analysis, one patient resulted as Bartter type I Syndrome, two as Bartter type II, seven as Bartter type III and fourteen as Gitelman Syndrome. Twenty four patients (33%) demonstrated only one mutation but the analysis is not concluded and the number of compound heterozygous patients could increase. Twenty four patients (33%) without alterations on screening of the first gene are in analysis for a second gene (*SLC12A3* or *CLCNKB* for adult patients, *SLC12A1* or *ROMK* for neonatal and antenatal Bartter clinical diagnoses). In two cases only one mutation was found at the conclusion of the screening performed by SSCP.

P631. The clinical findings in Friedreich's ataxia with very small GAA trinucleotide expansions (<150)- a case series.

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Friedreichs ataxia (FA) which is autosomal recessive is the commonest genetic cause of ataxia. The identification of the FRDA gene has broadened the clinic phenotype to include previous exclusion criteria like age of onset beyond 25 years and the presence of deep tendon reflexes. W e identify rare patients in whom the smaller allele expansion is <150 trinucleotide GAA repeats in size and describe their clinical features.The smaller allele has previously been shown to determine the phenotype.

From a cohort of 196 unrelated patients with genetically confirmed FA, we identified 8 patients with an expansion less <150 repeats (4%).The smallest expansion was identified had 73 repeats . The mean age of onset was 39.5 years with a range of 29 to 48 years. The oldest living patient was 63yrs.The mean time to diagnosis was 10.8 years.

All except one patient had early onset ataxia ,with 5 of the 8 patients having clinical evidence of spasticity with brisk reflexes and extensor plantar responses. In 3, spasticity was the most significant finding. One patient had severe bilateral sensoriineural hearing loss prior to the development of significant ataxia .We also report other significant clinical differences between these patients and more typical FA.

We conclude that Friedreich's ataxia should be considered in the differential diagnosis of all patients presenting with a spastic ataxia even if the age of onset is beyond 40 years and that the phenotype of patients with small GAA expansions is different to more typical FA and may delay diagnosis. .

P632. Friedreich's ataxia carrier screening in the population originating from the Paphos district of Cyprus

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A cluster of Friedreich's ataxia (FRDA) patients has been identified in two neighbouring villages of the Paphos district of Cyprus and has been reporter by Dean, Chamberalin and Middleton (1988). The frequency of carriers within the two villages was estimated to be 1 in 5 to 1 in 6. These and additional patients originating from the Paphos district of Cyprus have been studied at the molecular genetic level. All Cypriot patients had a homozygous expansion of the GAA trinucleotide repeat in the first intron of the frataxin gene. In order to better estimate the frequency of FRDA mutation carriers in the population of the Paphos district, we initiated a screening program based on volunteer participation. The program was carried out for 18 months and it included: a) preparation of a leaflet with the relevant facts about FRDA and its high prevalence in the region, b) many field trips for organised talks at city/village centres and blood collection and c) genetic counseling sessions at hospitals. One thousand and fifty individuals, above the age of 18 years old, originating from the Paphos district of Cyprus participated in the program after signed concent. Analysis of the GAA triplet repeat revealed that ninety-eight of the individuals were heterozygous carriers of the expansion accounting for 9.33% of the sample. Thus, the estimated frequency of FRDA mutation carriers in the greater region of the Paphos district of Cyprus is 1 in 11 to 1 in 10 individuals. This project has been supported by UNOPS.

P633. Evidence for a frataxin gene deletion in a family with Friedreich Ataxia and Neuropathy.

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Friedreich ataxia is associated with the expansion of a GAA repeat in intron 1 of the frataxin gene. Approximately 96% of patients are homozygous for this expansion mutation with 4% being compound heterozygotes for the repeat expansion and a point mutation.

The proband is a 21 year old male with ataxia, scoliosis and pes cavus from early childhood, as well as coincidental Marfanoid features. DNA analysis confirmed a diagnosis of Friedreich Ataxia due to an apparently homozygous expansion mutation. However, testing of his mother demonstrated an allele within the normal size range with no evidence for an expansion mutation by long range PCR, triplet primed PCR or Southern blotting. We hypothesized that she may be a carrier of a deletion mutation.

Heterozygosity for microsatellite markers flanking the frataxin gene in both mother and son excluded the possibility of a large deletion. A fluorescent dosage PCR method was developed to compare the ratio of PCR products generated using primers flanking the GAA repeat to a control amplicon. Comparison of peak areas by the dosage quotient (DQ) method showed mean DQs of 0.53 and 0.48 for the mother and heterozygous expansion carrier compared to homozygous normal controls (n=9 replicates). This result suggests that the mother is heterozygous for a frataxin deletion. To date, there are no published reports of frataxin deletions. The mother has a personal and possible family history of a predominantly sensory axonal neuropathy. Family studies are in progress to explore the possibility that this deletion may have pathogenic consequences.

P634. Friedreich ataxia with spastic paraplegia in the absence of point mutations in the *FRDA* gene

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OBJECTIVE: To report a patient with late onset, slowly progressive spastic paraplegia, in a Friedreich ataxia (FA) family with 2 affected

siblings and marked intra-familial phenotypic variability.
BACKGROUND: FA is an autosomal recessive disease with onset before age 25. Most patients are homozygous for the GAA repeat in the *FRDA* gene, while 4% have an expansion on one allele and a point mutation on the other. Point mutations (particularly D122Y and G130V) give rise to atypical FA phenotype with slow disease progression, minimal or no ataxia, and gait spasticity (Durr *et al*, 1999).
CLINICAL FINDINGS: A 43-year old FA patient (P1) developed progressive spasticity, bilateral lower limb weakness and mild ataxia at age 25 and was wheelchair-bound at age 40. His sister was diagnosed with FA at age 29, exhibiting ataxia, bilateral lower limb weakness and dysarthria. His brother (P3) with FA developed progressive ataxia and leg weakness in infancy, and died at age 31. Autopsy revealed slight atrophy of the cerebellum at the level of the vermis and atrophy of the dorsal roots.
RESULTS: P1 had expansions in both *FRDA* alleles, each with 750 GAA repeats. P2 had 850 and 450 repeats, while P3 had 1050 and 790 repeats.
CONCLUSIONS: Marked intra-familial phenotypic variability exists in this family despite average size GAA expansions, demonstrating the importance of modifying genes. Our proband is unusual in having atypical FA with spastic paraplegia with equal GAA expansions in both alleles and without point mutations D122Y or G130V.

P635. Hereditary corneal dystrophies in Ukraine: The clinical and major TGFBI gene mutations analysis.

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Mutations in the human transforming growth factor beta-induced gene (TGFBI) encoding kerato-epithelin are responsible for the group of autosomal dominant diseases of the cornea: granular (Groenouw type I), lattice type I, lattice type 3A, Reis-Bucklers and Avellino corneal dystrophies. Clinically, they are characterized by a progressive accumulation of protein deposits of different structure and visual deterioration.
Five previously reported mutations of the TGFBI gene: R124C, R124H, R124L (exon 4), R555W, R555Q (exon 12) were analyzed in 37 individuals from 19 families with different forms of corneal dystrophy. The exon 4 and exon 12 of the TGFBI gene were amplified by polymerase chain reaction (PCR) with following restriction digestion. The R124C mutation was detected in 1 unaffected 10-year old individual and in 23 patients from 11 families with lattice corneal dystrophy. Clinical diagnosis was confirmed by results of histological and ultrastructural analysis performed on corneal specimens obtained during keratoplasty. The R124C was detected in 1 patient with clinical diagnosed Reis-Bucklers corneal dystrophy. The R555W mutation was detected in 1 patient with granular corneal dystrophy. The R124H, R124L and R555Q mutations were not detected in other five families with lattice and in one family with granular corneal dystrophy. We have suggested that obtained mutation analysis results in all these families are connected with wide genetical heterogenety of corneal dystrophies. These results show that TGFBI gene mutations analysis is important for early differential diagnosis of corneal dystrophies and genetic consulting in high risk families, and in future development of effective preventive therapy.

P636. Autosomal recessive congenital nystagmus in a large eight generations Indian pedigree.

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Congenital nystagmus (CN) is characterized by bilateral uncontrollable ocular oscillations with a frequency of 1:1500 live births. Families with X-linked, autosomal dominant and autosomal recessive modes of inheritance have been reported. The genes responsible for X-linked (NYS1) and autosomal dominant (NYS2) CN have been mapped to chromosomes Xp11.4-p11.3 (Am J Hum Genet 64: 1141-46, 1999), Xq26-q27 (Am J Hum Genet 64: 600-7, 1999) and 6p12 (Genomics 33: 523-6, 1996), respectively, but no mutation causing gene is yet identified. We have studied a large eight generation Indian pedigree with isolated non-syndromic CN (OMIM 257400) in which the anomaly segregates as an autosomal recessive trait. The onset is during early infancy. The pedigree consists of 228 individuals including 45 affecteds (23 males/22 females). The age distribution of these affecteds is 6-90 years.
The family is highly consanguineous and over 15 consanguineous marriages were observed. Clinical examinations were performed on twenty-five selected affected individuals. Those included color vision testing and eye movement recordings. All the examined individuals showed asymmetric pendular eye movements with unidirectional jerky nystagmus. Spasmus nutans combined with asymmetric nystagmus was observed in 12 affecteds. No other associated anomalies such as decreased vision, strabismus, color blindness, ocular albinism or congenital stationary night blindness was present. Chromosomal analysis was done in two affecteds, which showed no anomaly. Linkage studies with markers closely linked to NYS2 will either confirm allelism to this locus or support evidence for genetic heterogeneity and may reduce the genetic interval encompassing the NYS2 gene. u_c_rao@hotmail.com.

P637. Dental Phenotype in Axenfeld-Rieger Syndrome

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Axenfeld-Rieger syndrome (ARS) (MIM 180500) is a phenotypically heterogeneous autosomal dominant disorder characterised by malformations of eyes, teeth and umbilicus. Members of one family, diagnosed with ARS (3 affected individuals A,B,C spanning three generations) and 3 unaffected relatives (D,E,F) were screened for mutation in the PITX2 gene. A novel missense mutation Arg43Trp (position 5 in the homeobox) was found.
The oral phenotype was analysed and compared with ocular and umbilical findings. Maxillary hypoplasia was seen with related mandibular prognathism. Missing teeth were observed in A, B and C (20 to 27 permanent teeth missing, severity of the oligodontia increased from B, C to A). Persistence of primary teeth was noticed in B, C. Patient B displayed abnormalities of tooth shape including microdontia, tapered upper left permanent incisor (21) and generalised short roots. Her molars exhibited a rounded, mulberry-like cusp pattern and the lower left permanent molar (36) was macrodont. Proband C had a notched permanent lower incisor (31). Dental anomalies were also present in one non-affected member of the family (D) who had a rounded, mulberry-like cusp pattern of upper first permanent molars (16, 26). Severity of the umbilical phenotype increased from B, C to A. Patient B had the best visual acuity followed by A, C. Only proband C experienced glaucoma.
The dental phenotype observed in this family is consistent with other case reports. Its severity appeared to correlate with other clinical observations. Hence dental phenotype could give important information related to the severity of this condition.

P638. Is C677T polymorphism of the MTHFR gene a risk factor for ROP?

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Background: Retinopathy of prematurity (ROP) is a vasoproliferative disorder of the eye affecting premature neonates, which can result in blindness. The risk factors for the disease include low birth weight, low gestational age and supplemental oxygen therapy (hyperoxia increases production of free radicals, which can accelerate vasoproliferation). Another factor stimulating free radicals production is hyperhomocysteinemia. Until now no correlation between ROP and

hyperhomocysteinemia has been demonstrated.
Aim of the study: To evaluate the role of the common C677T methyl enetetrahydrofolate reductase gene (MTHFR) polymorphism, leading to mild hyperhomocysteinemia, as a potential genetic risk factor for the occurrence of ROP.
Methods: DNA of 40 prematures with ROP (birth weight 520-1500g, gestational age 23-31 weeks) and of 40 prematures without ROP (birth weight 700-1470g, gestational age 24-32 weeks) was genotyped by means of polymerase chain reaction – restriction fragments length polymorphism technique and the prevalence of 677TT genotype in both groups were compared.
Results: 677TT genotype prevalence was significantly higher in the ROP-group (7 cases) than in the no-ROP-group (1 case) (p in Fisher's exact test=0.028; odds ratio=8.27; 95% confidence interval=1.25-54.45).
Conclusion: Although preliminary, the above data suggest a role of the 677TT genotype of the MTHFR gene as an additional, genetic, risk factor for the occurrence of ROP.The study was supported by the State Committee for Scientific Research, grant 0523/P05/2002/22.

P639. Using ABCA4 gene microarray to analizing Spanish STGD population

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Stargardt disease is a recessively transmitted disease caused by mutations in the ABCA4 gene. ABCA4 encodes a photoreceptor-specific ATP binding cassette transporter, responsible for distinct but related inherited retinal disorders, Stargardt disease, fundus flavimaculatus, cone rod dystrophy, retinitis pigmentosa and age related macular degeneration. A model of correlation between genotype and phenotype was proposed based on the severity of the mutation. According to this model, homozogosity for null mutations might be responsible for RP phenotype, a null mutation in combination with a moderately severe mutation could produce CRD, a null mutation with a mild mutation or two moderately severe missense mutations would cause STGD, and one mutant allele alone could predispose to AMD. AsperBiotech has designed a chip for the ABCA4 gene that includes the analysis of 372 described variants in the gene, allowed the detection of known ABCA4 variants by the APEX method with the 97% efficiency. We analysed DNA from 24 patients from STGD pedigrees. We detected both mutations in 11 of the STGD DNA´s (45.83%), in 29.1% only one mutation could be detected and in 6 samples no mutation could be found. We conclude that for an initial screening the ABCA4 microarray is very useful. We could inform 11 families and offered genetic counselling, but we couldn't rule out this gene as responsible for the STGD phenotype in the families where no mutation was found. This procedure allowed us to establish the more frequent mutations in ABCA4 gene in Spanish affected population.

P640. Mutation screening of OPA1 in optic atrophy patients

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Dominant optic atrophy (DOA) is an inherited optic neuropathy with a frequency of approximately 1:50,000. Although heterogeneous the major locus for DOA has been mapped to chromosome 3q28 and mutations identified in a gene encoding a large dynamin-related GTPase, designated OPA1. In this study we screened OPA1 in a panel of 15 optic atrophy patients to try and identify causative mutations. The 15 patients were either single cases with no available family history or from families too small to test for linkage. We used intronic primers to amplify the 30 coding OPA1 exons from genomic DNA and screened these by single strand conformational polymorphism and heteroduplex analysis (SSCP-HA). Any band shifts observed were sequenced on an ABI377 DNA sequencer to determine the DNA change. Four mutations and five polymorphisms have been identified in this patient panel to date. Two of the mutations are novel (2700insTTACAAAT and 532delT). The remaining two mutations are the recurrent exon 27 4-bp deletion (2708delTTAG) which has now been reported in 20 unrelated patients and families to date. [c.toomes@leeds.ac.uk]

P641. Investigation of mutations involved in Macular Corneal Dystrophy in Iranian patients

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Macular Corneal dystrophy (MCD) is an inherited autosomal recessive disorder that is clinically characterized by progressive corneal stroma clouding in both eyes. MCD is subdivided three immunophenotypes (MCD Types I, IA, and II), some mutations in the carbohydrate sulfotranseferase 6 genes (CHST6) were identified to cause MCD. The CHST6 genes mapped on chromosome 16q22 that encoded a protein with the same name. DNA was extract from 14 blood samples from suspected of MCD patients. PCR method was used to amplify regions of three common MCD point mutations which is hot spot for this disease. Sequencing method was used to identify new point mutations. We suggest that the sequence is the best method for the population which we do not know much about genetic background.

P642. Exclusion of COL8A1 as a candidate gene for two Corneal Endothelial Dystrophies

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Fuchs Endothelial Corneal Dystrophy (FECD) and Posterior Polymorphous Corneal Dystrophy (PPCD) are both disorders of the corneal endothelium in which endothelial decompensation can lead to stromal and epithelial edema with resultant visual loss. Mutations in COL8A2, the gene for the α_2(VIII) chain of type VIII collagen, have previously been described in 8% of patients with both FECD and PPD. The role of type VIII collagen in FECD and PPD is as yet unclear but may influence the differentiation of the endothelium since both conditions are thought to be caused by defects in neural crest terminal differentiation. The in vivo composition of type VIII collagen is poorly understood and may exist as homo- or heterotrimers of α_2(VIII) and α_1(VIII) chains. COL8A1, the gene for the α_1(VIII) chain of type VIII collagen, is thus an attractive candidate for both FECD and PPD. Analysis of the transcribed region of COL8A1 in 141 unrelated patients with either FECD or PPD revealed no possible pathogenic alterations. It is therefore unlikely that mutations in COL8A1 are a significant cause of either FECD or PPD. These results support the hypothesis that type VIII collagen exists as homotrimers of either α_2(VIII) or α_1(VIII) and that the two proteins may have separate functions.

P643. Mapping of arRP to the RP28 locus on chromosome 2p11-p15 in a second Indian family

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Retinitis pigmentosa (RP) is a heterogeneous group of disorders of the eye. RP is characterized by abnormalities of the photoreceptors or retinal pigment epithelium (RPE) leading to progressive loss of vision. Patients generally present with night blindness followed by constriction of the peripheral visual fields. In the advanced stages of the disease, the retina is characterized by the presence of intraretinal and preretinal clumps of black melanin pigments appearing like bone

spicules, attenuated retinal vessels, loss of the RPE and paleness of the optic nerves. RP can be inherited as an autosomal dominant, autosomal recessive, X-linked recessive, X-linked dominant, or, in rare cases, a digenic trait. There are a total of 22 known loci for arRP (autosomal recessive RP) (RetNet, http://www.sph.uth.tmc.edu/Retnet/). We have ascertained a consanguineous Indian family in which RP is segregating as an autosomal recessive trait. We have genotyped the family using markers from the candidate regions of RP20 (RPE65), RP25, and RP28 loci. The results suggest that this family is linked to the RP28 locus with a maximum multipoint lod score of 3.08 at D2S2397. The RP28 locus was previously mapped to a 16-cM region between D2S1337 and D2S286 in a single Indian family. The two families are from different ethnic backgrounds and are not known to be closely related. The present result confirms linkage of arRP to the RP28 locus in a second Indian family. We are currently in the process of refining this region.

P644. Autosomal dominant familial exudative vitreoretinopathy: evidence suggestive of a fourth autosomal dominant locus.

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Familial exudative vitreoretinopathy (FEVR) is a mendelian condition characterised by bilateral deficient vascularisation of the peripheral retina. Clinical features can be highly variable, even within the same family, with severely affected patients being registered blind from a young age, while mildly affected individuals may even have no symptoms. FEVR can be inherited in dominant, recessive and X-linked modes and four loci, one X-linked (EVR2) and three autosomal dominant (EVR1, EVR3 and EVR4), have already been mapped. The genes underlying EVR2 (NDP) and EVR1 (FZD4) have already been described but the genes mutated at the EVR3 and the recently mapped EVR4 loci remain to be identified. The aim of this study was to screen the three known autosomal dominant loci in a new pedigree originating from Mexico. Clinical analysis of this family suggested features typical of FEVR, with deficient peripheral retinal vascularisation as the common phenotype in all affected individuals. DNA samples from 18 family members were genotyped using fluorescently tagged microsatellite markers spanning 11q12-q14 (EVR1 and EVR4) and 11p12-13 (EVR3). Haplotype analysis revealed recombinants with all markers spanning the EVR1, EVR3 and EVR4 loci. Exclusion of the three known loci for autosomal dominant FEVR in this pedigree provides further evidence for a fourth autosomal dominant locus and implies the existence of at least 5 genes responsible for the phenotype. A whole genome search is currently being undertaken in this family in order to map this new FEVR locus. (c.toomes@leeds.ac.uk)

P645. Mapping of gene responsible for autosomal recessive Retinitis pigmentosa, frequent in Slovak Gypsy population

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Retinitis pigmentosa (RP) is a group of clinically and genetically heterogeneous retinal degenerations in which abnormalities primarily of rod photoreceptors lead to progressive visual loss, initially manifesting as night blindness and constriction of the peripheral visual field. RP can be transmitted as an autosomal dominant, autosomal recessive, X-linked, or digenic trait and to date 38 genes (mapped or cloned) have been identified in association with nonsyndromic form of the disease.

We collected 63 DNA samples (15 affected individuals and 48 close, healthy relatives) of family members from two large Gypsy pedigrees with occurrence of autosomal recessive RP. Due to high endogamy and specific demographic history of Slovak Gypsies, we assume existence of only one founder mutation occurring in all Slovak Gypsy patients with RP. Computer simulation, employing SIMLINK program, confirmed sufficient data for successful linkage analysis. Performed autozygosity mapping did not reveal any of 21 to date identified loci linked to autosomal recessive RP. Relatively high genetic distances between analyzed markers and candidate loci, as well as the existence of novel locus are liable. Subsequent linkage analysis, using calculation of two-point LOD scores by the Cyrillic and MLINK programs, excluded to date 12 of total 21 candidate loci. In case of excluding linkage to all of known loci, the whole genome analysis and searching for novel locus will take place.

P646. Molecular investigations in Achromatopsia

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Achromatopsia (ACHM) is an autosomal recessive stationary cone dystrophy which occurs in complete and incomplete forms. Individuals with complete ACHM have nystagmus; photophobia ;6/60 vision and are totally colour-blind, whereas individuals with the incomplete phenotype have a milder condition. ACHM is genetically heterogeneous. Mutations in CNGA3 and CNGB3 genes which encode the cone specific alpha and beta subunits of a cyclic cGMP channel, a crucial component of phototransduction, account for about 20-30% and 40-50% of cases respectively. Molecular investigation of a panel of consanguineous families with ACHM was undertaken. Initial investigations showed 6 families to link to CNGA3 and CNGB3. Mutation analysis was performed in these families at Moorfields Eye hospital and confirmed the linkage results. After excluding linkage to CNGA3 and CNGB3 in large consanguineous family linkage to CNGA3, autozygosity mapping was used to map a novel locus, ACHM4, to chromosome 1p13, and subsequently we identified a germline frameshift mutation in the GNAT2 gene. GNAT2 encodes the alpha subunit of cone transducin, a cone specific component of the phototransduction pathway. A novel homozygous missense GNAT2 mutation has been found in a further two families. Genotype-phenotype correlations for CNGA3, CNGB3 and GNAT2 mutations are being analysed. As mutations in CNGA3 have also been described in patients with progressive cone dystrophy, we are currently screening a panel of 31 families with progressive cone dystrophy for mutations in GNAT2 using DHPLC and direct sequencing.

P647. Mutations in Myocilin gene in families with Primary Open-Angle Glaucoma and Juvenile Open-Angle Glaucoma.

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We report our work aimed at investigating the prevalence of myocilin (*MYOC*) mutations in Italian glaucomatous families and its relationship with primary open-angle glaucoma (POAG), juvenile open-angle glaucoma (JOAG) and pigmentary dispersion glaucoma (PDS). Twenty six POAG patients were selected on the basis of a positive family history of glaucoma. All patients and 210 relatives had an accurate clinical characterization. Each index patient was screened by SSCP for mutations in the *MYOC* gene. A *MYOC* mutation was found in two families. In one family a previously reported p.K423E was transmitted from the POAG index case to both the two JOAG sons. In the second family a p.C25R change affecting the signal peptide, was transmitted from the POAG index case to the JOAG son, but not to the PDS son. Clinical characterization of the two families with *MYOC* mutations indicates that POAG and JOAG are the two sides of a continuous phenotypic spectrum due to a common molecular defect. On the other hand, our results confirm the different origin of PDS. Since *MYOC* mutations may be responsible for a fraction (2/26, 7.7%) of POAG/JOAG families, molecular genetic diagnosis should be included in the management of patients with glaucoma.

P648. RPGR gene mutations cluster upstream of an evolutionary diverged polypurine polypyrimidine minisatellite in exon ORF15.

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Mutations in the X-chromosomal *RPGR* gene account for up to 20% of all cases of retinitis pigmentosa, a figure higher than for any other single locus of the more than 20 different RP loci mapped throughout the genome. This high degree of sequence variation is due to a large 3´terminal exon (ORF15) of an alternatively spliced transcript predominantly expressed in retina. It contains a repetitive purine-rich sequence coding for an acidic tail (>250 glutamic acid residues). We performed dot-matrix, tandem-repeat and PCR analyses of the *RPGR* genomic sequence. Comparative analyses in mouse, cattle and pufferfish indicate that the repetitive sequence of exon ORF15 has evolved from a minisatellite which appears in the human genome as an evolutionarily diverged polypurine•polypyrimidine tract. We looked at the spectrum and distribution of mutations of four comprehensive mutational screens (486 xlRP patients, including 58 unpublished cases of our own) and found that the majority of the 106 disease-causing mutations (frameshifts caused by small deletions and nonsense mutations) cluster in the sequence adjacent to the 5' end of the diverged minisatellite. In contrast, in-frame sequence variations (~34% of controls) with consequent losses or gains of tandem repetitive units are located throughout the diverged minisatellite. From this observation we hypothesize the presence of two mutational mechanisms in exon ORF15: a) a slipped stranded mispairing mechanism leading to loss or gain of tandem repetitive units and b) a "gratuitous" DNA repair mechanism, triggered by the triplex-formation of the polypurine•polypurimidine tract and inducing enhanced mutagenesis in the flanking sequence.

P649. Detection of deletions in exons 7 & 8 of the SMN gene in SMA patients in Khouzestan region

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The term of SMA is used for a clinically and genetically heterogeneous group of neuromuscular disorders. Two types of disease occur prenatally; AMC and CAN. The patients on the basis of age of onset and severity of the clinical course as assessed by clinical examination, muscle biopsy and electromyography. Childhood proximal SMA has autosomal recessive pattern of inheritance and it is classically subdivided into three forms: acute, intermediate and mild. Up to now, SMN, NAIP, p44, H4F5 genes have been mapped to a 850kb interval on 5q13 which are involved in SMA in occurrence and aggravation of disease. SMN gene, telomeric copy (tel SMN) is highly homologous with centromeric copy (cen SMN). Both copies show identical sequences, except for five exchange of a base pair at the 3-end of the gene intron 6 to exon 8. However only deletion/ mutation in tel- SMN seen to cause SMA. Whereas homozygous deletions of cen-SMN was found in about 2-3% of carries and control, in this research, we have studied deletions of exon 7 & 8 of the SMN gene using PCR/RFLP technique. 33 individuals from 25 families in Khouzestan proving were studied for this research. The genetic counseling ,DNA extraction, PCR by specific primers of exons 7& 8, digestion with restriction enzymes and Polyacrilamide electrophoresis were perfumed.
87-71 percent of cases were positive for presence of the above deletions. Using the procedure described here will be able to detect the carrier individuals in order to decrease the incidence of disease.

P650. Molecular analysis of NAIP gene in Iranian patients suffering from Spinal Muscular Atrophy

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Spinal muscular atrophies (SMA) are of the most common autosomal recessive disorders characterized by degeneration of anterior horn cells in the spinal cord, and lead to progressive muscular weakness and atrophy.
At least three SMA- related genes have been identified; SMN (survival motor neuron) , NAIP(neuronal apoptosis inhibitory protein) and P44, all of located on chromosome 5q13 in two highly homologous copies(telomeric and centeromeric) within the SMA region .
The objective of this study was to investigate the NAIP gene deletion in SMA and families.
Here, we analyzed homozygous deletion in exons 5, 6 and 13 of NAIPt gene in 8 families (including 19 individuals) with SMA, which exon 7 of SMNt gene analyzed before and was being deleted.
In our study, we found homozygous deletion exon 5 of the NAIPt gene in 4 individuals (two affected and two unaffected), in which only exon 5 of NAIPt gene were deleted.

P651. Molecular Detection of SMNt Deletion in SMA Patients in Iranian Population Over a Five-Year Period

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Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder. One of the candidate genes for this disease is survival motor neuron gene (SMN) that exists in two nearly identical copies, telomeric SMN (SMNt) and centromeric SMN (SMNc). The two genes differ in their exons by only two base pairs, one in exon 7 and one in exon 8 that allow them to be distinguished by restriction site assay. 90%-95% of SMA patients carry homozygous deletions in SMNt affecting exon 7 only, or both exon 7 and exon 8.
DNA extracted from blood samples was amplified by PCR. The PCR products were digested by restriction enzymes , DraI and DdeI, and subsequently analyzed by polyacrylamide gel electrophoresis followed by silver staining. The ratio of band intensities (SMNt/SMNc) were indicative of carrier (heterozygous deletion of SMNt) or affected (homozygous deletion of SMNt) status of the samples.Over a five-year period, we have studied 88 clinically diagnosed SMA families for exon 7 and exon 8 deletions in SMNt gene. Among these families, 65 families were referred for carrier detection and 23 for prenatal diagnosis. We have been able to determine the carrier status of 49 families of 65 families referred for carrier detection. In 11 families we could confirm the carrier status only in one of the individuals. A total of 23 prenatal diagnosis were carried out, of which, 14, 3 and 6 were carrier, affected, and normal respectively.

P652. Practical approach to molecular diagnosis of myopathies and muscular atrophies in Republic of Moldova.

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Recent progress in molecular genetics has greatly improved our understanding of the molecular basis of many inherited neurological diseases and provided practical help for the clinical neurologist. In our Moldavian data base we have 110 families with DMD and 30 with SMA.
DNA analysis of the DMD families consists of detecting deletions in 13 different exons of the dystrophin gene and RFLP analysis (pERT87-8/TaqI, pERT87-15/BamH1 and intron 16/TaqI polymorphisms). About 76 % of probands were proved to be carriers of dystrophin gene deletion by MPCR. Our protocol for molecular analysis allows to define 93% of cases as informative.
Molecular analysis was applied to 11 first or second trimester fetuses. Five male fetuses were unaffected, two male fetuses were affected, in one case the risk remained at 50% and 3 cases were heterozygous carriers.

Molecular studies at the SMNI locus were performed in 25 families. The results of direct DNA diagnosis of SMA are: in 24 out of 25 families (96%) the diagnosis of SMA was confirmed at the molecular level by revealing homozygous deletion of exons 7 and/or 8 of the STN^T gene in the probands. In 4 cases we performed prenatal diagnosis (PD) for SMA. In 2 out of 4 fetuses we detected heterozygous deletion of the exons. Thus molecular diagnosis is increasingly important, because it may provide valuable information for the affected individuals and their families in order to make informed choices on life and family planning

P653. 'Oculopharyngeal muscular dystrophy-genotype studies in a Croatian population'

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Oculopharyngeal muscular dystrophy (OPMD; OMIM 164300) is a late-onset disorder characterised by progressive ptosis, dysphagia and proximal limb weakness. Dominant and recessive OPMD are caused by stable short $(GCG)_{7-13}$ triplet repeat expansions in exon 1 of the poly(A)binding protein 2 gene(PABP2); normal repeat length being $(GCG)_6$. The $(GCG)_7$ allele is a polymorphism that acts as a modifier of severity of dominant OPMD, or as a recessive mutation. According to Brais (2001) this mutation has 1-2% prevalence in North America, France and Japan. In recent study performed on 201 normal individuals from United Kingdom (Hill et al. 2001) no $(GCG)_7$ allele was detected. Our preliminary study of first six OPMD patients from Croatia enabled us to identify four different allelles with 7, 8, 9 and 11 (GCG) repeats. Based on these findings, we hypothesised that allele $(GCG)_7$ might be frequent in Croatia. To validate our hypothesis, we have screened a control population of 500 samples from individuals with no known family history of OPMD for an expansion of (GCG) repeat using PCR and polyacrylamide gel electrophoresis. One healthy heterozygote not related to previously detected patient with alleles $(GCG)_{7,8}$ was found suggesting the high frequency of 4% (2 /506 patients) of $(GCG)_7$ mutation in Croatia. In view of this, we may expect autosomal recessive homozygotes in our population. Patients with this form may be underdiagnosed because of a milder phenotype and the absence of clear family history. More extensive study should confirm these data.

P654. Identification of a novel mutation in LAMA2 gene in three Tunisian patients affected with MDC1A

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Congenital muscular dystrophies (CMD) are a heterogeneous group of autosomal recessive neuromuscular disorders; with severe manifestations consisting on muscle weakness and hypotonia at birth. Regarding classical CMD (MDC1A), a primary deficiency of the laminin α2 chain (merosin) was reported. Linkage data and positional cloning showed that MDC1A is caused by defects in the laminin α2 gene (LAMA2) on chromosome 6q22. Since, several mutations have been identified.

We have identified three patients affected with CMD and belonging to the same Tunisian family. In order to determine the deficient protein and the responsible mutation, we have performed an immunohistochemical and immunoblot analyses using two antibodies against 80 and 300 kDa fragments of merosin and antibodies toward α-sarcoglycan, β-dystroglycan and dystrophin. Genotyping was undertaken by radioactive detection system and determination of mutations was performed using an ABI 3100 system.

Our results showed that all patients had a total merosin deficiency. Alpha-sarcoglycan, β-dystroglycan and dystrophin immunostaining were normally present on their muscle fibres surfaces. Linkage analysis by homozygosity mapping was found to be compatible with linkage to the LAMA2 locus on 6q22. Mutation screening of all 64 coding exons and their intron-exon junctions of the LAMA2 gene in this family revealed a novel LAMA2 nonsense mutation different from all the mutations subsequently reported.

In this work, we describe evidence for linkage to the LAMA2 locus on 6q22 in a Tunisian family and identify a novel LAMA2 nonsense mutation in three Tunisian patients affected with MDC1A.

P655. 'DNA linkage analysis of losus D5S435 in Moldavian families with SMA'.

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DNA linkage analysis of losus D5S435 in Moldavian families with SMA

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Introduction. All three forms of spinal muscular atrophy (SMA) from severe to very mild are the result of mutations in the *SMN1* gene. The disorder has the autosomal recessive type of inheritance. Here, we report the data about DNA linkage analysis, which was performed in Moldavian families with SMA to evaluate its value for carrier detection.

Methods: DNA was isolated from peripheral blood using standard procedures. PCR's were performed for locus D5S435 allele variants detection in 15 families with SMA and in control group. The fragments were separated by 8% PAAG electrophoresis and stained by etidium bromide.

Results: The control group was consisted from 40 normal individuals (80 chromosomes). Locus D5S435 analysis exposed 8 alleles from 9 of Russian population (Shaghina, 2001). The most frequent allele in control group contained 140 bp length with a frequency of 0.38. The heterozygosity rate was determined and consisted 0,75. Ten Moldavian families with SMA, containing 30 DNA samples were analyzed. The same allele was the most frequent in affected individuals and had a frequency 0.42. Seven families (70%) were informative for this locus.

Conclusions: Thus, this result indicates that D5S435 permits to use for prenatal diagnosis. It should also be useful for carrier detection and, possibly, the prenatal diagnosis in families at risk of SMA, which is part of the prevention and control of this disease.

P656. Quantitative determination of *SMN1* and *SMN2* copy numbers using MLPA (multiplex ligation-dependent probe amplification).

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SMA, spinal muscular atrophy, is one the most common autosomal recessive disorders with an incidence of approximately 1:10,000 and a carrier frequency of 2%.

Based on the severity of the disease three types of SMA have been deliniated: type I (Werdnig-Hoffmann disease) is the most severe, type II is intermediate and type III (Kugelberg-Welander disease) is the mildest form.

The majority of the patients, > 95%, are homozygous for absence of *SMN1,* the SMA- determining gene.

It now generally accepted that *SMN2*, which almost identical to *SMN1*, can modify the severity of SMA and several reports support the hypothesis that the copy number of SMN2 is inversely related to the severity of the disease.

We have used the method MLPA (multiplex ligation-dependent probe amplification) to determine the copy number of both *SMN1* and *SMN2*. Using the MLPA kit (MRC-Holland) we have determined the copy number of *SMN2* in 70 patients known to be homozygous for absence of *SMN1*. The method turned out to be highly reliable, and all our patients could be unambiguously placed into four distinct groups with 1, 2, 3 or 4 copies of SMN2. The copy number correlated with the type of the disease. The copy numbers of both *SMN1* and *SMN2* (exons 7 and 8) are determined simultaneously and the method is therefore also very useful for carrier diagnosis. It is easier than previously reported methods using real-time LightCycler PCR, where *SMN1* and *SMN2* copy numbers are analysed separately.

P657. SMN1 atypical genotypes in parents of SMA patients and in general population

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Spinal muscular atrophy (SMA) is a common autosomal recessive disease. Homozygous deletion of *SMN1* is found in 98% of the patients linked to 5q13. Since the introduction of carrier detection, genetic counselling in SMA families has greatly improved.
However, SMN analysis has revealed some atypical genotypes and rare molecular mechanisms that should be considered when the recurrence risk is assessed. Studying SMA parents and normal controls by SMN quantitative analysis, we have established the frequency of carrier genotypes in a French population which is higher than previously estimated (1/34). Moreover, we have determined the frequency of chromosomes carrying *SMN1* duplications and assessed the prevalence of the carrier genotype associating a duplication of *SMN1* on one chromosome and its deletion on the other one.
Parents of SMA patients homozygous for the *SMN1* deletionshowed a clearly heterozygous status in only 95.6 % of cases (only one *SMN1* detected), while 4.5% showed a genotype with two *SMN1* genes: among them, *de novo* deletions (1%) and duplication of *SMN1* on a chromosome associated with the heterozygous deletion on the other one (3%) were detected. In addition, we report the first evidence for a *de novo* gene conversion in one family.
These results improve the interpretation of the SMN genotypes and allow the recurrence risk in SMA families and their relatives to be calculated more accurately.

P658. Microdissection and gene specific PCR on single chromosomes identify Spinal Muscular Atrophy (SMA) carriers

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Approximately 95% of all Spinal Muscular Atrophy (SMA)-patients carry a homozygous deletion of at least exon 7 of the survival motor neuron (SMNt) gene. The presence of (multiple copies of) a nearly identical centromeric homologue of this gene, SMNc, complicates the detection of this deletion in carriers.
So far, quantitative PCR or haplotyping were the only methods by which SMA carriers could be identified, but these methods are not fully reliable, because of the variable copy number of genes and pseudogenes that may be present.
We report the development of a new and direct approach to identify SMA carriers by microdissection of single copies of chromosome 5 followed by gene specific amplification of the SMN exon 7.
In this study we included nine individuals with or without a heterozygous deletion in one of the SMNt genes. In total, 48 chromosome 5 pairs were tested blindly (two complete chromosome sets for each individual). Of these, 36 (75%) were of sufficient quality to be further analysed. Data obtained revealed that allelic drop out (ADO) occurred in 3% of all single chromosome samples. Genotyping of individuals proved to be 100% accurate as no discrepancies were found between the determined genotype and the actual genotype in all individuals tested.
In conclusion, this new method allows reliable and efficient identification of SMNt exon 7 deletion carriers. However the described method is technically complex and it has to established if the method is sufficiently robust for routine diagnostics.

P659. Congenital muscular dystrophy with short stature, proximal contractures and distal laxity: a new CMD syndrome?

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The congenital muscular dystrophies form a very heterogeneous group of disorders which present early on in life with muscle weakness and wasting, generalised hypotonia and joint laxity or contractures. Over recent years the clinical characterisation of different phenotypes and the establishment of molecular diagnoses has led to a change in the classification of CMD and a recognition of more and more clinical entities. The association of CMD with proximal contractures, distal joint laxity and normal intelligence is felt to be indicative of the Ullrich's CMD phenotype, which has recently been shown to be caused by recessive mutations in any of the three genes encoding for collagen VI. Characteristically individuals with UCMD are of normal intelligence. Furthermore to date short stature has not been a recognised feature of any of the CMD syndromes.
We report 2 patients who share a diagnosis of congenital muscular dystrophy (CMD) in association with short stature, proximal contractures, rigidity of the spine and distal joint laxity as well as mild to moderate mental retardation. They also have very similar facial features and developed early respiratory failure. The expression of collagen VI was confirmed to be normal on muscle biopsies of both patients. These findings together with their unusual clinical phenotype suggest that this might represent a new CMD entity.

P660. Our Experience of Diagnosis of Muscular Dystrophies and Congenital Myopathies

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The muscular dystrophies and congenital myopathies belong to the extremely heterogenous group of diseases with overlapping clinical symptomalogy. The current classification is based on the biochemical defects of the muscle tissue, especially on the plasmatic membrane of the muscle fiber.
In our Neurogenetic Centre we have implemented the database of 98 patients with clinical and/or laboratory symptomatology of muscular dystrophy or congenital myopathy (hypotonia, myogenic syndrome, elevated CK, myogenic signs on EMG investigation). From this group of patients we have proven by molecular genetic testing the deletion of the DMD/BMD gene in 20 patients. To this date we were able to obtain the muscle biopsy from 35 patients. By immunohistochemical analysis 10 patients have been diagnosed as dystrofinopathies (nondeletious forms the DMD/BMD gene), 2 patients as dysferlinopathies, 2 as defficiency of merosin, in 1 biopsy the result was ragged red fibres and in 1 MELAS has been diagnosed and in 2 patients the final diagnosis was multicore disease.
As we expected the most frequent cause of mentioned disorders are dystrophinopathies, but the proportion of the deletions of the DMD/BMD gene seems to bee lower then 2/3 in our populations. We are presenting here 4 illustrative cases with complete investigation.
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P661. The first non Japanese case of Fukuyama-type congenital muscular dystrophy (FCMD)

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Fukuyama-type congenital muscular dystrophy (FCMD), Walker-Warburg syndrome, and muscle-eye-brain disease are clinically similar autosomal recessive disorders characterized by congenital muscular dystrophy, cobblestone lissencephaly, and eye anomalies. FCMD is frequent in Japan, but no FCMD patient with confirmed *fukutin* gene mutations has been identified in a non-Japanese population. Here we describe a Turkish FCMD patient with severe brain and eye anomalies with congenital muscular dystrophy. Sequence analysis of the patient's DNA identified a homozygous 1-bp insertion mutation in exon 5 of the *fukutin* gene. This is the first case worldwide in which a *fukutin* mutation has been found outside the Japanese population.

P662. Molecular-genetic analysis of Fukuyama-type congenital muscular dystrophy in Russian families

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Fukuyama-type congenital muscular dystrophy (FCMD, MIM

253800), one of the most common autosomal recessive disorders in Japan (incidence 0.7-1.2 per 10,000 births), is characterized by congenital muscular dystrophy in combination with cortical dysgenesis (micropolygyria) and ocular abnormality. In Japan, about 87% of FCMD-bearing chromosomes are reported to contain a 3kb retrotransposon insertion in the 3' non-coding region of the *fucutin* gene. Point mutations in the *fucutin* gene found as a compound heterozygote with the founder insertion resulted in more severe phenotypes. FCMD patients with two point mutations have not yet been detected. Probably inactivation of both *fucutin* gene alleles by point mutations is embryonic-lethal. This could explain the rare occurrence of FCMD in non-Japanese populations.
Six FCMD patients from four Russian families have been investigated by clinical and molecular-genetic methods. All these patients have typical clinical features such as weakness of facial and limb muscles, general muscle hypotonia and atrophy, areflexia manifested from the first days of life, polymorphic convulsions, thorax deformity, microcephaly. Cortical atrophy and micropolygyria are revealed during autopsy. We have examined DNA samples of these patients using primers flanking the 3kb insertion in the 3' non-coding region of the *fucutin* gene. This insertion was not found in any of the FCMD patients studied. Probably other *fucutin* gene mutations or even another gene(s) are involved in the pathogenesis of FCMD in European patients.

P663. A benign congenital myopathy in an inbred Samaritan family

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We describe a novel form of myopathy in a mother and her two daughters from an inbred Samaritan family. The patients displayed severe neonatal hypotonia, lethargy and dysmorphic features. Motor milestones were delayed, however, the hypotonia and muscle weakness gradually improved during the first two years of life and independent walking was achieved by 18 months. The mother at the age of 23 years shows myopathic facies and minimal proximal weakness. Her intelligence is normal. Her muscle biopsy revealed central nuclei, and disruption of the intermyofibrillary network with moth eaten and spiral fibres.
We suggest this is a new benign form of congenital centronuclear myopathy. Inheritance is probably autosomal recessive (pseudodominant).

P664. A punctuated (CTG)n expansion associated with myotonic dystrophy

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The mutational spectrum of myotonic dystrophy 1 (DM1) is extremely homogeneous: so far, only pure (CTG)n untranslated triplet expansions at the 3' UTR of the DMPK gene have been reported. During routine diagnostic testing for DM1, employing the triplet repeat-primed PCR (TP-PCR) assay, we came across a discontinuous pattern indicative of regular interruptions in an at least 1.5 kb long -by Southern blotting analysis- expansion associated with myotonic dystrophy in a 50 year-old patient. Clinical manifestations included ECG-detectable vasovagal syncope, bilateral cataract, muscular weakness and EMG-documented myotonia. The change was mapped at the second position of the CTG triplet and partial sequencing revealed CCG interspersions. A modification of TP-PCR enabled penetration of the proximal edge of this atypical expansion to a depth of seven interruptions, verifying a modular structure with CCG triplets every 8 to 11 repeats. To our knowledge, this is the first punctuated DM1 expansion to be described and may be informative both for the mechanism of repeat instability and the RNA pathology underlying DM1. Ongoing work is focusing on the detailed characterization of the expansion structure, determination of its haplotype background and evaluation of its expression. A comprehensive study of the wider family is planned that may provide insights into the evolution of this novel DM1 allele while outlining a genotype-correlation. Quite apart, our data confirm TP-PCR as a robust, highly informative and flexible assay for diagnostic purposes.

P665. How common is the DM2 mutation as the cause of clinical Myotonic dystrophy?

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Myotonic Dystrophy is usually the result of a CTG repeat expansion in the 3' untranslated region of the DMPK gene on chromosome 19q13.3 (DM1). Recently, a second mutation (DM2), a CCTG expansion in intron 1 of the ZNF9 gene on chromosome 3q21, has been found to be responsible for some cases of myotonic dystrophy, as well as for the allied clinical disorder Proximal Myotonic Myopathy (PROMM). We have previously found only one family with the DM2 mutation in a research series of 95 myotonic dystrophy families
In a series of 205 diagnostic referrals to the Cardiff laboratory between 1992 and June 2000, 101(49.3%) were positive for the DM1 expansion. Of the 104, DM1 negative patients, 40, unrelated, adult patients, with no family history of the DM1 mutation, referred from either Neurologists or Geneticists, were analysed using a combination of direct PCR and QP-PCR for the CCTG expansion at the 3q21 locus. Three patients were positive, suggesting a frequency of around 1.5% for the DM2 mutation in all cases of clinically suspected myotonic dystrophy in Wales. All three cases with the DM2 mutation showed clinically atypical features, suggesting that analysis of the DM2 mutation in UK patients will be of greatest value in suspected cases of myotonic dystrophy that are atypical or where the DM1 mutation has proved normal.

P666. Mutation detection in one family affected with Hereditary Inclusion Body Myositis (HIBM) from Iran.

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Hereditary Inclusion Body Myositis is a chronic progressive muscle disorder. Inheritance is autosomal recessive. It begins with insidious onset in the second or third decade of life and is associated with distal muscle weakness in the upper extremities and proximal muscle weakness in the lower extremities.
The patient is a 24 year of age girl that suffers from gait disturbances. She was evaluated for muscle weakness.
The clinical pattern is typical for the quadriceps sparing type of hereditary inclusion body myopathy (HIBM).
Laboratory findings in this patient showed: increased CPK levels in serum, EMG/NCV: myopathic feature and muscle biopsy: muscular atrophy, neurogenic type.Her parents are unrelated but both of them are from Iranian Jewish ancestors.The candidate gene mutation is in the 3'UTR region of CNTFR gene from ggccgg to gggcgg. (about 50 bp)
The PCR product size is about 305 bp. With RFLP we obtain different size fragments from affected vs normal.This is the first time that a mutation of the HIBM gene has been detected in our country. Because this type of myopathy is prevalent in Iranian Jews diagnosis using PCR/RFLP will be a quick and useful method to confirm HIBM.

P667. LGMD2A caused by a large deletion: clinical, histochemical and molecular analysis

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Limb-girdle muscular dystrophies (LGMD) are a heterogeneous group of inherited diseases characterized by progressive symmetrical atrophy and weakness of the proximal limb muscles. LGMD2A is an autosomal recessive form of LGMD caused by mutations in the calpain 3 gene (CAPN3). In contrast to other types of LGMD, most of which are caused by defects in structural components of the muscle tissue, the gene product of CAPN3 is an enzyme with proteolytic activity. The pathological mechanism of CAPN3-defects is not well understood. The study of CAPN3 mutations and their effect may

therefore help to elucidate its role in the pathogenesis of LGMD2A. We describe a female patient presenting with clinical signs of LGMD. Histological and histochemical analyses were performed for classification. The results showed normal localization and staining for dystrophin, alpha-, beta-, and gamma-sarcoglycan, laminin alpha2 and merosin. Immunoreactivity to delta-sarcoglycan, however, was reduced. The molecular genetic analysis revealed a homozygous deletion of exons 2-8 of CAPN3, predicted to yield a truncated product. To our knowledge this is the largest deletion yet described in this gene. The vast majority of mutations identified in LGMD2A patients are point mutations or small deletions/insertions. Only one other large deletion spanning exons 4-7 is known. The molecular genetic analyses are still ongoing in order to identify the exact extent of the deletion, which will be presented and related to the detailed clinical, histological and immunohistochemical findings.

P668. Selective screening for genetic prophylaxis of LGMD2C among Bulgarian high risk Gypsy group

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Limb-girdle muscular dystrophy type 2C (LGMD2C) is caused by mutations in the gamma-sarcoglycan gene localized on 13q12, where a “private” Gypsy mutation C283Y is detected.
The geographic distribution of C283Y mutation among Gypsies in Bulgaria was found to be heterogeneous- very high carrier frequency was detected mainly in North-East Bulgaria (2.25%).
In Senovo, a town situated in North-East Bulgaria, two unrelated Gypsy LGMD2C patients were registered. For that reason, a selective screening for genetic prophylaxis of LGMD2C was performed there. We used direct amplification on dry blood spots followed by SSCP assay. Heterozygotes were confirmed by RsaI restriction digestion. Our study on 126 volunteers (31 children, 88 at reproductive age, 7 over reproductive age) showed that 22 of them (17.46%) were heterozygouse for C283Y mutation. A second genetic counseling and written result of the DNA assay were given to each volunteer. The most important group was the individuals at reproductive age- 15 of them (17.05%) occurred heterozygous. Considering the high consanguinity, carriers were informed about the risk of having an affected progeny. In addition, the first prenatal diagnosis of LGMD2C was performed in Senovo. Two of the detected carriers occurred couple and after genetic counseling they referred to our laboratory for performing a prenatal diagnosis.
Considering the high carrier and/or disease frequency in some regions and the high consanguinity the performance of selective screening program for genetic prophylaxis of LGMD2C in endogamous Gypsy community at high risk seems reasonable. Such regions should be with priority in the Bulgarian healthcare system.

P669. LGMD2A in Bulgarian patients - mutations and polymorphisms in CAPN3 gene

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Limb-girdle muscular dystrophy type 2A (LGMD2A) is due to mutations in the muscle specific calcium-activated neutral protease 3 (CAPN3) gene, located in 15q15.1-q15.2. The translational product of this gene is calpain 3. To date, more than 100 independent pathological mutations have been reported in this gene. The clinically significant mutations are scattered along the whole gene sequence. They are mainly private sequence changes, although there are some common ones. Recently, the mutation 550delA in exon 4 of the *CAPN3* gene has been reported as very frequent in Russian, Ukrainian as well as in Turkish patients. The published results are in favour of a common founder effect, rather than the presence of recurrent mutations.
So far, we have 12 families with genetically proved diagnosis of LGMD2A. The screening for mutations was performed by direct sequencing of the whole gene. We found that 9 patients (75%) were homozygous or heterozygous for a common mutation 550delA in exon 4 of *CAPN3*. Two patients were heterozygous for another mutation 967G>T, Glu323X in exon 7. Additionally, the unique mutations 505C>G, Arg169Gly in exon 4; 352A>G, Arg118Gly in exon 2 and 1981-84delATAG in exon 17 were also detected in our sample. During mutation screening we also found some polymorphisms: 606T>C, Ser202Ser in exon 4; 706G>A, Ala236Thr in exon 5 and 1752T>C, Val584Val in exon 14.
In conclusion exons 4 and 7 of *CAPN3* seem to be hotspots for mutations in our sample of Bulgarian patients.

P670. Interspersions in the OPMD triplet repeat expansion

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Oculopharyngeal muscular dystrophy (OPMD) is a late onset autosomal dominant disease that usually presents with dysphagia, ptosis, and proximal limb weakness. The causative genetic basis of this disease is the expansion of a 6(GCG) repeat in exon 1 of the poly A binding protein 2 gene (PABP2) on chromosome 14q11. Expansion causes an increase in the number of alanine residues in PABP2 and results in the deposition of characteristic nuclear filament inclusions in skeletal muscle fibres.
Repeat expansion sizes range from 7(GCG)s to 13 (GCG)s. Most published reports describe the repeat as pure GCG. However we have analysed the nature of the repeat in over 70 patients with OPMD and have found approximately 35% to have a repeat that contains interspersions of GCA. Four different types of GCA interspersion have been identified in OPMD patients, $(GCG)_6(GCA)(GCG)_2$, $(GCG)_6(GCA)(GCG)_3$, $(GCG)_6(GCA)(GCG)_4$, $(GCG)_6(GCA)(GCG)_5$ and $(GCG)_6(GCA)_3(GCG)$. We have been unable to find interspersions in 80 unaffected control samples with the normal 6(GCG) repeat allele.

P671. Proximal D4Z4 deletions in facioscapulohumeral muscular dystrophy (FSHD): clinical phenotype, size and detection

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Facioscapulohumeral muscular dystrophy (FSHD) locus at 4q35 is closely linked to D4F104S1 (p13E-11), a probe that recognizes the pathognomonic FSHD deletion involving the subtelomeric D4Z4 tandem repeat array. Extended deletions that include both the more proximal D4F104S1 region and the D4Z4 repeat are difficult to interpret in the diagnostic setting. A novel method has been developed to devise a means to determine the true frequency of proximally extended deletions in FSHD individuals. Three families selected for this study were originally identified during routine analysis on the basis that the affected individuals in each family had failed to exhibit a small (<38kb) EcoRI fragment. High molecular weight DNA from these families was analysed with both conventional and pulsed-field gel electrophoresis using DNA markers p13E-11, 9B6A, B31, 4qA and 4qB. Large genomic deletions were identified involving both D4Z4 and D4F104S1. The precise number of D4Z4 repeat units borne by the p13E11 deletion allele was established by the use of an additional restriction enzyme (MseI) digest. All three cases carry different sizes of deletion proximal to the D4Z4 repeat units. Using telomeric probe 4qA, a method was developed which identifies large genomic deletions involving both D4Z4 and D4F104S1 using conventional gel electrophoresis. Proximally extended deletions can be found in patients with a normal spectrum of the disease, thus ruling out the possibility of a contiguous gene syndrome. This assay promises to allow estimation of the true frequency of proximally-extended deletions and should improve the accuracy and reliability of molecular diagnostic testing for FSHD.

P672. Mutation analysis of Dp71 in Duchenne and Becker muscular dystrophies.

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The dystrophin gene has been shown to encode 19 transcripts from 8 different promoters over a total of 2.4Mb at Xp21.1. Patients with mutations in the muscle transcript (Dp427m) present with Duchenne or Becker muscular dystrophy. This full length 14Kb mRNA codes for a structural protein of 3686 amino acids over 79 exons. The protein has distinct functional regions and is crucial in maintaining the link between actin and the extracellular matrix. The other dystrophin transcripts have differing size, functional properties and expression. One of these: Dp71 (comprising the 17 C-terminal exons of Dp427m) is the most abundant gene product in the brain; its function remains unknown. Studies have shown an increase of Dp71 mutation in cases of DMD/BMD with severe mental retardation - suggesting a neuropathological role.
We have developed a dHPLC protocol for screening the coding regions of Dp427m exons 63-79 for small mutations. Direct sequencing in a subset of 10 patients has validated this method.
A total of 51 patients with DMD/BMD were selected for analysis by dHPLC. These patients had a diagnosis with a variety of clinical presentations and had tested negative for gross mutation on a PCR multiplex screen. In total 7 mutations were found in the coding sequence, plus one possible splice site mutation; no further gross mutation was detected. The predicted molecular effects and clinical associations of these mutations with severe mental retardation and its relation to an increase in transmission are discussed.

P673. Short D4Z4 repeat arrays on 4qB chromosomes do not cause FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominantly inherited myopathy mainly characterized by progressive weakness and wasting of the facial, shoulder girdle and upper arm muscles. FSHD is caused by contraction of the polymorphic D4Z4 repeat array in the subtelomere of chromosome 4q. Recently, two allelic variations were identified, 4qA and 4qB, that differ by few insertions and deletions distal to D4Z4. Both variations are almost equally common in the population, but FSHD alleles are exclusively of the 4qA type. Since both variations are equally susceptible to rearrangements in the control population, the exclusive linkage of FSHD with 4qA chromosome ends may best be explained by a functional difference between both chromosome ends. However, the studies in anonymized control individuals could not distinguish between contractions and expansions and could not formally rule out that short D4Z4 repeat arrays on 4qB chromosomes are non-pathogenic.
We have now identified 2 independent FSHD kindreds in which each proband carries in addition to the familial pathogenic D4Z4 repeat array on chromosome 4qA, a FSHD-sized D4Z4 repeat array on a 4qB chromosome. Further analyses of these 4qB chromosomes reveal normal segregation in healthy carriers. These results confirm a functional difference between 4qA and 4qB chromosomes in which 4qA chromosomes carry in addition to a contraction of D4Z4 characteristics necessary for the development of FSHD. Alternatively, 4qB chromosomes carry additional elements preventing FSHD pathogenesis. Therefore, the recently proposed transcriptional repressor model for FSHD needs refinement since it does not explain this allelic variation.

P674. Genetic and Molecular Study of Myotonic Dystrophy Type 2

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Myotonic dystrophy type 2 (DM2, MIM #602668) is a dominantly inherited disorder similar to adult-onset myotonic dystrophy type 1 (DM1, MIM #160900). The DM2 mutation is an untranslated CCTG expansion in intron 1 of the *ZNF9* gene on chromosome 3q21.3. This gene encodes a highly conserved protein of 19kDa whose biological function and role in the disease pathogenesis are still unclear. As part of a multidisciplinary project, we developed a genetic test to reveal expanded *ZNF9* alleles, which combines a long-PCR and Southern blot .We analyzed 20 DM1-negative patients and identified the DM2 mutation in 12 of them, confirming the existence of at least another locus involved in the DM phenotype. To analyze the subcellular localization and the expression of the ZNF9 protein, we performed immunofluorescence (IF) experiments on human and rat skeletal muscle. In longitudinally sectioned myofibres of both species, IF reactivity for ZNF9 showed a regular transverse banding pattern throughout the fibre width. The transverse bands width were similar to sarcomeric I-bands, and showed in some instances a beaded appearance. In double IF experiments ZNF9 and the sarcoplasmic reticulum (SR) Ca/Mg ATPase (SERCA1) localized to the same transverse elements, but the two signals didn't show a superimposition in merged images. These data indicate that ZNF9 localized to I-band associated elements, other than the SR terminal cisternae. This distribution does not exactly match that observed for DMPK (product of the *DM1* gene), suggesting different functions for these proteins. Work supported by the Italian Ministry of Health

P675. Estimation of penetrance in transthyretin amyloid neuropathies

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Transthyretin amyloid neuropathies are dominant diseases, first described in northern Portugal, now recognized across the world. Depending on the geographic origin, wide phenotypic variations have been observed. Previous genealogical studies suggested that penetrance was incomplete and variable, but this parameter has never been estimated accurately. We performed a genetic study of transthyretin amyloid neuropathies in two separate European populations. A method of estimation was developed based on maximum likelihood using a survival analysis approach. Sixty two cases of French origin and 48 of Portuguese ancestry entered the study. Genealogical investigations, clinical information on symptomatic cases and results of genotyping voluntary family members were obtained in relatives of 46 French and 33 Portuguese kindreds, respectively. Up to 12 different pathogenic transthyretin variants were detected in the French, including the Val30Met, whereas the Val30Met was the only variant in the Portuguese families. The penetrance curves were found to be quite different between the two populations, with an age of onset much lower in Portuguese than in French cases (34±10.5 years vs. 58.9±12.3 years) and a penetrance by age 80 years slightly higher in Portuguese (91% vs. 86%) than in French. Surprisingly, the risks were found lower in the French Val30Met carriers than in the carriers of other mutations ($p < 0.02$). Our results provide an important input for the management of asymptomatic carriers in the Portuguese and French families.

P676. Molecular Basis of Charcot-Marie-Tooth type 1 (CMT1) disease in a cohort of Turkish patients

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Charcot-Marie-Tooth disease, also known as Hereditary Motor and Sensory Neuropathy, is a group of inherited peripheral neuropathies with a common phenotype and a prevalence of 1:2500. At least 11 genes and 24 loci responsible for the disease have so far been identified. CMT type 1 (CMT1) is the demyelinating dominant form of the disease characterized by distal weakness and atrophy of the

extremities with reduced nerve conduction velocities (<38 m/s).
In this study, we performed a molecular genetic study in 84 unrelated patients clinically diagnosed as CMT1. The patients were screened for the CMT1A duplication by Southern and STR analyses. In patients tested negative for the duplication, the *Cx32*, *MPZ*, *PMP22* and *EGR2* genes were screened for mutations by SSCP and subsequent sequencing analyses.
The CMT1A duplication was identified in 21 out of 84 (25%) patients. Eighteen of the duplications were present in familial cases whereas the other three were *de novo* mutations. Six patients had mutations in *Cx32*, four in *MPZ*, and two in *PMP22* genes. The clinical severity was consistent with the type and location of the mutations in all cases. The patients tested negative for mutations in the *EGR2* gene and twenty-five cases (30%) negative for mutations had consanguineous parents. The low frequency of the CMT1A duplication compared to that in other populations (70%) might be due to the high incidence of the demyelinating autosomal recessive form (CMT4) in our cohort of patients. Also, the possibility of an acquired neuropathy cannot be ruled out among these patients.

P677. A molecular genetic study of Charcot-Marie-Tooth disease type 2 in the Turkish population

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Charcot-Marie-Tooth type 2 (CMT2) is the axonal form of the disease and less common than CMT1 with normal or slightly reduced motor and sensory nerve conduction velocities (>38m/s). Six chromosomal loci and two genes (*KIF1Bβ*, *NF-L*) have been associated with autosomal dominant CMT2 (ADCMT2). The autosomal recessive CMT2 (ARCMT2) loci have been assigned to chromosomes 1q21.1-q21.3 (*Lamin A/C* gene), 8q21.3 and 19q13.3.
In the framework of this study, the molecular basis of CMT2 in the Turkish population was investigated. Eighteen ADCMT2 patients were screened for the presence of mutations in the *Cx32*, *MPZ*, and *NF-L* genes using SSCP and subsequent sequencing analyses. A E186K mutation was identified in the *Cx32* gene. Two mutations identified in the *NF-L* gene, S5R and G426S, are the third and fourth novel mutations reported according to our knowledge. Patients had similar phenotypes although the mutations affected different domains of the NF-L protein.
Linkage to known loci responsible for ARCMT2 was investigated in two consanguineous Turkish CMT2 families using homozygosity mapping. Haplotypes of the affected individuals revealed heterozygosity and lod-score analysis confirmed these results. Screening of the *GDAP1* gene, which was previously linked to CMT4A, revealed a 967-980del mutation in one of these families. Exclusion of linkage to all known loci in the other family provides evidence for further genetic heterogeneity in ARCMT2.
Mutation screening of the *Lamin A/C* and *GDAP1* genes in 19 ARCMT2 patients is being performed and so far a homozygous G151V mutation has been identified in the *GDAP1* gene.

P678. Autosomal recessive forms of axonal Charcot-Marie-Tooth (AR-CMT) disease in the Mediterranean basin: relative frequencies and founder effect of the mutations in the LMNA and GDAP1 genes.

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CMT is a pathological and genetic heterogeneous group of hereditary motor and sensory neuropathies. Two major types have been distinguished: demyelinating and axonal CMT. Only 3 loci account for axonal ARCMT on chromosomes 1q21, 8q13 and 19q13. Recently, mutations in the LMNA (1q21) and GDAP1 (8q13) genes have been identified.
We selected 60 consanguineous families with axonal ARCMTand perform a GenScann for 15 microsatellites markers covering the 3 loci. Sequencing of the LMNA and GDAP1 genes was performed in all the families with putative linkage to 1q21 and 8q13 loci. In families with putative linkage to 1q21, the R298X mutation in the LMNA gene was predominant (74%), highly suggesting a founder effect in North Africa. Indeed, a common haplotypes with flanking markers segregated in these families. Among the families with putative linkage to 8q13, the S194X mutation was found in all Moroccan families (8/8) in the GDAP1 gene. These results, together with the finding of common haplotypes with 8q13 markers, also highly suggest a founder effect of this mutation in Morocco. In addition, we identified a new mutation (R318G) in a compound heterozygote (S194X / R318G) of Moroccan origin.
The R298X and S194X mutations found in the LMNA and GDAP1 genes, respectively, are probably the result of founder effects in North Africans. Given their high frequency in this population, it is therefore worth searching these mutations in families with positive linkage to the corresponding regions.

P679. A genetic study of early onset demyelinating neuropathy

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Demyelinating autosomal recessive forms of CMT are classified as CMT type 4 (CMT4). The phenotype is more severe and manifests earlier than the classical CMT1 form. To date, seven loci have been shown to be associated with CMT4 and five genes have been identified.
Twenty-three early-onset CMT4 cases with mean NCV of 13.7 m/s were screened by SSCP and subsequent sequencing analyses for the mutations in *EGR2* and *PRX* genes. A novel recessive R1070X mutation in the *PRX* gene was identified in one patient. No mutations were detected in the *EGR2* gene. Two patients were further analyzed for *NDRG1* gene mutations since they were of Gypsy origin and R148X mutation was identified in one of them. The patients tested negative for *EGR2* and *PRX* mutations are still being screened for the mutations in the *MTMR2* and *GDAP1* genes.
The R1070X mutation in the *PRX* gene hypothetically creates a premature translational stop codon at 28 amino acids upstream of the acidic domain of the L-periaxin. Immunofluorescence and Western blot analyses of a sural nerve biopsy of the patient detected production of a truncated protein.
This novel mutation provides evidence for the suggested role of the acidic domain of the protein in the stabilization of the myelin sheath. Absence of the acidic domain may block interaction of the protein with downstream proteins that are required to transmit the extracellular signals to the nucleus. Our findings also illustrate genetic heterogeneity in patients with an early-onset demyelinating neuropathy.

P680. PMP22 gene duplication in a family with Dejerine-Sottas and Charcot Marie Tooth 1A polyneurophaties

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Charcot Marie Tooth 1 (CMT1) polyneuropathy is a demyelinating

peripheral neuropathy characterized by symmetrically slowed motor nerve conduction velocities (NCV, <38m/s) and onion bulb formation. CMT1 presents in the second or third decade and is associated with distal muscle weakness and atrophy and absent stretch reflexes. Different subtypes of CMT1 can be delineated by genetic analysis. CMT1A is due to several molecular defects at the *PMP22* locus. Dejerine-Sottas (DS) is a more severe early onset demyelinating neurophathy. Several genes have been implicated in the etiology of DS, including *PMP22*. Most CMT1A patients have duplication of *PMP22* while most DS patients show point mutations in *PMP22*. We used FISH and DNA sequence analysis of the PMP22 gene in a family of 4 members with CMT1A (3 males and one female) and 1 with DS. Onset of CMT1A was in the second and third decade with slow progression of symptoms. The DS patient was affected at age 3 with a more severe phenotype. CMT1A patients presented upper limb NCV of < 30 ± 2.1 (SEM) while the DS subject showed a upper limb NCV of < 15 m/s. In all cases there was duplication of the *PMP22* region and a normal sequence of the *PMP22* gene. We concluded that the presence of two copies of the *PMP22* gene gives rise to the spectrum of CMT1A and DS in this family. These data indicate the presence of epigenetic or environment factors associated with these entities.

P681. PMP22 polymorphisms in 3 unrelated females with CMT1A

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Charcot Marie Tooth (CMT) is the most frequent type of inherited neuropathy with a prevalence of 1 in 2500. Genetic studies have identified around nine loci for CMT1. *PMP22* gene defects have been associated with several polyneuropathies. The presence of gene duplications of this region results in CMT1A, Dejerine-Sottas and Roussy-Levy phenotypes. Point mutations in the *PMP22* gene have also been observed in these conditions. Most CMT1A patients show duplication of the PMP region while the rest have point mutations. These mutations include missense amino acid substitutions, deletion of a single amino acid and frameshift mutations. Most mutations occur in amino acids comprising the putative transmembrane domains. CMT1A is due to overexpression of the peripheral myelin protein-22 gene, resulting in a gain of function. The affected region of the gene is sometimes associated with the severity of the phenotype. In the present study, we analyzed the PMP22 and MPZ genes in 3 unrelated females with CMT1. We did not detect any mutation by *MPZ* gene sequence analysis. Three different polymorphisms in the *PMP22* gene were found: G45C (Val15Val), T159C (Cys53Cys) and G471C (Arg157Arg). Although these polymorphisms do not produce amino acid substitutions, the absence of mutations in the *MPZ* gene suggest that these changes could be involved in the genesis of the CMT1A in these patients.

P682. Two STR-markers analysis for 17p11.2-12 duplication screening in autosomal dominant CMT-families from Ukraine

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Charcot-Marie-Tooth neuropathy (CMT) is one of the most common hereditary disorders, affecting 10-40:100,000 individuals. CMT is a heterogeneous group of disorders characterized by chronic peripheral motor and sensory neuropathy. The mode of inheritance can be autosomal dominant, autosomal recessive, or X-linked. Autosomal dominant CMT type 1A results from duplication of a 1.5 Mb segment on the short arm of chromosome 17 (17p11.2-12), or other mutations in peripheral myelin protein-22 gene (*PMP22*). We have collected 88 DNA samples from 28 unrelated CMT families with different modes of inheritance. In this study we screened the 17p11.2-12 region for duplications in 6 unrelated families from Ukraine with autosomal dominant CMT using two (CA)n repeat markers localised in the duplicated region: *D17S921* and *D17S1358*. The different alleles of these polymorphic loci were amplified by PCR with Cy5 labelled primers and analysed using ALF-express 6% denatured short gel system. We considered a CMT1A duplication was present in a CMT patient if three distinct alleles were detected for at least one STR marker, or if clear dosage differences were seen for two different alleles. Duplication of the 17p11.2-12 region (CMT1A) was detected in patients from 3 of the 6 families with autosomal dominant CMT. These results show that CMT1A duplication analysis is important for early differential diagnosis of CMT and genetic counselling of high risk families.

P683. A new insertion of CC in exon 4 of PMP22 gene in a patient with Herehitary Neuropathy with Liability to Pressure Palsies (HNPP).

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Hereditary neuropathy with liability to pressure palsies (HNPP) is an autosomal dominantly inherited disorder characterized by recurrent painless episodes of focal neuropathy often precipitated by minor trauma. Electrophysiological studies demonstrate an underlying generalized neuropathy with moderate slowing of conduction velocities, predominant over entrapment sites and prolonged distal latencies. Sural nerve biopsies show segmental de- and remyelination with focally thickened myelin sheaths, called tomacula. HNPP is genetically homogeneous, usually caused by the functional loss of one allele of the Peripheral Myelin Protein 22 (PMP22) gene: this loss results from a 1.5 Mb deletion on chromosome 17p11.2-p12, region where the PMP22 gene is located. Also point mutations in PMP22 gene are responsible for HNPP phenotype. These mutations usually result from frame shift or nonsense mutations causing a premature translational stop codon or from abnormal splicing. Here we present a case of a man referred to our laboratory with hereditary neuropathy with liability to pressure palsies. Molecular analysis by using microsatellite markers excluded the deletion of 1.5 Mb on chromosome 17. The analysis of coding region of PMP22 gene by Denaturing High Performance Liquid Chromatography (DHPLC) revealed an abnormal profile of exon 4. The direct sequencing of exon 4 of PMP22 gene by Big Dye Terminator on ABI Prism 377 sequencer showed an insertion CC at codon 116 that produces a frame-shift with mistranslation and an early stop at codon 120. Until now no evidence of this variation has been found in our patients and in 50 normal chromosomes analyzed.

P684. Dejerine Sottas Syndrome –heterozygosity in different peripheral neuropathy genes ?

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Dejerine Sottas Syndrome (HMSNIII #145900) is a severe peripheral neuropathy of HMSN1 type with onset around 2 years. Genetic aetiology described include a homozygous 17p11.2 duplication, or dominant/recessive mutations in one of the peripheral neuropathy associated genes Myelin Protein Zero (159440), PMP22 (601097), EGR2 (129010) and periaxin (605725).

We describe a case of DSS in an Irish male who presented at the age of 3 with delayed motor milestones, mild proximal and distal weakness in both upper and lower limbs, and areflexia. NCV studies of peroneal/sural/median/ulnar nerves failed to evoke reproducible responses, sural nerve biopsy showed segmental demyelination and onion bulb formation consistent with HMSN1. Muscle biopsy showed evidence of denervation atrophy. The patient was wheelchair bound at 6 yrs and exhibited mild bilateral hearing loss at 7yrs. There is no family history of muscle disorder or neuropathy.

Molecular genetic analysis of the patient revealed a 17p11.2 duplication using both quantitative multiplex dosage and 17p11.2 microsatellite marker analysis. Mutation analysis of MPZ revealed

a novel CGC to CAC base change in codon 67 (R67H) in the extracellular domain functional in myelin compaction. This base change has not been detected in UK control samples. Mutation screening of connexin 32 and PMP22 revealed no additional abnormalities.
To our knowledge this is the first report of DSD associated with heterozygosity for mutations in two different peripheral neuropathy genes. Family studies in the patient's clinically normal mother and sister are currently underway and will be presented.

P685. Mutation of the SBF2 gene, encoding a novel member of the myotubularin family, in Charcot-Marie-Tooth neuropathy type 4B2/11p15

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Autosomal recessive Charcot-Marie-Tooth neuropathy (CMT) is a severe childhood-onset neuromuscular disorder. Autosomal recessive CMT is genetically heterogeneous with one locus mapped to chromosome 11p15 (*CMT4B2*). The histopathological hallmarks of CMT4B2 are focal outfoldings of myelin in nerve biopsies. Homozygosity mapping, in a Turkish inbred family with four children affected by CMT characterised by focally folded myelin, provided linkage to the *CMT4B2* locus. We identified a large, novel gene, named SET binding factor 2 (*SBF2*), that lies within this interval and is expressed in various tissues, including spinal cord and peripheral nerve. *SBF2* is a member of the pseudo-phosphatase family of myotubularins and was an obvious candidate for *CMT4B2* by virtue of its striking homology to myotubularin-related protein 2 (*MTMR2*), that causes another form of autosomal recessive CMT with outfoldings of the myelin sheaths in peripheral nerve biopsies. Molecular study of the *SBF2* gene in the CMT family demonstrated the presence of a homozygous inframe deletion of exons 11 and 12 in all four affected individuals. On the protein level, this mutation is predicted to disrupt an N-terminal domain that is conserved in SBF2 and its orthologues across species. Myotubularin-related proteins have been suggested to work in phosphoinositide-mediated signalling events that may also convey control of myelination. Localisation of *SBF2* within the candidate interval, cosegregation with the disease, expression in the peripheral nervous system, and resemblance of the histopathological phenotype to that related to mutation in its paralogue *MTMR2* indicate that this gene is the *CMT4B2* gene.

P686. Mutations in the GJB2 and GJB3 genes among patients with inherited nonsindromic deafness from Bashkortostan.

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Congenital deafness is a relatively common human disorder, occurring in one per 1000 newborns on average. Also GJB2 gene, GJB3 gene mutations associated with inherited hearing loss, but the patients with GJB3 mutations had progressive age at onset, raging from mild-moderate to profound hearing loss.
We have analyzed a total of 58 families/unrelated patients with recessive or sporadic deafness from Bashkortostan (Russia) and 35 (60%) of them were found to carry the 35delG mutation. Five different GJB2 gene mutations, including 3 novel mutations (254C→A, 313-314delAA, 314delA, 360delG, 235delC) and one GJB3 novel mutation (557A→T) were identified. We also analyzed 55 unrelated families, were collected from individuals who had no known hearing loss.
In order to explore the ancestry of GJB2 mutations in the patients with non-syndromic inherited deafness, we estimated linkage disequilibrium between 35delG mutation, other GJB2 gene mutations and flanking microsatellite markers D13S143, D13S292, D13S175. In patients, 35delG mutation was in significant disequilibrium with allele 13 of D13S143, which relatively uncommon among hearing controls (ΔSt_{13}= -0,6304). The chromosomes with 35delG mutation have the lowest haplotype diversity (0.6875) and the mutant chromosomes without 35delG mutation, the highest (0.9632). Haplotype 13-18-15 was observed on 10% of all mutant chromosomes without 35delG mutation, and haplotypes 13-20-17 and 13-17-16 were detected on 10% chromosomes with 35delG mutation. Haplotype analysis of the markers D13S292, D13S175 and D13S143 in the pericentromeric region of chromosome 13 helped to optimize DNA diagnostics of non-syndromic deafness in patients from Bashkortostan.

P687. The variable expression of non-syndromic hearing loss associated with GBJ2 gene mutations in an isolated population.

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EM is a village of 8,600 inhabitants founded some 250 years ago by three brothers, which has been isolated because of the preference of consanguineous marriages. Several genetic diseases are frequent in the village, in particular profound deafness, which was expected to result from founder mutation. A single locus was first found by linkage analysis (DNFB1), but three different frequent mutations were characterized in the GBJ2 gene coding for connexin 26. Screening of a random sample of 400 adults confirmed the high incidence of the three mutations in the village. A total of 17.7% of the healthy individuals screened were either carrier of 35Gdel (10.4%), W77R (3.1%) or V37I (4.2%) mutations.
While Gdel35 and W77R both lead to inactive connexin 26, V37I has been often considered as a polymorphism. We were able to confirm the clinical variability of homozygotes for 35Gdel, for W77R and of compound heterozygotes 35Gdel/W77R. All the cases had profound to moderate deafness and none had mild deafness. In fact, neither during the screening of healthy family members of the probands nor of the random sample did we find an affected individual. For the V37I mutation the variability was wider, since one of the compound heterozygotes had a profound prelingual deafness while several had mild defect, detected only during adulthood. In addition, while we did not detect any homozygote or compound heterozygote for V37I in the screening of the random sample, we did find an asmptomatic adult among the healthy family members of deaf individuals.

P688. Connexine 26 M34T variant is a frequent polymorphism in France

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Mutations in *GJB2* are the most common cause of congenital non syndromic hearing loss. More than 60 different *GJB2* mutations have been reported yet as recessive (DFNB1) and dominant (DFNA3) hearing loss alleles. The controversial allele variant M34T has been hypothesized to cause autosomal dominant or recessive nonsyndromic hearing impairement. The results of several in vitro data concerning the functionnal consequences of the M34T have shown contradictory results. We present the clinical and genotypic study of 11 families in which the M34T *GJB2* variant has been identified. The M34T mutation did segregate with the deafness in only one of the 7 familial forms of NSSNH. Eigth persons with normal audiogram presented a heterozygous M34T variation and five normal hearing patients were composite heterozygous for M34T and an other *GJB2* mutation. Screening a french control population of 116 subjects, we have found a frequence of the M34T allele in the french population of 1,72%. This percentage was not significatively different from from the frequence of M34T variant in the deaf population which

is 2,12%. All these data suggest that the M34T variant has no clinical effect in human and is a frequent polymorphism in France. These results have important implications for genetic counseling.

P689. The expression pattern of the USH1C protein in the mouse eye suggests differences in Usher type 1 multi protein complex formation between the ear and the eye.

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Usher syndrome is an autosomal recessive disease with pathology of the ear and eye. Three clinical subtypes exist that are linked to 11 different loci, and it has been shown in the ear that at least in type 1, the proteins interact to form a functional complex. An indirect immunofluorescence system has been used to examine the localisation of USH1C in the murine eye, identifying the protein as a distinct layer within the photoreceptor outer segments. Expression studies carried out in *waltzer* and *shaker-1* null mutant mice, (models for USH1D and USH1B respectively), indicate that there is no alteration of the expression pattern of the USH1C protein in the absence of myosin VIIA (USH1B) and cadherin 23 (USH1D) in the eye. This suggests that, type 1 proteins may not physically interact in the visual system, although USH1C may in fact be important in the anchorage of other type 1 proteins, as has been suggested in the cochlea. The onset of expression of Rhodopsin and other photoreceptor transcripts is known to coincide with outer segment disk production at postnatal day 6. This study has used an RT-PCR method to show USH1C protein expression prior to this point, suggesting that USH1C may have an alternative role in the eye, possibly a role in organisation of signalling complexes.

P690. Detection of some mutations in Iranian deaf families

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Hearing impairment is the most prevalent inherited sensory disorder, affecting about 1 in 1000 children. Half of all cases has a genetic origin. Approximately 70-80% of genetic hearing loss are autosomal recessive, 15-20% are autosomal dominant and 2-3% are X-linked or mitochondrial. Among hereditary examples, 30% are syndromal, associated with other anomalies.
In this research, 1000 Iranian deaf families were studied. According to their pedigree, 35% of those had a genetic etiology. 50 families of autosomal recessive nonsyndromic hearing loss group were selected for studying 35delG and 167delT mutations of connexin26 gene (DFNB1) by PCR-RFLP method. 6% of these families had 35delG mutation but none of them had 167delT mutation.
Among 346 families with genetic hearing loss, 15 families suspect to mitochondrial hearing loss were studied for A3243G mutation in tRNA(leu) gene, A7445G, 7472 insC , T7510C and T7511C mutations in tRNA(ser) gene and A1555G ototoxic mutation in the 12SrRNA gene, by PCR-RFLP and PCR-SSCP methods. No mutation was detected in our patients.
According to the results of this study, a majority of hearing loss in this population is caused by environmental factors similar to other developing countries.
The frequency of the 35delG mutation is lower than the report of European and American populations (70%). But rare prevalence of mitochondrial mutations that cause hearing loss is similar to American and European populations.

P691. Frequencies of mitochondrial DNA mutations among the Turkish people with prelingual nonsyndromic hearing Loss

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Recent studies have shown that the mtDNA delT961Cn, A1555G, A7445G, T7510C and T7511C mutations are associated with nonsyndromic hearing loss. To determine the frequencies of these mutations in our population, we have screened 210 students with prelingual onset nonsyndromic sensorineural hearing loss from five schools for the deaf located in different parts of Turkey. The subjects were included in the study after the connexin 26 gene was studied and found to be negative for mutations. The mitochondrial mutations were screened using PCR/RFLP-based methods and the presence of delT961Cn was confirmed with direct DNA squencing. The results revealed three probands with the A1555G mutation (1.43%), all of whom presumably had been exposed to aminoglycosides during infancy. Another 12S rRNA mutation, delT961Cn, was found only in one proband (0.47%), suprisingly in a consanguineous family with autosomal recessive inheritance, but without a history of aminoglycoside exposure. The T961G polymorphism was also detected in two probands (0.94%). The A7445G (as well as substitutions at positions 7443 and 7444), T7510C, and T7511C mutations were not detected in any of the samples in our study group. These results show that A1555G is the most common mitochondrial mutation in the Turkish population causing prelingual nonsyndromic hearing loss. The importance of the delT961Cn change as a deafness-associated mutation has to be evaluated further, especially when a history of aminoglycoside exposure is absent.
This study was supported by the Ankara University Research Projects (2001-08-09-076) and the Turkish Academy of Sciences to M.T. (MT/TUBA-GEBIP-2001-2-19).

P692. A novel M163L mutation in GJB2 gene associated with autosomal dominant isolated hearing loss

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Mutations in the GJB2 gene acount for up 50% of cases of autosomal recessive hearing loss. However, a few mutations have been reported related with autosomal dominant forms of hearing loss.
In the present work, we report a novel heterozygous A→C mutation at nucleotide 487 resulting in a methionine to leucine substitution at codon 163 (M163L). This mutation was found in a branch of a large family exhibiting intrafamilial variability for the onset and severity of hearing loss. All the affected individuals of the three generations present bilateral, moderate to severe hearing impairment and some of them are heterozygous for 35delG.
The new mutation M163L was detected by SSCP analysis of the GJB2 coding sequence and subsequently identified by direct sequencing in both directions. The remaining coding sequence showed no SSCP anomaly. M163L mutation affects an aminoacid which is highly conserved among β connexins of mammals and lies in the EC2 domain of Cx26, which is involved in interactions between connexons of adjacent cells. Restriction endonuclease digestion with *Pst*I did not revealed any carrier for M163L in a control sample of 100 unaffected unrelated individuals.
These data strongly suggest that M163L mutation might be a new GJB2 mutation implicated in non-syndromic autosomal dominant hearing loss.

P693. Mutations of connexin 26 in Iranian population with autosomal recessive sensorineural hearing loss (SNHL)

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Hereditary hearing loss is a very common disorder that affects one out of 1000-2000 living newborns. 70% of these hearing disorders is in the form of Non-Syndromic deafness, while 85% of these cases have an Autosomal Recessive pattern of inheritance. Estimations show that more than 100 loci are involved in this defect. In populations studied so far, mutations of Connexinn 26 (GJB2) is reported as the most prevalent. These mutations cause Autosomal Recessive Non-Syndromic Hearing Loss (ARNSHL). We studied the frequency of these mutations in Iranian Population. Five-hundred and ninety chromosomes (295 patients) were studied for 35delG mutation in GJB2 gene, where only 12% showed this mutation. 35% of patients

had been studied by SSCP and 8 of them showed these shifts in their DNA sequencing results; R127H, W24X, V52V, V27I, E114G, V153I, A171T and R184P. Recently 5% of remaining patients are studied for new mutations and only Slice Site Mutation -3170G>A is detected. Comparing these all it shows that other loci might be the probable major contributors to ARNSHL in Iranian Population.

P694. Mutations of connexin 26 in Iranian population with autosomal recessive sensorineural hearing loss (SNHL)

M. Mohseni[1], *M. Malekpour*[1], *s. arzhangi*[1], *R. A. Cucci*[2], *A. Ebrahimi*[1], *A. Daneshi*[3], *M. Farhadi*[3], *K. Khahrizi*[1], *R. J. H. Smith*[2], *H. Najmabadi*[1];
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Hereditary hearing loss is a very common disorder that affects one out of 1000-2000 living newborns. 70% of these hearing disorders is in the form of Non-Syndromic deafness, while 85% of these cases have an Autosomal Recessive pattern of inheritance. Estimations show that more than 100 loci are involved in this defect. In populations studied so far, mutations of Connexinn 26 (GJB2) is reported as the most prevalent. These mutations cause Autosomal Recessive Non-Syndromic Hearing Loss (ARNSHL). We studied the frequency of these mutations in Iranian Population. Five-hundred and ninety chromosomes (295 patients) were studied for 35delG mutation in GJB2 gene, where only 12% showed this mutation. 35% of patients had been studied by SSCP and 8 of them showed these shifts in their DNA sequencing results; R127H, W24X, V52V, V27I, E114G, V153I, A171T and R184P. Recently 5% of remaining patients are studied for new mutations and only Slice Site Mutation -3170G>A is detected. Comparing these all it shows that other loci might be the probable major contributors to ARNSHL in Iranian Population.

P695. Mutations in GJB2 gene in patients with non-syndromic hearing loss in Poland

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Hearing loss is the most frequent disease of human senses. At least 60% of all cases are attributed to genetic factors in developed countries. Non-syndromic hearing loss accounts for about 70% of all cases and in this group most frequent is autosomal recessive mode of inheritance. Until now several genes causing hearing loss have been discovered and the most important one is GJB2 gene coding connexin 26. Connexin 26 is a component of gap junctions in the organ of Corti.
Having regard to the frequency of the mutation obtained in pilot study (data not published), a new method was elaborated permitting to display the three most common mutations in the coding region of GJB2 at the same time. This method consists in simultaneous amplification of the regions 12-72 and 306-464 of the coding region of GJB2 with the use of labelled primers. Products thus obtained are next subject to size analysis with the application of ABI 377. 1629 patients with hearing loss were examined with this new method. 327 patients were homozygotes for the 35delG mutation and 189– heteorozygotes for this mutation. 314del14 mutation was found in 25 compound heterozygotes for 314del14 and 35delG, and in one case – a homozygote for 314del14. Therefore 314del14 mutation is second most frequent mutation in the coding region of GJB2 in patients with hearing loss in Poland.

P696. Search and exclusion of candidate genes within the DFNB32 locus

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We have previously identified the DFNB32 locus in a large consanguineous family with congenital profound autosomal recessive non-syndromic deafness. This locus is mapped to chromosome 1p13.3-22.1 and contains the *COL11A1* gene responsible for 2 syndromic deafness : Marshall and Stickler syndromes. The entire coding region of this gene was screened and no mutation was observed (Masmoudi et al. in press 2003). Moreover, The DFNB32 critical region overlap with DFNA37 locus. A search of the deaf mouse mutants, mapped to the orthologous region of chromosome 1p13.3-22.1, revealed 3 mutations (Chondrodysplasia: "*cho*", Osteopetrosis colony stimulating factor 1 KO : "*op*" and varitint-waddler: "*va*") producing hearing impairment and affecting inner ear. In September 2002, Kim et al have localized the human ortholog of *va* between markers D1S3449 and D1S2252, which map approximately 8 Mb telomeric to the DFNB32 locus. The responsible genes for *cho* and *op* mutations are respectively *Col11a1* and *Csf1*. The *CSF1* gene was considered as a candidate gene. Using the Human Cochlear cDNA and EST database, we have identified 10 genes in the DFNB32 region (in order from centromere to telomere) : *COL11A1, GPR88*, *SLC35A3*, *SNX7, PTBP2*, *CNN3, ABCD3, GCLM, CGI-100*, and *RPL5*. Mutation screening was performed on the *SLC35A3* and *PTBP2* genes but no mutation was observed. We are investigating the rest of candidate genes in the DFNB32 candidate region in order to identify the causative gene.

P697. Refined localization of autosomal recessive non-syndromic deafness DFNB13 locus using 17 novel microsatellite markers and exclusion of 8 known genes in the region

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We have identified 2 large consanguineous Tunisian families with congenital profound autosomal recessive non-syndromic deafness. These families are originated from the same region. In order to map the responsible gene(s), we have performed linkage analysis with microsatellite markers corresponding to the previously identified loci. Linkage was found with markers surrounding the DFNB13 locus in both families. DFNB13 is an autosomal recessive non-syndromic deafness locus. It was previously mapped to a 17 cM interval of chromosome 7q34-36 between D7S2468 and D7S2439 markers. In order to refine the genetic interval, we have analyzed a total of 34 polymorphic markers in 7q34-36, comprising 17 published and 17 new markers, on 11 affected and 15 unaffected individuals. Our results lead us to refine the DFNB13 locus from 17 cM to an area of 2Mb. All affected individuals from both families shared the same haplotype suggesting that a single founder mutation is responsible for the deafness in these families. A search of the Human Cochlear cDNA Library and EST Database have revealed 2 known genes within the 7q34 area. The region syntenic to the DFNB13 interval is located on the murine chromosome 6. No deaf mouse mutant has been reported in this region. In total, eight known genes in this region have been screened and eliminated as candidates for DFNB13. Other known and predicted genes found in the DFNB13 interval are being screened for deafness-causing mutations.

P698. Mapping of a new autosomal dominant non-syndromic hearing loss locus (DFNA43) to chromosome 2p12

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Hearing loss is a common form of sensory impairment, affecting millions of individuals worldwide. Deafness can be due to genetic

or environmental causes or a combination of them. Approximately 1 out of 1000 infants is affected by either severe or profound deafness at birth and during early childhood, i.e. in the prelingual period. About 60% of these cases are considered to have genetic bases, with deafness mostly occurring as a non-syndromic defect. Non-syndromic hearing loss is characterised by extensive genetic heterogeneity. More than 70 loci have been mapped and 29 genes identified so far. We have performed genetic linkage studies in a three-generation Italian family segregating an autosomal dominant form of non-syndromic hearing impairment (NSHI). Onset of hearing impairment (HI) in all affected subjects occurred in the second decade of life with subsequent gradual progression from moderate to profound loss. HI was bilateral and symmetrical, involving all frequencies. After excluding known DFNA loci using markers, listed on the Hereditary Hearing Loss Homepage (URL: http://dnalab-www.uia.ac.be/dnalab/hhh), a genome wide scan was conducted with 358 highly informative microsatellite markers using the ABI PRISM™ Linkage Mapping Set (PE Applied Biosistem,USA). Significant linkage (Zmax= 4.21; q=0) was obtained with chromosome 2p12 markers. The results were confirmed by multipoint analysis (Zmax=4.51), using the location score method. Haplotype analysis defined a 9.6 cM disease-gene interval on chromosome 2 without overlap with the other identified loci. Fine mapping and identification of candidate genes are in progress.
Work supported by Italian Ministry of Health

P699. No evidence for association of COL1A1 and COL1A2 with otosclerosis in Spain patients

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Otosclerosis is one of the most common causes of hearing loss in white adults. The COL1A1 and COL1A2 genes coding for the proα(I) chain of the collagen type I have been proposed as candidate genes involved in the development of otosclerosis. In this connection, the COL1A1 gene was recently reported to be associated with otosclerosis based on a population-based case-control study. We report here an independent study of association between polymorphisms in COL1A1 and COL1A2 genes and otosclerosis, in a case-control sample derived from a population of caucasian individuals living in the Northwest of Spain. Specifically, we have tested two polymorphisms in COL1A1 which were previously shown to be associated with otosclerosis, and six polymorphisms in the COL1A2 gene. We performed multiple association analyses based on allele, genotype and haplotypes frequencies. Overall, we observed no indication that COL1A1 and COL1A2 are significant susceptibility genes for otosclerosis. Failure to replicate previous findings call for further solid evidence supporting that genes coding for the proα(I) chain of the collagen type I are actually involved in susceptibility to otosclerosis.

P700. Molecular detection of novel WFS1 mutations in 19 Italian patients with Wolfram syndrome by a DHPLC-based assay

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Wolfram syndrome (WS) is a recessively inherited mendelian form of diabetes and neurodegeneration also known by the acronym DIDMOAD from the major clinical features, including Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy and Deafness. Affected individuals may also show renal tract abnormalities as well as multiple neurological and psychiatric symptoms. The causative gene for WS (WFS1) maps to chromosome 4p16.1 and consists of eight exons, spanning 33.44 Kb of genomic DNA. In this study we report on the mutational analysis of the WFS1 coding region in 19 Italian WS patients and 25 relatives, using a DHPLC-based protocol. The entire WFS1 coding region was amplified in 14 fragments of 220-400 bp and analyzed by DHPLC, using the WAVE Maker Software version 4.1.40. Each anomalous elution peak was then subjected to direct sequencing.A total of nineteen different mutations in WFS1 were found in 18 of 19 patients (95%). All these mutations, except one, are novel, preferentially located in WFS1 exon 8 and include deletions, insertions, duplications, nonsense and missense changes. In particular, a sixteen base-pair deletion in WFS1 codon 454 was detected in 5 different unrelated nuclear families, being the most prevalent alteration in this Italian group. Nine neutral changes and polymorphisms were also identified. Overall, this study represents the molecular characterization of the largest cohort of Italian WS patients studied so far, increases the mutational spectrum of WFS1 allelic variants worldwide and provides an efficient, cost-effective and reliable detection protocol for mutational screening of WS patients and unaffected relatives.

P701. Detection of 35delG mutation Frequency in Deaf families in Hamedan province of Iran using ARMS-PCR

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Mutations in the GJB2 gene at the DFNB1 locus on chromosome 13q12 are associated with Non syndromic hearing loss (NSHL) in many populations. A single mutation, at position 35 (35delG), accounts for approximately 30-63% of mutations in white populations with a carrier frequency of 1.5-2.5% in the most European, North American and Mediterranian populations. In this study we have investigated the prevalence of the GJB2 gene mutations using ARMS-PCR and direct sequencing in the Hamadanian population.
Method: ARMS-PCR test was used for 35delG mutation screening.
Results: We studied 35 children from 35 families with NSHL who did not have a well-defined etiology for their hearing loss. Of 35 probands studied, 23 (65.71%) had the 35delG mutation in their Cx26 gene; 5 (14.28%) were homozygous, 18 were heterozygous (51.43%) and 12 (34.29%) were normal for 35delG. Because of the high incidence of heterozygotes (51.43%), it seems that frequency of 35delG is higher than other populations previously studied in Iran.
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P702. Pendred syndrome: Clinical and molecular caracteristics

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Pendred Syndrome (PS) is an autosomal recessive disease characterized by congenital sensorineural hearing loss combined with goitre. The hearing loss is associated with temporal bone abnormalities that range from dilated vestibular aqueduct (DVA) to Mondini dysplasia. The thyroid dysfunction due to iodine deficient cellular transport can be revealed by the perchlorate test. Variable phenotypes have been recognized in PS patients even in the same

family and inconstant results have been obtained with the perchlorate test. Recently mutations in the *PDS* gene (*SLC26A4*) have been observed in PS and in non syndromic deafness patients (DFNB4). In order to improve PS diagnosis, we develop the molecular analysis of *PDS*. We studied 31 patients from 24 PS families. Patients were 17 children and 14 adults. Hearing loss, temporal bone and thyroid status were recorded in patients. The entire *PDS* coding sequence was studied by a DGGE/sequencing method. We identified mutations in 20/24 PS families. Two mutated alleles were present in 15 families. The most common mutations were Y530H, G209V, T416P, L445W, 1614+1G/A, V138F, 1001+1G/A, and L597S. Four novel mutations were observed : S133X, S137P, S552I and 2089+1G/A. S552I was present in a multiplex family and segregate with the disease. In the four families without a *PDS* mutation, neither mutations in the non coding-sequences of *PDS* nor the involvement of another gene can be excluded. This study shows that the existence of common mutations in *PDS* will be helpful for the *PDS* screening in PS and non syndromic deafness patients.

P703. A genotype-phenotype correlation for hearing impairment caused by TECTA mutations

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Alpha-tectorin is a noncollagenous component of the tectorial membrane playing an essential role in auditory transduction. In several DFNA12 families mutations in *TECTA*, the gene encoding alpha-tectorin, were shown to cause hearing impairment (HI) with different phenotypes depending on the location of the DNA change. Here we report a Turkish family displaying inherited autosomal dominant HI. Linkage analysis revealed significant cosegregation of the disease with markers on chromosome 11q23.3- q24. This region contains the *TECTA* gene, which was subsequently sequenced. A nucleotide change in exon 13, 4526T>C, was detected leading to a substitution from cysteine to glycine at codon 1509 of the TECTA protein. This cysteine is located in the vWFD4 domain, which is thought to be involved in disulfide bonds and protein-protein interactions.

It is notable that the phenotype in this family correlates with other families that also display mutations within the vWFD domains. In all families these mutations result in HI involving high frequencies (2000-8000 Hz). In contrast, mutations which do not affect the vWFD domains seem to provoke mid frequency sensorineural HI (500-2000 Hz).

The identification of an additional family displaying a mutation in the vWFD domain of alpha tectorin underlines the phenotype-genotype correlation based on different mutations in *TECTA*.

P704. Spectrum of GJB2 mutations amongTurkish individuals with prelingual onset sensorineural hearing loss

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Considerable differences exist for the spectrum of *GJB2* mutations in different populations. Screening for the 35delG mutation in 256 independent probands (154 familial and 102 sporadic) with severe to profound, prelingual-onset, sensorineural nonsyndromic hearing loss coming from different regions of Turkey revealed 37 (14.5%) homozygotes. The overal allele frequency of 35delG was 18.5% (22.1% in familial and 13.2% in sporadic cases), ranging from 5% to 53% in different cities. Parental consanguinity was noted in 34% of 35delG homozygotes, yet it was 55% in 35delG negatives (p=0.034). Screening of *GJB2* for other changes in familial cases revealed three previously reported mutations, including E120del (2 alleles; 0.6%), 167delT (1 allele; 0.3%), L90P (detected in the father of a proband who was homozygous for 35delG), and a novel complex mutation 236_239delTGCAinsAGATCCG (1 allele; 0.3%), as well as two previously reported polymorphisms: V27I (5 alleles; 1.6%) and E114G (1 allele; 0.3%). The E114G polymorphism was detected in a proband who also was heterozygous for V27I. Assortative mating was a significant factor predicting detection of biallelic mutations in the *GJB2* gene. These results confirm the overwhelming majority of 35delG in the Turkish individuals with prelingual onset sensorineural hearing loss as well as the presence of other changes detected in Caucasian and Asian populations.

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P705. Most frequent GJB2 mutations among Slovak Caucasian and Slovak Gypsy patients with non-syndromic hearing loss (NSHL)

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Mutations in the connexin 26 (*GJB2*) gene 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 50 % of genetic non-syndromic childhood deafness. In populations of non-European ethnic background other *GJB2* gene mutations are occasionally common, e.g. 167delT in Ashkenazi Jews, R143W in Africans, 235delC in Koreans.

DNA samples of 55 and 54 unrelated NSHL patients from the Slovak Caucasian and Slovak Gypsy populations were screened for the most common *GJB2* mutation, 35delG. The coding region of the *GJB2* gene of heterozygous patients was sequenced, and so far mutations W24X, R127H and 333-334delAA have been identified.

In the Slovak Caucasian population 35delG accounts for 50.0% of the cases, 333-334delAA for 0.91% and W24X mutation was not present, which is consistent with most European countries. However, in the Slovak Gypsy population the most common mutation seems to be W24X, as it accounts for 22.22% of the cases, while 35delG and R127H account for only 7.40% and 0.92%, respectively. Because W24X has been found in India and Pakistan we assume that this mutation may also be present in other Gypsy populations.

The carrier frequency of 35delG was estimated as 3.28 % for the Slovak Caucasian population and 0.83% for the Slovak Gypsy population. The carrier frequency of W24X varied in different Slovak Gypsy subpopulations from 0% up to 26.09%.

P706. Connexin-26 (GJB2) missense mutation in Keratitis-Ichthyosis-Deafness (KID) syndrome

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KID syndrome is an autosomal dominant ectodermal dysplasia characterized by vascularizing keratitis, erythrokeratodermia and prelingual sensorineural hearing loss. Other features include progressive visual loss due to cornal epithelial defects, hair, teeth and nail dystrophy and susceptibility to mucocutaneous infections. Squamous cell carcinoma is a rare complication. Fewer than 100 cases of KID have been reported.

We present a 24-year-old female patient with classical KID syndrome, diagnosed at 4 years of age. She was born at term after an uneventful pregnancy, of nonconsanguineous parents. At birth, she had an erythroderma that disappeared within a few days; large hyperkeratotic plaques progressively developed on arms and legs. She had diffuse palmoplantar hyperkeratosis with a characteristic stippled surface and severe pachyonychia. Profound sensorineural deafness was diagnosed at 8 months. From birth, the patient has had ophthalmological problems including photophobia and repeated episodes of infectious keratoconjunctivitis leading to bilateral corneal

opacities and severe visual loss. Since the age of 20, she has presented recurrent scalp folliculitis with chronic suppurative cystic lesions and areas of scarring alopecia. No squamous cell carcinoma has been observed.
We looked for connexin gene mutations in this patient and identified a heterozygous missense mutation in the connexin 26 (GJB2) gene, S17F (50C>T). The mutation was not present in the unaffected parents. This mutation has been described in a further patient by Richard et al. (Am J Hum Genet 2002 70: 1341-8), and shown to be unable to form functional gap junctions.

P707. Neurosensory Non Syndromic Deafness: Analysis Of Connexins 26 And 30 In Italian Population.

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GJB2 is the gene most often involved in non-syndromic recessive deafness (NSRD) and encodes the gap-junction protein connexin 26 (Cx26).
More than 60 different mutations are described (http://www.iro.es/cx26deaf.html), but one is particularly common in our population, the 35delG that accounts for up to 60% of mutated *GJB2* alleles. Almost all mutations are located in the coding region of the gene (exon 2), with the exception of a splice site mutation located at the end of the non-coding exon 1.
We analysed 270 NSRD patients and identified mutations in 181/540 chromosomes; 23% (124/540) showed 35delG, while the remainder showed different mutations: 3170 G>A, 31del14, G12V, 35insG, W24X, M34T, V37I, A40G, E47X, W77R, L90P, V95M, H100L, 167delT, 290insA, 310del14, delE120, W133X, E147K, C174R, D179N, and R184W. We also found three allele variants: V27I, V153I and G160S. 35delG was present in about 68% of all Cx26 mutations identified. Five out of these mutations were novel: H100L, A40G, W133X and C174R were recessive while the D179N mutation showed a dominant segregation. Since other connexin genes have been involved in nonsyndromic deafness, we investigated the *GJB6* gene, encoding Cx30, that is tightly linked to *GJB2* at 13q12. A recent report has identified a large deletion, including exon 1 of *GJB6*, del(GJB6-D13S1830), that is the cause of deafness in patients carrying one recessive mutation in the *GJB2* gene in *trans*. We have found two compound heterozygotes both carrying del(GJB6-D13S1830) in association with the 35delG and the 167delT *GJB2* mutations, respectively.

P708. A novel mtDNA point mutation in the tRNAVal is associated with Melas

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We report the case of a 33 year old female who came to clinical attention for recent onset dyspnea, peripheral edema and weight loss and who was diagnosed at entry with hypertrophic cardiomyopathy and pericardial effusion. The patient's symptoms suggested a mitochondrial disease, most likely MELAS syndrome.
Family members: mother, father and one brother, underwent echocardiographic screening. A mild hypertrophy was only found in the mother. The mtDNA of the patient was fully sequenced and showed (besides A2706G, T14766C, T1W6086C non pathological mutations) a novel G1644A transition affecting the tRNAVal . This mutation seems to be pathological because: a) the G at 1644 position is a conserved base which is also maintained in those tRNAVal where the base sequence of the region greatly varies; b) the G ý A mutation at 1644 np of the tRNAVal is located in the unique tRNAVal , two bases 5' apart from G1642A transition, described as causative of MELAS syndrome1; c) the mutation, as indicated both by sequencing and agarose gel electrophoresis of the mismatched amplified mtDNA fragment digested with MbOI, is heteroplasmic.
Finally very few pathological mutations have been described up to now (http://www.mitomap.org) in the tRNAVal and no changes have been observed at position 1644 in our series of about 170 patients and 150 controls.

1. de Coo, I.F., Sistermans, E.A., de Wijs, I.J et al. (1998) « Neurology 50 (1): 293-295.
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P709. When is prenatal diagnosis useful in mtDNA disease?

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Heteroplasmy impedes prenatal diagnosis of mtDNA disease. In patients with mutations at bp 8993 (but not in the commonest mtDNA diseases), mutant mtDNA is 1) correlated with disease severity 2) uniformally distributed in all tissues 3) constant over time.
Studies on control placentae
(i) 3 mtDNA polymorphisms were uniformly distributed among 12-16 samples from 2 placentae.
(ii) There is a close correlation between the load of heteroplasmic variants between mother and placenta (R^2 = 0.94, n=19)
(iii) Paternal mtDNA contributes <0.001%
These data are consistent with animal studies and suggest that CVS should reflect the load of mutant mtDNA.
MtDNA analysis on CVS
Two women each lost a child with mtDNA disease
Case 1. Pearsons syndrome due to a mtDNA rearrangement, mother had a CVS for maternal age.
Case 2. Leigh's disease due to a mtDNA mutation at bp T9176C. We gave a 5% recurrence risk as mutant mtDNA was 0% in mother's blood but detected in 2/20 oocytes.
Results: No mutant mtDNA was detectable in either CVS and both pregnancies were normal.
Discussion. The level of heteroplasmic mtDNA variants is usually similar in mother and placenta. Paternal mtDNA is not commonly detectable in placenta.
CVS was the option favoured by 2 women in whom the recurrence risk approached the risk of the procedure (~1%). CVS may be useful in low risk pregnancies in which the likelihood of obtaining intermediate levels of mutant mtDNA is low, facilitated by PGD and/or preconception oocyte sampling.

P710. Investigation of A3243G mutation and 5 kb mtDNA deletions in Iranian diabetes mellitus patients(type 2)

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The A3243G mutation in the mitochondrial tRNA Leu has been reported in patients with diabetes type II. A 5 kb deletion has also been reported in these patients' mtDNA. Since there is no study about this topic in Iran, we assessed the frequency of the A3243G and 5kb mtDNA deletion in Iranian diabetes mellitus type II patients. DNA was extract from blood of 50 diabetic type II patients. PCR-RFLP and SSCP were used to detect the 3243 or other mutations in the mitochondrial tRNALeu gene. Standard and Multiplex PCR were used to check for the 5 kb deletion in the patients' mtDNA. We could not identify any deletions or 3243 point mutation in our cases, but 2 new patterns of PCR fragments were identified by SSCP, which may be new point mutations in the patient's mitochondrial tRNA Leu. Sequencing is needed to identify the exact position of the mutation and confirm our results.

P711. HVRI and HVR II mtDNA sequence polymorphism - a population genetic study of Romanian population

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The aim of this study is to detect sequence variation of hypervariable segments of the mtDNA control region in the Romanian population. The aim of this study is to determine the relationships between the Romanian population and other European populations and to establish the origins of Romanians. EDTA blood samples were obtained from 100 unrelated donors from all historical regions of

Romania and DNA was extracted using different techniques and protocols in order to determine the most suitable one for PCR amplification and sequencing. Two pairs of primers were designed in order to cover the first and the second VHR regions respectively. PCR reactions were carried out in a Perkin Elmer 2400 thermocycler and the PCR products were first purificated with Invisorb Spin PCRapid kit and then sequenced. 90 sequences, including the authors ones were compared, aligned and manually checked. We find that the Romanian population contains a moderate number of closely related mtDNA lineages. A comparison with data already published indicated that there are no important differences between Romanian and other European populations.
Our results are interpreted against the background of historical events, which have played an important role in changing the genetic structure of the Romanian population.

P712. Study of association of J,K,and M mtDNA haplogroups in Iranian LHON and MS patients

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LHON is a mitochondrial disorder that is exclusively maternally inherited, while multiple sclerosis is a complex disease that develops due to interaction between genotypes and environmental factors. Several reports imply an association between mitochondrial haplogroup J and some subsets of MS and LHON. We studied 38 MS patients and 110 normal healthy individuals for haplogroups J and K. Our results indicated that 9 out of 38 (24%) MS patients and 3/110 (2.7%) of the control group had haplogroup J, and 7/38 (18%) in the MS group and 5/110 (4.5%) in the control group were positive for haplogroup K. We also studied 13 LHON patients and 110 normal controls for haplogroups J and M. In contrast with other reports we have not seen any association between MTND4*LHON11778 mutation and haplogroup J. In this study none of LHON patients were in J group compared to 2.7% of normal controls, and no patients were in haplogroup M compared to 1% of normal controls. Interestingly, the frequency of this Asian marker among Iranian people was not significant.

P713. A novel single large-scale mtDNA deletion associated with congenital cataract

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Maturity onset cataract has already been presented in the literature in association with mitochondrial disease: retinitis pigmentosa, non-congenital cataracts, and glaucoma was found in a patient with disrupted anticodon loop of the tRNALeu(UUR). Congenital cataracts as element of possible mitochondrial diseases have also been presented as clinical descriptions, however, the underlying mtDNA abnormalities have not been determined. The patient reported here was born with appropriate anthropometric parameters and his only clinical symptom was bilateral congenital cataracts with strabism at birth, both lenses were removed surgically at the age of eight months. After an uneventful perinatal and infantile period the characteristic progressive hallmarks of mitochondrial disease developed at the age of 6 years. These included neuropathologic features of Leigh disease, ataxia, stroke-like hemiparesis, ophthalmoplegia and ptosis. Analysis of the mtDNA of the patient revealed a heteroplasmic single 6.7 Kb large-scale deletion harboring between 7,817 and 14,536 bp location, affecting nine protein coding regions and six tRNA genes, which has not been reported elsewhere. This case represents the first report of a verified mtDNA mutation associated with congenital cataracts as first clinical sign of a later developing progressive neuromuscular disease presented with combination of Leigh neuropathology, ragged-red fibres histopathology and stroke-like attack.

P714. Leigh syndrome with cytochrome c oxidase deficiency (LDCOX-) as a result of the SURF1 mutations. Genotype-phenotype correlation in Polish patients.

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COX deficient Leigh syndrome, inherited as an autosomal recessive trait, is one of the most common neurodegenerative disorders of infancy or childhood. Clinical symptoms include developmental delay, psychomotor retardation, brainstem abnormalities and respiratory problems. Elevated lactate concentration in cerebrospinal fluid and serum is observed. Bilateral, symmetric necrotizing lesions in the basal ganglia, thalamus, brainstem and spinal cord are typical pathological changes. Recently SURF1 gene mutations were identified as a cause of the disease.
The aim of our study was to identify mutations in SURF1 gene and to correlate clinical coarse and genotypes of Polish patients with COX deficient Leigh syndrome. 23 classical Leigh patients were investigated. Sequence analysis revealed the presence of five different mutations (845delCT, 758delCA, Y274C, M235T, 312insATdel10) in Polish population. The mutation 845delCT was observed in homozygous or heterozygous form in all patients. In the group of patients with two frameshift mutations (845delCT, 758delCA or 312insATdel10) the clinical coarse was classical, severe with onset between 6 and 18 months and death before 4 years. In the group of the patients with the second missense mutation (Y274C, M235T) the clinical coarse was significantly slower with onset between 10 and 24 months. Some of the last patients are still living at the age of 8-15 years.
The study was partially supported by KBN Project no. 6PO5E05420.

P715. Novel mitochondrial tRNA tyrosine pointmutation A5836G in a myopathic family

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A novel mutation in the mitochondrial tRNA tyrosine gene, A5836G was found in a 48 year old woman presented with exercise intolerance, muscle weakness, ptosis and bilateral gradual visual loss. One of her three sons experienced sudden bilateral visual loss at the age of 18 with minimal visual recovery. To search for mitochondrial alterations DNA was isolated from peripheral blood leukocytes of the mother, her affected son and her two not affected sons according to standard methods. Automated sequencing of the total mitochondrial DNA revealed, besides numerous silent polymorphisms, two secondary Leber's hereditary optic neuropathy (LHON) mutations (T4216C and G13708A) and a novel pointmutation in the tRNA tyrosine gene: an A-G transition at np 5836. No primary LHON mutations were found. According to our investigations this novel mutation proved to be homoplasmic in the mother, in her affected son suffering from bilateral optic neuropathy, but in the two, so far asymptomatic children, as well, and it was not found in 250 normal control DNA samples. Muscle biopsy in the affected son showed several ragged red fibers. Although the mutation in the tRNA tyrosine was homoplasmic, the pathogenic role of this mutation in the optic neuropathy as an auxilliary factor besides the two secondary Leber mutations might be a possible explanation. It might account for the presence of ragged red fibers in the son and the symptoms seen in the mother, as well. Further functional, biochemical and clinical investigations are needed to support the role of A5836G in the disease pathomechanism.

P716. Novel mitochondrial tRNA Ile mutation in a patient with encephalomyopathy

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Point mutations localized in mitochondrial tRNA Ile gene can result isolated disorders (PEO, hypertrophic cardiomyopathy) or systematic dysfunctions e.g. systematic encephalocardiomyopathy. Our patient had progressive visual failure at left side, concentric constriction of his visual field at right, disturbed eye movements, polyneuropathic sensory disturbance, discreet pyramidal signs at left side, occlusion in left arteria carotis interna and in right arteria vertebralis and itellectual decline. Based on the appearance of his symptoms (no attack) and on the results of his examinations
(MRI including MRA, ENG, ERG, VEP, AEP, carotis Doppler sonography), we assumed that besides vascular causes, mitochondrial DNA damage (LHON or mitochondrial encephalomyopathy) can also be responsible for his disease.We have examined 5 primary (G3460A, G11778A, G14459A, T14484C, G15257A) and 7 secondary (C3275A, G3316A, T3394C, T4216C, G7444A, T9101C, G13708A) Leber-mutations and all of them were normal. Besides these LHON-mutations, sequencing of mtDNA between 3091-3450 and 4152-4516 np was performed. We have found a base substitution in tRNA Ile gene at np 4314. We have also shown the presence of this mutation with restriction enzyme digestion. The base substitution is localized in TΨC arm of tRNA molecule. In this gene 10 different point mutations have already been described, but T4314C mutation hasn't been published yet. The possible pathogenetic role of this mutation needs further investigations.

P717. Research on Mitochondrial DNA in Iran.

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Analysis of the mtDNA haplogroups is, however, not only restricted to the studies of human origin and evolution. It is also very important for studies of human pathologies. Indeed, recent studies have shown that mtDNA haplogroups can play an important role in modulating disease/phenotype expression. Such as Multiple Sclerosis (MS) (haplotype K and J); Leber Hereditary Optic Neuropathy (LHON) (haplogroup J); Wolfram or DIDMOAD (Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy and Deafness) syndrome (haplogroup B); Neonatal mortality rate (non-B group) …….
For analysis of MtDNA haplogroup in Iranian population we started with 11778, 3460, 14484, 14459 mutations in 34 LHON. 13 patients had one of mutations. We detect 11778 mutation in 77%, G3460A in 8%, T14484C in 7.5% and
G14459A in 7.5% LHON patients.
Deletion in mtDNA of 29 CPEO, KSS, IBM patients were investigated by standard and multiple PCR. We were found 24 patients with mitochondrial deletions. 5, 7, 7.5, or 9 Kb deletions.
Haplogroup J is only about 9% of general Eroupian population but it was found in 37% of LHON patients harboring the LHON mutation. we have found 24% haplogroup J in MS patients compairing to 11% in Healthy controls. Haplogroup K was found in 24% of MS patients but 16.5% in healthy controls.
In LHON Patients we had found in 8% compairing to 11% in Healthy controls.

P718. Genetic investigation of Iranian LHON patients

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Leber Hereditary Optic Neuropathy (LHON) is a maternally form of central visual loss that occurs subacutely in young persons. The aim of this study was to define the prevalence of a panel of mitochondrial DNA (mtDNA) mutations associated with Leber Herediraty Optic Neuropathy (LHON) in Iranian LHON population. We studied four well-known LHON associated primary point mutations (at nucleotide positions 11778, 3460, 14484 and 14459) in 29 Iranian LHON patients. On the basis of our study 9 affected patients were positive for one of four primary LHON point mutation, 8 patients were males (89%) and one was female (11%). The G11778A was found in all the females (100%) of the patient's family and no one showed the LHON phenotypes. We were detected 78% for the G11778A mutation. 11% for the G3460A mutation, 11% for the G14459A point mutation and the T14484C point mutation has not been detected in our patients. Our results showed similarity of Iranian LHON families with Russian, Europe and North America. So, 10 new point mutations were detecte in the rest of patients who had not any primary LHON point mutations.

P719. Respiratory chain complex I deficiency associated with recurrent mutations of mitochondrial DNA

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Respiratory chain deficiencies are genetically heterogeneous as both mitochondrial (mt) and nuclear mutations have been reported. Mutations in complex I deficiencies have been reported in nuclear and mitochondrial genes, encoding structural proteins of complex I. We describe two novel mutations, T10158C and T14487C, in the mitochondrial ND3 and ND6 genes, and two other mutations, T10191C and T12706C in the ND3 and ND5 genes respectively, in patients with isolated complex I deficiency associated with Leigh syndrome. All these mutations were recurrent *de novo* mutations, and were found in at least two unrelated patients (except the T14487C mutation). To understand the recurrence of these mtDNA mutations, a detailed analysis of the primary and secondary structures was performed around the mutations. We found that all the mutations occurred in a TCC or TTCC motif but no specific secondary structure could account for the recurrence of the mutations. These mutations were all associated with specific neurological features, namely Leigh syndrome or Leigh-like syndrome. Since these mutations were recurrent in Leigh or Leigh-like syndrome, we suggest that they should be included in systematic screenings of complex I deficiency.

P720. The mitochondrial DNA G13513A MELAS mutation in the NADH dehydrogenase 5 gene is a frequent cause of Leigh-like syndrome with isolated complex I deficiency

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Leigh syndrome is a subacute necrotizing encephalomyopathy frequently ascribed to mitochondrial respiratory chain deficiency. This condition is genetically heterogeneous, as mutations in both mitochondrial (mt) and nuclear genes have been reported. Here, we report the G13513A transition in the ND5 mtDNA gene in three unrelated children with Leigh syndrome and complex I deficiency. Variable degrees of heteroplasmy were found in all tissues tested and a high percentage of mutant mtDNA was observed in muscle. Asymptomatic mothers presented low levels of mutant mtDNA in blood leukocytes. This mutation, which affects an evolutionary conserved amino acid (D393N), has been previously reported in adult patients with MELAS or LHON/MELAS syndromes, emphasising the clinical heterogeneity of mitochondrial DNA mutations. Since the G13513A mutation was found in 21% of our patients with Leigh syndrome and complex I deficiency (3/14), it appears that this mutation represents a frequent cause of Leigh disease, which should be systematically tested for molecular diagnosis in affected children and for genetic counseling in their maternal relatives.

P721. Novel mitochondrial tRNA^{Trp} mutation associated with Kearns-Sayre Syndrome: Identification using Polyacrylamide gel electrophoresis coupled with matrix-assisted laser desorption/ionization mass spectrometry

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More than 70 different point mutations in human mitochondrial tRNA genes are correlated with severe disorders, including fatal cardiopathies, encephalopathies, myopathies, and others. So far, investigation of the molecular impact(s) of mutations has focused

on the affected tRNA itself by seeking structural and/or functional perturbations capable of interfering with synthesis of the 13 mitochondrion-encoded subunits of respiratory chain complexes. Here we report a fast and simple method for the structural analysis of newly identified tRNA mutations. In analogy to two-dimensional analysis, the mobility shift in native polyacrylamide gel electrophoresis (PAGE) due to a nucleotide substitution of a single-stranded transfer ribonucleic acid (tRNA) fragment serves as the first dimension for tRNA mutation analysis. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), as the second dimension, allows precise determination of the mass of the tRNA fragments resolved by native PAGE. Off-line combination of native PAGE with MALDI-MS is demonstrated for high-resolution analysis of mitochondrial tRNAs and its mutants. Using this method, we characterised a novel mitochondrial $tRNA^{Trp}$ (T5553C) mutation associated with Kearns-Sayre Syndrome (KSS).

P722. Chromosome 19q-linked "familial infantile convulsions plus", FIC+, represents a distinct novel inherited epilepsy: implications for linkage studies and syndromology in the seizure disorders

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A large family is presented in which a seizure phenotype appears to follow autosomal dominant inheritance. Among 24 individuals with seizures in two generations, the predominant features were onset of seizures within the first two years of life, occasionally followed by remission, with frequent recurrence in later childhood or adulthood. Genome-wide linkage analysis gave a LOD score of 3.1 at 19q13 (no other genomic regions showing a LOD score of >1.5), although possible phenocopies were detected. This is the third familial epilepsy to map to this region of the genome. Candidate genes have been sequenced; so far a mutation has not been found. The phenotype within the family is highly variable. 2/19 confirmed haplotype carriers experienced infantile seizures only (resolving by 2y, similar to benign familial infantile convulsions, BFIC); 12/19 had onset in infancy with persistence to later childhood; 3/19 had later seizures without a history in infancy; 2/19 remained asymptomatic to adulthood. Where available, most individuals have had normal electroencephalograms, but several (5/14) have shown mild non-specific instability. Affected subjects tend to have slightly lower IQs than unaffected siblings. This pattern of seizures within a large family is likely to represent a previously-undescribed inherited seizure disorder, which we have termed „familial infantile convulsions plus", FIC+. Given the degree of clinical variability and presence of obligate phenocopies, it is probable that there is more than one genetic cause for epilepsy in this family.

P723. Neurodevelopmental and physical morbidity in children exposed to antiepileptic drugs in utero

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Objective: The purpose of our study was to determine the prevalence of cognitive impairment in children exposed to AEDs in utero and to explore its association with the dysmorphic features seen.
Methods: In a retrospective study of 218 mothers recruited from regional epilepsy clinics in Manchester and Liverpool, we assessed 375 children exposed to different AEDs. Structured interviews, clinical examination and psychometric tests(WISC) were used to assess exposure, dysmorphic features and IQ (intelligence quotient).Blind assessment of photographs was used to categorise children as mildly, moderately and severely affected based on dysmorphic features.
Results: The mean full scale IQ in children exposed to VPA(87.2,CI 81.9 to92.5) was lower than that in the NE group(89.5, CI 85.5 to 93.4) and other monotherapy groups(CBZ,91.1, CI 86.4 TO 95.8;PHT, 97.6,CI 90.3 to 105.0).The mean verbal IQ(VIQ) was significantly lower in the VPA group(83.61, CI 78.2 to 89.0) compared to NE(90.9, CI 87.2 to 94.6) and other monotherapy groups(CBZ,94.08,CI 89.6 to 98.5; PHT, 98.48, CI 90.6 -106.3).
Dysmorphic features were more commonly seen in children exposed to VPA, with 44% having moderate to severe dysmorphic features in contrast to those exposed to CBZ(9.2%) and the NE(2.2%). 55% of those with moderate to severe features had a very low IQ(<79). There was a significant correlation between VIQ and dysmorphic features in the VPA group(Spearman's rho-0.436).
Conclusion: The retrospective nature of our study precludes the ascertainment of absolute risks. Nonetheless, these results suggest that prenatal VPA exposure in particular may carry an increased risk of neurodevelopmental problems.

P724. Foetal valproate syndrome (FVS): experiment of the consultation of genetics in Rennes (France)

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Over a 6 years period (1996 to 2002), 38 files related to the association «pregnancy and sodium valproate» with 42 examined foetuses or children. Recruitment was made by the means of the consultations of foetal medicine or dysmorphology (children addressed for developmental delay, dysmorphy more or less malformations). This population was divided into 2 categories: 1) 14 medical abortions for major malformation, concerning 12 families, with 11 cases of spina bifida (including 2 recurrences) and 3 major cardiac malformations. 8 pregnancies out of 13 proceeded under folic acid; 2) 28 examined children presenting of some signs compatible with the FVS. The reason for consultation was for 16 of them a delay +/- a dysmorphy +/- a malformation; a malformation for 5 new-born children, a neonatal withdrawal for 4 new-born babies, and 3 sibs of affected children. The malformations observed in these children were a spina bifida not diagnosed during the pregnancy, a craniosynostosis (trigonocephaly), a cleft lip and palate , 4 cardiac malformations, 3 limbs, 7 urogenital, and 4 corpus callosum anomalies. All these children had a developmental delay except one. Two children were too young to conclude and one was deceased at 8 days of life. The delay of language was noted for all the oldest children (maximum 11 years old) and some have behavioural problems or an associated epilepsy (1 patient). An history of neonatal hypotonia was often present. Common teratogenic mechanisms with the foetal alcohol syndrome, the mitochondrial respiratory chain deficiencies, the toluene embryopathy are discussed.

P725. Clinical Experience with Preimplantation Genetic Diagnosis (FISH) at Sheba Medical Center, Israel

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The ability to detect abnormal genetic traits among embryos before conception is an important breakthrough in modern reproductive medicine. Although it is easy to foresee the potential impact of PGD on our life in the near future, many factors still hinder its clinical application in many countries. It is estimated that only several hundreds babies have been born so far following this diagnostic approach in no more than 50 centers worldwide.
During 2001-2002 we have invested considerable effort in order to incorporate this technology in our center as a clinical service. We have performed PGD in 32 couples with the following indications. In 21 couples there were abnormal genetic traits: 10 with X-linked diseases, 5 couples were with chromosomal mosaicism and 6 couples with balanced reciprocal translocations. The rest of the patients were diagnosed due to the following indications: 3 for recurrent pregnancy losses, 4 due to recurrent implantation failures and 4 due to previous non-disjunction.
59 embryos (2.1±1.2 per embryo transfer) were replaced and 11 implanted (18.6%). Seven (21.8%) pregnancies were established, out of which 4 resulted in delivery of 7 healthy babies and one is an ongoing twin pregnancy. The pregnancy losses were related to patients with normal genetic traits.
In conclusion, our preliminary results demonstrate that PGD is a reliable technique for prevention of genetic diseases. We hope that in the near future the crude method of terminating affected pregnancies will be replaced by pre selection of normal embryos for replacement by PGD.

P726. Diagnosis of febrile seizures on the basis of HLA-DQ haplotypes.

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Objective: The aim of this study was to compare the frequencies of HLA -DQA1 and DQB1 haplotypes in the patients with febrile seizures (FS) and in control group.
Methods: Haplotypes for HLA-DQA1 and HLA-DQB1 were determined using RLFP-analysis in 68 patients with FS and in 70 individuals from the control group. Statistical analysis used stepwise discriminant analysis.
Results: In patients with FS, haplotypes HLA-DQB1*0504 in heterozygous condition were more frequent ($p < 0.05$), as well as HLA-DQB1*0503 also in heterozygous condition ($p < 0.1$). In the control group HLA-DQA1*0501 heterozygotes ($p < 0.1$) and homozygotes ($p < 0.05$) were more frequent. More informative were six haplotypes: HLA-DQA1*0501, DQB1*0201, DQB1*0504, DQA1*0401, DQB1*0503 and DQB1*0402. The results of stepwise discriminant analysis showed that on the basis of the above mentioned haplotypes the diagnosis of FS can be established in 67.65% of cases.

P727. Identification of a new mutation in the EPM2A gene in Lafora's Disease

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Lafora disease (LD) is an autosomal recessive progressive myoclonus epilepsy (PME) that presents in teenage years with myoclonias, seizures, progressive neurological deterioration and the presence of glycogen-like intracellular inclusion bodies (lafora bodies) in the brain, spinal cord, heart and liver of affected patients. A gene for PME, *EPM2A*, has been mapped to chromosome 6q23-q25. *EPM2A* is composed of four exons.
In this paper we describe a patient with PME. The patient, a 17-year old man, was born to healthy unrelated parents coming from a small town in Sicily. He had normal psychomotor development, but at age 11 years became affected with occipital lobe seizures. The course of this disease was characterized by worsening epilepsy with myoclonus and neurologic deterioration. In addition to clinical features, the detection of glycogen-like intracellular inclusion bodies at skin biopsy suggested a diagnosis of Lafora disease. Genetic analysis of the *EPM2A* gene detected a new missense mutation Arg91Pro in exon 1. Mutations in the exon 1 have previously been decribed in other patients and these mutations were associated with an atypical Lafora's disease.

P728. PKD2 mutations in Czech population with autosomal dominant polycystic kidney disease

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Autosomal dominant polycystic kidney disease (ADPKD) is a genetically heterogeneous disease caused by mutations in at least three different loci. Mutations in the *PKD2* gene are responsible for about 15% cases of the disease, based on linkage analysis. *PKD2*-linked ADPKD is supposed to be a milder form of the disease, with a mean age of end-stage renal failure (ESRF) approximately 20 years later than PKD1.
We screened all coding sequences of the *PKD2* gene in 116 Czech patients. 53 patients who reached ESRF after 63 years of age and 10 patients who were not undergoing renal replacement therapy at that age were selected from dialysis centers from the Czech Republic and from the Department of Nephrology of the General Hospital in Prague. Age 63 years was used as the cutoff value because it is between the ages of onset of ESRF for PKD1 and PKD2 published in recent studies. A further 53 patients were selected from the PKD families who could be linked either to the *PKD1* or *PKD2* genes according to linkage analysis.
We detected 22 mutations (six new mutations): 14 mutation in the 63 patients (22%) with a mild clinical course and 8 mutations in 53 families (15%) with possible linkage to both PKD genes. We describe two cases with the severe form of ADPKD and PKD2 mutation.
We identified nonsense mutations in 8 patients (36%), frameshift mutations in 12 patients (55%) and 2 new missense mutations (9%).
This work was supported by the grant IGA MZ CR

P729. Molecular study of PKD1 & PKD2 genes in three Iranian families with Autosomal Dominant Polycystic Kidney Disease by linkage analysis.

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common
hereditary nephrological disorder affecting 1 in 400-1000 of people. It accounts for 8-10% of the cases of end-stage renal disease worldwide, thus representing a serious medical, economical and social problem. ADPKD is in fact a systemic disorder, characterized by the development of cysts in the ductal organs (mainly the kidneys and the liver), also with gastrointestinal and cardiovascular abnormalities.. Three genes are implicated in causing ADPKD , one on chromosome 16p13.3 , PKD1 , account for 85 – 90 % of all cases , and the other, PKD2 gene, on chromosome 4q21-23 is estimated to be responsible for 10- 15 % of the cases ADPKD . Very rare cases relate to the third gene , PKD3, that has not been mapped yet . We studied heterozygosity rate and PIC for markers 16 AC2.5, KG8 and SM7 linked to PKD1 gene and also D4S423 , D4S231 and D4S1534 linked to PKD2 gene in 30 unrelated healthy individuals . Our results have shown that two marker , KG8 and SM7 for PKD1 gene and D4S423 and D4S231 for PKD2 gene are informative in our population .secondly we performed linkage analysis on three affected Iranian families. our data indicate , that two of them were linked to PKD1 gene ,whereas the other one was related to PKD2 gene .

P730. Mutation screening in autosomal recessive polycystic kidney disease (ARPKD/ PKHD1)

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Autosomal recessive polycystic kidney disease (ARPKD/ PKHD1) is an important cause of childhood renal- and liver-related morbidity and mortality. We have recently shown, with others, that mutations in the PKHD1 gene on 6p result in ARPKD. By now, a total of 63 different PKHD1 mutations have been described (www.humgen.rwth-aachen.de). Most of them have been identified in a study recently conducted by us in a cohort of 90 ARPKD patients. A mutation detection rate of 61% was yielded by SSCP and subsequent sequencing. Mutations were found to be scattered throughout the gene's 67 exons without evidence of clustering at specific sites which makes analysis extremely cumbersome. About 45% of the changes were predicted to truncate the encoded protein, polyductin. All missense mutations were non-conservative with the affected amino acid residues found to be conserved in the murine orthologue. Preliminary genotype-phenotype correlations could be established for the type of mutation rather than for the site of the individual mutation. All patients carrying two truncating mutations displayed a severe phenotype with perinatal or neonatal demise.
We now report improvement of PKHD1 mutation screening by denaturing high-performance liquid chromatography (DHPLC) using the Wave Fragment Analysis System (Transgenomics). PKHD1 mutation analysis by DHPLC has been demonstrated to be an efficient and effective means to establish the molecular cause of ARPKD. Direct identification of mutations allows an unequivocal (prenatal) diagnosis and accurate genetic counselling even in families displaying diagnostic challenges.

P731. Comparison of the CFTR mutation spectrum in three cohorts of patients of Celtic origin from Brittany (France) and Ireland

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This study aims to compare the spectrum of the mutations identified in the gene responsible for cystic fibrosis in three cohorts of patients of Celtic origin from Brittany and Ireland. It included 389 patients from Brittany, 631 from Dublin and 139 from Cork. The CFTR gene analysis relied on the detection of the most common mutations, followed by a complete gene scanning using DGGE or D-HPLC. High mutation detection rates were obtained in each cohort: 99.6%, 96.8% and 96.0% respectively. A high frequency of the F508del mutation (74.8% to 81.3%) and of the „Celtic“ mutation (G551D: 3.7% to 9.7%) was observed in each population. Apart from this, the mutation spectrum observed in each region differed. In Brittany, the most common abnormalities were: 1078delT (3.6%), N1303K (1.4%), W846X2 (1.0%) and 1717-1G>A (1.0%), whereas in the cohort of Dublin, the most frequent mutations were: R117H (3.0%), R560T (2.4%) and 621+1G>T (1.7%). Finally, in the Cork area, only the R117H mutation reached a frequency of 1%. This collaborative study highlights the similarities of the CFTR alleles in the Breton and Irish populations, but also the disparities that exist between these populations, despite their common origin. Each population has its own history, with its mixture of founder effects and genetic drifts, which are at the origin of the current mutation distribution. The molecular study of the CFTR gene provides new tools for retracing European populations‘ histories. This improved knowledge of CF epidemiology allows the improvement of diagnostic strategies and the refinement of genetic counselling.

P732. A Decade of Rare Cystic Fibrosis Mutation Screening

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We have been offering a rare cystic fibrosis (CF) mutation scanning service since 1993. The service is aimed at detecting mutations not covered by routine CF mutation testing in diagnostic laboratories. Mutation scanning throughout this period has been by combined SSCP/heteroduplex analysis. In order to reduce costs and give shorter and predictable turn-round times for the service, testing is stepwise. Level 1 comprises screening of exons 3, 4, 7, 10, 11, 12, 13, 19, 20 & 21. Level 2 comprises screening of exons 2, 5, 9, 14a, 14b, 16, 17a, 17b, 18 & 23. The remaining 7 coding exons are only screened on special request. The composition of exons was chosen to maximise mutation detection rate whilst minimising workload using information from a retrospective screen of the whole CF gene in 300 known CF affected patients from the UK. Strict quality control has been maintained by including several control mutations alongside each exon tested. Analyses where all control mutations were not distinguished were rejected. During the 10 year period we have screened 391 samples at either levels 1 or 2. We have detected 202 mutations either reported as or presumed to be pathogenic. Of the 202 mutations 16 had not been previously reported to the International CF Consortium. In addition 19 rare polymorphisms of unknown effect were also identified. The reasons for referral have been very diverse, ranging from patients with clinically confirmed CF where one or both mutations were unidentified after routine testing to patients with male infertility.

P733. Prevalence of Mutations delF508, G551DM, N1303K, G542X, and W1282X in the CFTR Gene of Iranian Cystic Fibrosis Patients

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Abstract:

Cystic Fibrosis (CF) is an autosomal recessive disorder caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene, which may cause a common lethal disease. CF is characterized by obstruction pulmonary disease, pancreatic insufficiency , and increase in sweat chloride concentration. The incidence of cystic fibrosis and the frequency of disease-causing mutations vary among different ethnic groups and geographical regions around the world.
Identification of the disease-causing mutations enables carrier screening in the general population.
In this study 56 chromosomes of 28 unrelated CF families were screened for the five common mutations including ΔF508, G542X, N1303K, G551DM, and W1282 using ARMs-PCR.
ΔF508 covered 14.3% of the alleles, which is one of the lowest frequencies detected world wide so far, and is in agreement with the hypothesis of a decline of the frequency of this mutation in a northwest to southeast gradient. Mutations G542X and W1282X accounted for 5.2% of CF alleles with the frequencies of 3.4% and 1.8% respectively.
However, the two other mutations, N1303K and G551DM, were not detected at all. Thus we have identified 3 mutations in the CFTR gene in Iranian CF families, accounting for 19.5% of CF alleles. More investigation is being carried out using SSCP/HD analysis and sequencing to identify the other possible mutations in Iranian CF patients.

P734. The IVS8 poly-T analysis in Latvian cystic fibrosis patients

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The CFTR gene contains a polymorphic site located in the intron 8 consisting of polypyrimidine tract (5T, 7T, 9T), the IVS8-5T variant causes an improper splicing of the mRNA. In homozygosity or compound heterozygosity with other CF mutations the resulting pathology is Congenital Bilateral Absence of the Vas Deferens (CBAVD). The aim of our work was to introduce the method of IVS8 poly-T DNA analysis into our laboratory, to detect the prevalence of each variant among patients of cystic fibrosis, and the association of the mutation dF508 with the poly-T alleles. Objects of the study were 28 cystic fibrosis patients. DNA was obtained from venous blood by standard phenol/chloroform extraction protocol. The PCR and restriction enzyme HpaI digestion was used, and the product analyzed on PAA gel. The results were as follows: of 56 alleles tested 17 were 7T, 39 were 9T, allele 5T was not found. The combinations of alleles were: 7T/7T - 4 patients, 7T/9T - 9 patients, 9T/9T - 15 patients. Most of dF508 chromosomes were associated with the 9T variant, however the 4 7T/7T patients were heterozygous for the mutation dF508. The further studies will be directed to reveal the prevalence of the IVS8 poly-T variants in healthy Latvian population.

P735. Localization of the SCA23 gene on chromosome 20p12-p12.2 by linkage analysis in Dutch ataxia families

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Autosomal dominant cerebellar ataxias (ADCAs) comprise a group of disorders leading to invalidity, usually with an adult age of onset. They are characterized by gait and limb ataxia, and disturbances of speech and oculomotor control, with additional variable features. The estimated minimal prevalence of ADCA in the Netherlands is 3: 100,000. To date, 21 different SCA (spinocerebellar ataxia) loci have been identified in ADCA. The corresponding gene has been cloned

for nine of these loci. Screening of the SCA1-3, 6 and 7 genes has yielded a diagnosis for 60% of the Dutch ADCA families. However, screening of the SCA 8, 10, 12 and 15 did not explain the remaining 30% of ADCA families.
We have recently identified a novel SCA locus in a large Dutch ADCA family by linkage analysis. Multi-point linkage analysis has localized the disease-causing gene, designated SCA23 (approved by the HUGO Nomenclature Committee), to chromosome region 20p13-p12.2. The size of the interval is approximately 27 cM. Because several SCA mutations display a strong founder effect, we have used a haplotype-sharing analysis (SHA) to narrow down this interval.
In an additional 22 smaller ADCA families, we searched for the identical haplotype of the disease gene region. Four families partly showed this identical SCA23 haplotype. This result suggests that the disease-causing mutation in these four families arose from the same founder, and secondly, it has led to a refined <10 cM interval between the markers D20S889 and D20S95. At present, we are analyzing potential candidate genes in this refined region.

P736. Consequences of polyglutamine and polyalanine expansions in the context of the protein Ataxin7

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Spinocerebellar ataxia type 7 (SCA7) is an autosomal dominant polyglutamine disorder caused by the expansion of a translated (CAG)n repeat in the gene encoding Ataxin7.
Polyglutamine diseases are neurodegenerative disorders characterized by the presence of neuronal intranuclear inclusions (NIIs) due to the inherent ability of proteins containing large polyglutamine stretches to aggregate. However, whether these inclusions, the monomeric protein, or the aggregation process itself are responsible for the toxicity is a matter of debate. Interestingly, there is some evidence that stretches of polyalanine, similarly to stretches of polyglutamine, can aggregate.
To determine the role of the nature of the repeated amino acid versus the protein context and the role of aggregation in the pathological process, we produced SCA7 cellular models with polyalanine (Ataxin7-90A) instead of polyglutamine expansions and compared them with corresponding models with polyglutamine expansions (Ataxin7-100Q).
Ataxin7-90A and Ataxin7-100Q were transiently expressed in HEK 293 cells and in primary cultures of rat embryonic mesencephalic neurons. In both cases (Ataxin7-90A and Ataxin7-100Q), the formation of nuclear and perinuclear aggregates was observed. In addition, the presence of molecular chaperones and components of the Ubiquitin-Proteasome system in these aggregates points to an abnormal folding of the expanded proteins. However, the aggregates formed by Ataxin7-90A, as visualized using optic microscopy, are morphologically distinct from the Ataxin7-100Q NIIs. Furthermore, at the ultrastructural level, amorphous deposits of Ataxin7-90A but no fibrillar inclusions as observed with Ataxin7-100Q were detected. Finally, preliminary results indicate that Ataxin7-90A is more toxic than Ataxin7-100Q in primary cultures of mesencephalic neurons.

P737. A novel R1347Q CACNA1A missense mutation causes phenotypes of spinocerebellar ataxia with hemiplegic migraine in a Portuguese family

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The *CACNA1A* gene encodes the highly conserved brain specific P/Q-type calcium channel a1A-subunit, mainly expressed in the cerebellum. Mutations in this gene cause a channelopathy mainly characterized by attacks of episodic gait and limb ataxia, hemiplegic migraine or progressive cerebellar ataxia, which can be isolated or coexist in the same family. We have previously described a large Portuguese family presenting slowly progressive ataxia associated with hemiplegic migraine in whom the disease-causing mutation was an R583Q mutation located on the 4th segment of protein domain II. We have now ascertained another family presenting a similar clinical pattern precipitated by physical effort, hemiplegic migraine and coma, in which ataxia and aura symptoms began simultaneously and during childhood. We have (1) assessed CAG repeat size, and (2) screened our family for mutations in the *CACNA1A* gene. Mutation detection was performed by PCR amplification, single strand conformational polymorphism (SSCP) and sequencing. Expansions of the CAG repeat were not present in any of these patients. SSCP and sequencing analysis detected a G-to-A substitution in exon 25 of the *CACNA1A* gene, resulting in a new arginine to glutamine change at codon 1347, located at the S4 voltage sensor segment of protein domain III. In conclusion, this newly described mutation causes slowly progressive ataxia with hemiplegic migraine and coma. The finding of a new arginine to glutamine mutation in this segment indicates that the positively charged residues located in the voltage sensor segment are extremely important in the maintenance of the correct gating properties of calcium channels.

P738. A French family with a distinct phenotype linked to SCA14

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Spinocerebellar ataxia 14 is a pure autosomal dominant cerebellar ataxia (ADCA) mapped to chromosome 19q in a single Japanese family. We have identified a large French kindred segregating with an ADCA in which 14 patients and 10 at risk individuals were sampled. Age at onset ranged from childhood up to 60 years. All patients showed cerebellar ataxia and the majority showed increased reflexes, without Babinski sign and intellectual impairment (8 out of 16).
The index patient tested negative for nucleotide repeat expansions in the SCA1-3, 6-8, 10, 12 and 17 genes, and microsatellite typing excluded linkage to the SCA4-5, 11, 13, 16 and 21 loci. Lod scores above the threshold of 3 were, however, obtained with markers *D19S180*, *921* and *924* mapped to the *SCA14* locus. Multipoint linkage analysis using markers *D19S571*, *921* and *926* gave a maximum lod score of 5.75 at marker *D19S921*. Haplotype reconstruction restricted the candidate region to a 10 cM interval between flanking markers *D19S571* and *D19S926*. In conclusion, we have identified a third SCA14 family that allowed the reduction of the initial candidate interval from 3.4 to 2.4 Mbases. The phenotype of the French and Japanese families differ by the associated neurological signs and the broader range of age at onset observed in the kindred of French ancestry.

P739. Eight novel mutations revealed by means of DHPLC mutational analysis of the neurofibromatosis type I (NF1) gene in NF1 patients from Sicily and Calabria (southern Italy).

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Neurofibromatosis type 1(NF1) is an autosomal dominant disorder with a prevalence of 1 in 3500 individuals in the general population and is characterized by pigmentary changes, neurofibromas, and systemic complications. We performed molecular analysis in 74 consecutive NF1 patients from southern Italy by means of denaturing high performance liquid chromatography (DHPLC) and DNA sequencing. DHPLC is largely automated heteroduplex-based technique optimised, in this study, for the rapid screening of mutations in exons 5, 19, 20, 21, 22, 23, 24, 25, 26, 27, 29, 34 and 37, respectively, of the NF1 gene. We detected twelve mutations of whom four represented known mutations (R1276X, K1423E, 3456delACTA, 6488 insA) and eight were novel mutations previously unreported. Of the eight novel mutations, two created a stop codon (Q1360X, E1192X), two were nucleotide substitutions (L1109F,

N1394D), two were small insertions (1504insA, 2263insC), one was a small deletion (1503delG) and another was a splice site mutation (IVS24+1 G->A).
These novel mutations contribute to the germline mutational spectrum of the NF1 gene. The use of DHPLC appears to be a rapid efficient tool for NF1 mutational analysis.Our study suggests that the combination of DHPLC and DNA sequencing can be used as a powerfull method for characterizing mutations in disorders whose the responsible gene is extremely large .

P740. A genome wide scan reveals a locus for primary ciliary dyskinesia (PCD) in the Druze population

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PCD is an autosomal recessive disorder characterised by respiratory tract infections, sinusitis, bronchiectasis and subfertility. It affects 1: 20,000 live births. The clinical phenotype results from dysmotility of the cilia, which is associated with a variety of structural abnormalities. The core or axoneme of cilia comprises a bundle of microtubules and associated proteins including dyneins, nexin links and radial spokes. About 50% of patients exhibit laterality defects, commonly *situs inversus*, known as Kartagener syndrome. We have studied four families with an inner dynein arm ciliary defect from the isolated Druze population. Parents of three of the four families are first cousins and there are a total of seven affected (six of whom have *situs inversus*) and twenty-two unaffected individuals. A genome wide scan identified a region consistent with linkage in three of the four families on chromosome 15q13.3. Using GENEHUNTER, a maximum multipoint lod score of 3.2 was obtained between D15S165 and D15S1042. This critical region spans approximately 4 megabases of genomic DNA , 70% of which is available as finished genomic sequence. Work is in progress to refine the localisation of this gene by identificatuin of allelic association. One of the 12 known genes in this region, *CKTSF1B1* (Gene Cystine Knot Superfamily 1), encodes the protein *gremlin* which belongs to the DAN group of proteins known to play a role in left-right axis determination. The gene is expressed in the lungs and testes and plays a role in lung morphogenesis. *CKTSF1B1* therefore represents an excellent candidate gene for PCD.

P741. PON1 genotyping in a southern italy association study in subjects with carotid artery disease: a linkage disequilibrium analysis of Gln192Arg, Met54Leu and –108C/T polymorphisms revealed a novel Lys to Asn 786 gene variant

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The PON1 genotypes Q192R and Met54Leu show a strong relationship to coronary artery disease (Serrato and Marian, 1995; Garin et al.1997; Brophy et al. 2001). A linkage disequilibrium is present between the mutation giving rise to leu54 and arg191 and this, in turns, leads to susceptibility to cardiovascular and coronary artery disease. More, the -108C/T polymorphism is responsible itself of the 20% of the observed variability in PON1 expression levels. We here report an association study in a southern italy population of 70 subjects who underwent coronary atherectomy and 70 control. We detected a LD between leu54 and arg191 (P=0.0003); also leu54 was in LD with the –108C polymorhism (P=0.0001) but, susrprisingly, the serum levels either of cHDL and the paraoxanase were in line with the control population. To explain this discrepancy we carried out a sequencing analysis of the PON1 gene: one additional gene variation leading a lys to asn substitution in position 786 ($C^{786}G$) was found. This mutation was present in the 8% of the controls and in the 80% of the patients. In vitro studies showed a 30% increased mRNA stability in cells transfected by $C^{786}G$ construct and 35% increased levels of transcription in PON1 wt and PON1 carrying the $C^{786}G$ constructs done by Real Time PCR ($p<0.0001$). In conclusion we characterised a novel mutation localised in the coding sequence leading to a increase of PON1 gene expression by increasing the transcription efficiency conferring to the carriers a protection effect to carotid arthery disease.

P742. Linkage disequilibrium analysis of the human adenosine deaminase (*ADA*) gene in two major ethnic groups.

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The linkage disequilibrium (LD) pattern within the adenosine deaminase (*ADA*) gene was analyzed by studying 13 polymorphic loci in 137 families from Europe and Africa. Evidence for the presence of a 12 kb meiotic crossover hotspot, spanning part of the first and the second intron and flanked by regions of reduced recombination activity, was observed. Moreover, segregation analysis of 113 informative meioses revealed two recombination events which are internal or overlap the 12 kb region, thus suggesting a recombination rate for the hotspot region about fifty fold higher than the mean rate across the human genome. The possible correlation between the 12 kb hotspot region and the 3.2 kb region that is deleted in some patients affected by autosomal severe combined immunodeficiency (SCID), will be discussed.

P743. The Landscape of Linkage Disequilibrium in the human MHC Region

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Linkage to chromosome 6p has been established for several inflammatory and autoimmune diseases. The extent and structure of linkage disequilibrium (LD) in the region is unclear. The human genome has been suggested to be organized into haplotype blocks, characterized by high internal levels of LD between SNPs, separated by recombination hot-spots. Such a structuring would have significant impact on association mapping strategies.
We investigated the distribution of haplotype blocks in a 20 Mb region using 920 SNPs, followed by a more detailed investigation of 3.53 Mb covering the MHC, using 294 SNPs. Samples from four Caucasian populations, comprising between 45 and 550 individuals each, and one African-American population (45 individuals) were studied. Clustering of Lewontin's D' and correlation between LD and physical distance between SNPs were implemented.
In contrast to previous assumptions, our data suggest that the genome is not uniformly structured into haplotype blocks and that recombination hot-spots are only present in parts of the genomic regions of interest. High LD levels and clear haplotype block structures were found to intermingle with areas of low or medium LD, both inside and outside the MHC. All Caucasian populations show the same pattern and characteristics of LD structure. The African-Americans, despite exhibiting a similar distribution of LD over the region, are nevertheless characterized by a higher degree of haplotype diversity. These findings are likely to have a strong impact upon the future design of systematic association studies of chromosome 6p-linked human diseases.

P744. The Tylosis with Oesophageal Cancer (TOC) locus: A SNP of a region on 17q25.

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Tylosis (focal non-epidermolytic palmoplantar keratoderma) is associated with the early onset of squamous cell oesophageal cancer in two large families from the UK and US and a smaller German pedigree. The familial cancer association is rare in the general population, but the gene is also implicated in the development of sporadic squamous cell oesophageal cancer. Our recent haplotype analyses using novel microsatellite markers have reduced the minimal region on chromosome 17q25 from ~500kb to ~65kb, between markers D17S2239 and D17S2244. This region is covered

entirely by 1 fully sequenced RPCI-11 BAC clone and contains at least 6 candidate genes.
Mutational analysis carried out on the coding regions of the candidate genes did not identify any disease associated mutations.
A recent collaboration with the Wellcome Trust Sanger Institute in Cambridge UK, has enabled us to sequence the entire non-repetitive portion of the ~65kb region in four TOC family members (an affected and unaffected member from each of the UK and US families). This analysis identified 58 single nucleotide polymorphisms (SNPs) in one or both of these families, 52 of which proved to be previously undescribed. Further fine mapping of the TOC disease locus by haplotype analysis of 23/58 SNPs has allowed the reduction of the minimal region to 42.5kb. One known and two putative genes are located within this region and are under investigation. SNP genotyping of additional family members has highlighted 3 out of the 58 SNPs for further investigation as potential disease causing alterations.

P745. Application of the Enzyme Mismatch Cleavage Method using T7 Endonuclease I for Screening the Duplicated Region of Polycystic Kidney Disease Type 1 Gene.

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The Enzyme Mismatch Cleavage (EMC) method utilises a resolvase, which recognises and cleaves mismatched double stranded DNA, such as occurs in heteroduplexes generated in the presence of mutations, polymorphisms, insertion/deletions.
We designed a protocol using the T7 Endonuclease I enzyme for screening the duplicated region of Polycystic Kidney Disease Type 1 gene (PKD1). Long Range PCR amplicons were designed and amplified in 70 affected individuals from 70 PKD families. Nested PCR was performed with oligos designed for exons 11 to 34. The PCR products were digested with T7 Endonuclease I and run on a 12% Polyacrylamide gel. The gel was stained with the Silver Staining method for greater resolution of the DNA fragments.
Concurrently, we tested exons from the same 70 samples using Single Strand Confomation Analysis (SSCA) with radioactive oligos, and direct sequencing.
We present data on the optimisation of the EMC method, optimal PCR fragment length, and its accuracy and cost effectiveness when compared to SSCA and direct sequencing. For example, in exon 17, all methods were equally effective at identifying single base substitutions (7412C>T, P2471L and 7441C>T, L2481C/T) when present in a heterozygous state. In other exons, EMC identified novel variants, which were not detected by SSCA. EMC also identified heterozygous deletions of various length such as 7735-7737del3GGC in exon 20 (DeltaG2579), and 7972-7979del8 (Frameshift after 2657) in exon 21. The fragments generated by the EMC also correlated accurately with the position of the substitution / deletion as identified by sequencing. Funded by Cyprus Research Promotion Foundation.

P746. The cytochrome P4501A1 (CYP1A) and microsomal epoxide hydrolase (mEPHX) genes polymorphisms associations with lung disease severity at cystic fibrosis patients

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Cystic fibrosis (CF) is an autosomal recessive disease characterized by progressive obstructive lung disease and pancreatic. While some of clinical variations may be explained by the type of mutations in the CFTR gene, but the severity of clinical disease in CF modified by secondary genetic and environmental factors.
The aim - to determine the possible role of Ile462Val (CYP1A1 gene) and Tyr113His, His139Arg (mEPHX gene) polymorphisms on the severity of CF airway disease. Blood samples were taken from 60 patients with CF (32 middle CF, 28 severe CF) and 164 control subjects.
The frequency of Val/Val genotype of CYP1A1 gene was significantly higher in patients with severe CF (0.07) compared to control (0.00) and middle CF (0.00) patients ($p<0.01$). The frequency of Ile/Val genotype was higher in middle CF patients (0.160 versus 0.06 in control) (OR= 2.82) and in severe CF patients (0.11). These appears to be high association between severity of CF phenotype and the mutation of CYP1A1 gene which increase enzyme activity (OR=27.64).
The mEPHX is an enzyme which plays an important role in metabolizing highly reactive epoxide intermediates in the lung. We observed assosiation of severe CF airway disease with the very slow metabolysing form of mEPHX (OR= 19.32) (0.11 versus 0.006 in control and 0.00 in middle CF; $p<0.01$).
The combination of high enzyme activity CYP1A1 and very slow metabolysing form of mEPHX has been associated with increased risk of severe lung disease at CF patients (OR= 12.3; $p<0.05$).

P747. Haplotype Structure of Angiotensinogen Gene in Chinese Hans Essential Hypertension

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The renin-angiotensinogen system plays a major role in regulating blood pressure, blood volume homeostasis and maintaining electrolyte. Previously, the angiotensinogen (AGT) gene, which encodes the key substrate for renin in this system, has been reported to be associated with essential hypertension in Europeans Cacasian, Africans Caribbeans and Japanese. Therefore, We examined the association between variants of the angiotensinogen gene and the risk for hypertension in Chinese people. 345 patients with documented essential hypertension and 189 control subjects without hypertension were selected and frequency matched by age and sex. We have genotyped seven polymorphisms in the 5‘ flanking region (corresponding to G-217A, G-152A, A-20C and A-6G), exon2 [Thr174Met(C3889T), Thr235Met (C4072T)] and the 3‘UTR of exon5(C11535A). The frequencies of haplotyes combined these seven SNPs was estimated by Clark‘s algorithm and five frequent haplotypes, which coded as A(A-217, G-152, A-20, A-6, M174, T235, C11535), B(G-217, G-152, A-20, G-6, T174, M235, C11535), C(G-217, G-152, A-20, G-6, T174, M235, A11535), D(A-217, A-152, C-20, A-6, T174, T235, C11535) and E(G-217, G-152, C-20, A-6, T174, T235, C11535), accounted for 90% of those observed in this study. Only C(G-217, G-152, A-20, G-6, T174, M235, C11535) haplotypes frequency was significantly increased in hypertension subjects, suggesting that this haplotype is associated with a hypertensive effect. These results have important implications for the usefulness of LD approaches in the mapping of genes underlying susceptibility to complex disease.

P748. No evidence for an association with the serotonin transporter gene polymorphisms (5-HTTVNTR and 5-HTTLPR) and autism

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The serotonin transporter gene is a likely candidate in autistic disorder. The aim of this study is to determine a possible association between polymorphisms 5-HTTVNTR and 5-HTTLPR in serotonin transporter gene and autism.
Materials and Methods. 59 individuals with idiopathic autism, 47mothers, 33 fathers (28nuclear families), 59 controls. Ages between 3.6 and 41y. We studied the two polymorphic regions : promoter(5-HTTLPR) with a long or short allele and intron 2 (5-HTTVNTR) with fragments of 9, 10, 12 repeats.
Results. There were no differences between the genotype and allelic frequencies of autistic individuals and control group in both polymorphisms. With both polymorphisms in patients and controls there was no prevalence of any specific genotype, but there was a predominance of the long allele (12 and L).There was no difference in genotype and allelic frequency between the autistic group versus mothers, fathers, or mothers and fathers. Confirming previous study we observed a preferential 12/12 genotype in patients with mothers carrying the same genotype. In both populations 5-HTTLPR and 5-HTTVNTR were in concordance, and there was a predominance of long allele (L and 12), but although not significative, the difference

between frequency of L and S in patients was bigger.
Conclusions. In this study we did no found any association of autism with both polymorphisms in serotonin transporter gene. The existence of a preferential 12/12 genotype in autistic individuals with mothers carrying the same genotype was confirmed. There is a possible predominance of the allele L in patients although actually still not significative.

P749. Detection of Genotyping Errors Using Hardy-Weinberg Equilibrium Testing

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Genotyping data sets may contain errors which, in some instances, lead to false conclusions. Deviation from Hardy-Weinberg Equilibrium (HWE) in random samples may be indicative of problematic assays. This study has analysed 144,000 genotypes generated by TaqMan, RFLP, sequencing or mass spectrometric methods from 547 single nucleotide polymorphisms (SNPs). One hundred and ninety of the SNPs map within four large (780-2900 kb) genomic regions. The remaining 357 SNPs map within 34 candidate genes. The minor allele frequencies of all 547 SNPs ranged from 0.002 to 0.491. Four hundred and eleven SNPs had a minor allele frequency greater than 0.05, and this subset was examined for genotype distribution deviation from HWE. Genotype distributions for 39 assays (9.5%) deviated from HWE (P< 0.05), which is approximately two times more than would be expected by chance. Some of the possible reasons for this deviation were explored: assays for five SNPs proved non-specific and genotyping errors were identified in 23 SNPs. No explanation for deviation from HWE was determined for the other 11 SNPs. By including a more rigorous test for assay specificity, and introducing an accurate standardised high-throughput genotyping system, our process has been successfully improved to address issues of non-specificity and genotyping error.

P750. A locus for Jeune asphyxiating thoracic dystrophy, JATD, maps to chromosome 15q13

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Jeune asphyxiating thoracic dystrophy (JATD; MIM number 208500) is an autosomal recessive multisystem developmental disorder. JATD is characterised by abnormal skeletal development, with typical radiographic findings that include a long, narrow "bell shaped" thorax with short, abnormal ribs, metaphyseal irregularities, abnormal pelvis and short long bones (involving predominately ulnae, radii, fibulae and tibiae). Renal, hepatic, pancreatic and retinal abnormalities are common features of JATD and polydactyly of both hands and/or feet has been reported. JATD demonstrates wide phenotypic variability and cases have been classified into lethal, severe, mild and latent forms. Most cases are severely affected and die from asphyxia caused by a small thorax and hypoplastic lungs, in the perinatal period. We have performed a genome-wide linkage search using autozygosity mapping in a cohort of four consanguineous families with JATD, three of which originate from Pakistan and one from southern Italy. We localised a novel JATD locus (*JATD*) to chromosome 15q13, with a maximum cumulative two-point LOD score at D15S1031 (Z_{max} = 3.12 at θ = 0.00). Investigation of a further four European kindreds, with no known parental consanguinity, revealed evidence of an ancestral haplotype. Both severe and mild forms of JATD mapped to 15q13, suggesting that phenotypic variation in JATD reflects allelic heterogeneity and not locus heterogeneity. However, preliminary work on further JATD families (both consanguineous and non-consanguineous) suggest that not all of them are linked to chromosome 15q13, indicating that JATD is likely to be genetically heterogeneous.

P751. Evidence for further locus heterogeneity of Meckel-Gruber syndrome.

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Meckel-Gruber syndrome (MKS), the most common monogenic cause of neural tube defects, is an autosomal recessive disorder characterized by a combination of renal cysts and variably associated features, including developmental anomalies of the central nervous system (classically prosencephalic dysgenesis, occipital encephalocele and rhombic roof dysgenesis), hepatic ductal dysplasia and cysts, and polydactyly. Locus heterogeneity has been demonstrated by the mapping of the *MKS1* locus to 17q21-24 in Finnish kindreds, *MKS2* to 11q13 in North African-Middle Eastern families, and our recent mapping of *MKS3* to 8q24 in a cohort of eight consanguineous kindreds, originating from the Indian sub-continent. We now report the ascertainment of a further eight consanguineous kindreds, with a MKS-like phenotype. This cohort appears to have the classic features of MKS, in comparison to those of *MKS3*-linked kindreds in which polydactyly (and possibly encephalocele) appear to be less common. In all eight families, we have used haplotype analysis to exclude linkage to the known MKS loci. To localize the gene(s) that underlies MKS, we have therefore adopted an autozygosity mapping approach, and are currently completing a genome-wide linkage screen. The identification of *MKS* genes will enable the development of molecular diagnostic tests to facilitate genetic counselling, carrier testing and prenatal diagnosis, and may also provide important molecular insights into fundamental developmental pathways.

P752. The intron 18/BclI polymorphic site in locus Xq28 used for linkage analysis of hemophilia A: both variants of this locus in affected man have been revealed

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Hemophilia A is a common X-linked bleeding disorder affecting approximately 1 in 10 000 males. Severity and frequency of bleeding are dependent on the degree of residual factor VIII activity. In humans, the gene for factor VIII maps to the distal long arm of the X chromosome (Xq28). It consist of 26 exons and spans 186 kb, coresponding to about 0,1% of the whole X chromosome. Noteworthy in this gene are the large exon 14, which codes for the B domain, and a large intron between exons 22 and 23. The most common intron 22 - inversion is responsible for about 40% of the severe hemophilia A cases while large delections, point mutations and small deletions or insertions are responsible for the disease in the rest of patients. Polymorphic restriction sites (RFLP-restriction fragment lenght polymorphism) can be utilized for molecular genetic diagnosis of hemofilia A. A genetic testing is to combine the PCR analysis of the intron 13 and intron 22 dinucleotide repeats with the PCR analysis of the intron 18 BclI marker. This protocol provided informative results in approximately 88% of families. We performed linkage analysis for family with two Hem A patients by using previously described

polymorphic systems (intron 13, intron 18 and intron 22). Unexpected result has been revealed in one of tested patients. Restriction analysis determined status of heterozygozity in intron 18. This result have been verified by sequence analysis on ABI PRISM 310 genetic analyzer.

P753. MTHFR genotype in hemodialysis patients

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Methylene-tetrahydropholate reductase (MTHFR) is an important factor in homocysteine metabolism, playing a role in the remethylation of Hcy to methyonine. MTHFR activity has genetic determination: C677T polymorphism of MTHFR gene, causes synthesis of thermolabyle form of enzyme, with decreased enzyme activity and consecutive hyper-Hcy-emia. Number of studies analyzed C677T frequency in vascular diseases, due to established toxicity of the Hcy to vascular endothelium. These investigations are important for nephrological patients too, trying to give answers about a role of genetic factors in hyper-Hcy-emia in renal failure. The aim of our study is to establish MTHFR C677T frequency in 105 hemodialysis patients, and to make correlation between MTHFR677 genotypes and Hcy plasma levels. Specific regions of MTHFR gene were amplificated by PCR method and C677T polymorphism was detected by restriction enzyme Hinf I. The frequencies of alleles in our patients were: 0.652 for allele C and 0.348 for allele T. The frequencies of genotypes were: 41.3%, 47.83% and 10.87% for CC, CT and TT genotype, respectively. There was no significant difference of genotype and allele frequencies between hemodialysis patients and control group. The patients had elevated mean level of plasma Hcy, and TT genotype showed significantly higher pHcy. The results have practical implementation, indicating etiology of hyper-Hcy-emia and possibilities for vascular disorders prevention in nephrological patients.

P754. A Search fo Genetic Markers in Disease Linkage Loci of distal Xq28 region

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The cytogenetic region q28 of the human X chromosome has great health relevance in Medical Genetics because it contains several disease loci whose causative genes are still unknown. In particular, two hereditary diseases of the central nervous system (CNS) map in the distal Xq28, X-linked polymicrogyria (PMGX) and a form of non specific X mental retardation (MRX72)
The difficulty to isolate new Xq28 disease genes is remarkable because of the paucity of polymorphic markers available for linkage study.
Thus, we have initiated a systematic search of new short tandem repeats (STRs) and single nucleotide polymorphisms (SNPs) in 1.6 Mb of distal Xq28, between G6PD gene and the telomeric repeat TTAGGG.
Starting from the nucleotide sequence, performed in our laboratory, we have selected new microsatellites. Sequence analysis of the genomic clones RP11-211L10, RP11-196H18, RP11-273L24, RP11-405N23 and RP11-53A12 was performed by CENSOR software looking for DNA repeated elements. Specific primer pairs were designed for each potential STRs and used in QF-PCR reactions. DNA pools from males of 40 CEPH families were genotyped to establish the number of alleles and their frequencies in the Caucasian population. Based on this strategy, we are able to establish the index of heterozigosity and consequently the power of each new Xq28 marker to be used in linkage studies.
We found significant evidences of the presence of several new STRs that we are currently using to narrow down the MRX72 locus.
Furter study are in progress to construct haplotypes of distal Xq28 to evaluate linkage disiquilibrium.

P755. Localisation of the gene for lacrimal/salivary gland dysplasia

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Lacrimal/salivary gland dysplasia (LSGD) is a rare autosomal dominant trait. The disorder is characterized by absent or dysplastic lacrimal and/or salivary glands. In some cases the absence or reduction in size of the glands is accompanied by absence of lacrimal puncta. The disorder has a variable expressivity and the diagnosis may be difficult in mild cases. A few families segregating for LSGD have previously been described but the molecular mechanisms behind the disease are yet unknown. We have identified a four-generation family from Southern Sweden with LSGD. The family comprises 25 individuals of which at least 12 are affected. No abnormalities in addition to LSGD are observed in the family. In order to localise the gene behind LSGD we performed a genome-wide linkage analysis with DNA samples from family members. Polymorphic microsatellite markers with an average spacing of 10 cM were used. Preliminary results indicate linkage to two chromosomal regions. These two chromosomal regions are currently being mapped at a higher resolution in order to define the candidate gene locus for LSGD.

P756. Locus heterogeneity of Bardet-Biedl syndrome (BBS) in consanguineous Pakistani families.

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Bardet-Biedl syndrome (BBS; MIM number 209900) is an example of a complex disorder with extreme heterogeneity that is characterized by mental retardation, pigmentary retinopathy, polydactyly, obesity, and hypogenitalism. Seven *BBS* loci are known (*BBS1* to *BBS7*), with approximately 23% of BBS patients thought to be unlinked to any of these loci. We have ascertained six affected probands from three large consanguineous families originating from the Indian subcontinent. Clinical assessment supports a diagnosis of BBS, with typical features. In two families, we have used haplotype analysis to exclude linkage to the known *BBS* loci, using a total of 32 microsatellite markers. In the third family, linkage could not be excluded from *BBS6*. To identify novel loci linked to BBS, we have therefore adopted an autozygosity mapping approach and have initiated a genome-wide linkage screen. The mapping of novel *BBS* loci will enable molecular genetic testing to be offered to large kindreds. This is particularly important for British families, as the phenotypic spectrum for BBS appears to be broad and could create problems in genetic counseling and prenatal diagnosis in the absence of a reliable diagnostic test.

P757. Association of angiotensinogen polymorphism M235T and essential hypertension in patients from the Republic of Macedonia

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Association of angiotensinogen polymorphism M235T and essential hypertension in patients form the Republic of Macedonia
ABSTRACT
Angiotensinogen polymorphism M235T is the most common genetics risk factor for essential hypertension. According to literature, the prevalence of this polymorphism in Europe is 30-60% among patients with Essential hypertension. European studies suggest 15-40% prevalence of this polymorphism in healthy population.
The aim of this study was to determine the prevalence of angiotensionogen polymorphism M235T in patients with essential hypertension and in healthy population from the Republic of

Macedonia, to determine whether there is a statistically significant difference and to give some clinical aspects.
The study was designed as a retrospective-prospective case-control study. We analyzed 40 patients and 40 healthy individuals. We postulated strict inclusion criteria for patients regarding to strict diagnosis of essential hypertension, family history of essential hypertension, the level of blood pressure or previous therapy. The polymorphism was identified with PCR-RFLP method, using specific primers followed by digestion with Tth 111 I restriction enzyme.
We found 55% prevalence of M235T in patients with essential Hypertension and 32,5% in the control group. There was a significant difference in M235T prevalence in both groups ($p<0.04$), and a positive association was observed between angiotensinogen polymorfism M235T and essential hypertension. This results suggest the role of angiotensionogen polymorphism M235T in ethiopatogenesis of essential hypertension in patients from the Republic of Macedonia.
Key wards: Essential hypertension, angiotensinogen, M235T

P758. Linkage analysis of Vesicoureteral Reflux using a candidate gene approach.

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Vesicoureteral reflux (VUR), the retrograde flow of urine from the bladder into the ureter and towards the kidneys, is a common disorder, found in 1-2% of newborn caucasians. 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. VUR occurs frequently in families, suggesting that it is inherited, but the mode of inheritance is still unknown. We have collected 480 DNA's from 97 families with more than one child affected with primary VUR. We are using this resource to assess candidate VUR susceptibility genes selected based on literature reviews.
The uroplakins (UPK) are integral membrane proteins that physically strengthen the bladder wall. Based on knockout studies in mice, Hu et al., 2000 suggested UPK3 as a candidate for primary VUR. Three of the 4 uroplakin genes have been investigated. Other genes investigated were GFRA1, the GDNF alpha-receptor essential for neuronal survival and renal morphogenesis; KAL 1, mutated in patients with Kallman syndrome, which is associated with VUR and BMP4 or bone morphogenetic protein 4 which co-ordinates ureteric growth with other secreted factors. We have excluded UPK3 and a region on chromosome 10q (25.3) as major candidates. We are currently screening more promising candidates for pathological mutations.

P759. Absence of mutations in genes AGTR2 and UPK3 in patients with vesico-ureteric reflux

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Primary vesico-ureteric reflux is a common anomaly of the urogenital tract in children. It has a strong association with urinary tract infections and permanent kidney damage. The reflux is in most patients of genetic origin but so far only mutated *PAX2* gene has been found to cause the vesico-ureteric reflux in a rare renal-coloboma syndrome. Several genetic loci were linked to vesico-ureteric reflux but candidate genes have not been identified as yet. Knock-out mice for genes *AGTR2* (encoding angiotensin II type 2 receptor) and *UPK3* (encoding uroplakin type 3) are prone to develop different anomalies of the kidney and ureter including vesico-ureteric reflux. Therefore it appears plausible to assume a role for these genes in the the development of vesico-ureteric reflux. The aim of this study was to screen the coding regions of the *AGTR2* and *UPK3* genes for potential mutations in patients with primary congenital vesico-ureteric reflux.
In 82 patients with primary congenital reflux genomic DNA was extracted from whole blood samples. The coding regions of *AGTR2* and *UPK3* were screened for mutations with heteroduplex analysis and subsequent sequencing.
In the coding region of *AGTR2* (exon 3) no mutations or single nucleotide polymorphisms were detected and in 6 exons of *UPK3* only silent
single nucleotide polymorphisms or polymorphisms in flanking intronic sequences were found.
These results indicate that mutations in *AGTR2* and *UPK3* are not the cause of the disease in our patients with vesico-ureteric reflux.

P760. Construction of a linkage disequilibrium map in a schizophrenia locus on 15q15 with microsatellite markers

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We earlier reported significant evidence for linkage on chromosome 15q15 in periodic catatonia (PECA), a sub-phenotype of schizophrenic psychoses. Recently we were able replicate this initial finding in a second independent set of families. Linkage and haplotype analysis in a set of exceptionally large multiplex families each showing linkage to this locus revealed an 11 cM critical region. In order to aid the positional cloning effort, we set out to construct a linkage disequilibrium (LD) map of this region using 57 microsatellite markers.
We genotyped these markers in 189 individuals from 44 families, 27 trios and 17 extended families with PECA. Next we determined phase using Simwalk2 and extracted the non-transmitted haplotypes. We assessed LD using the HaploXT algorithm as implemented in the GOLD software package (Abecasis and Cookson, 2000). We detected significant LD ($D'>0.3$; $p>0.05$) for a broad range of inter-marker distances up to 630kb. Three marker pairs showed $D'>0.5$ and strongest LD ($D'=0.65$) was seen for one marker pair 87kb apart. Though LD tented to decline with inter-marker distance no clear correlation could be observed. Using haplotypes with a frequency of $p>0.15$ only, a block-like pattern of LD over longer distances interrupted by short stretches without any detectable LD was discernable. Although this pattern resembles those generally seen for regions with high density SNP genotyping, resolution of LD block structure was imprecise. More dense typing of markers including SNPs is therefore necessary to be useful for disease oriented LD studies.

P761. The androgen receptor CAG repeat and the risk of Alzheimer's disease in men

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In elderly men, low serum testosterone has been associated with poor cognitive performance and with Alzheimer's disease (AD). Testosterone exerts various neuroprotective actions, all via the androgen receptor (AR). AR isoforms are the main, perhaps sole receptors for testosterone. The AR is expressed at high density in the human brain, eg in the hippocampus. The glutamine (CAG) repeat polymorphism has been associated with transcriptional activity of the AR and with several brain disorders.
In a Caucasian cohort from the Oxford region of 101 men with sporadic, 'definite' or 'probable' AD (mean onset age: 71 years) and 140 elderly male controls (mean age: 76 years), we divided the data into short (20 CAG repeats) and long AR alleles. Short alleles were associated with AD: odds ratio = 2.5 (95% CI: 1.2-5.0), adjusted for age and carrier status of the genes, APOE (4 and BCHE-K). This association was particularly strong in early-onset cases (< 65 years). In a subset of 79 cases and 128 controls, both short AR alleles and low serum levels of total testosterone were associated with AD, with odds ratios > 2 ($p < 0.05$) in each case. The odds ratio of AD for men with short alleles and lower tertile testosterone versus those with long AR alleles and upper tertile testosterone was 4.2 (1.4 – 13)
These results suggest that short AR alleles are associated with increased risk of AD in men and that low serum testosterone may interact with short alleles in that association.

P762. Physical map of a translocation breakpoint in two unrelated Tourette syndrome cases within a region previously linked to the disorder.

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Tourette Syndrome (TS) is a complex neuropsychiatric disorder characterized by both motor and vocal tics. The complete etiology of TS is not well understood, but evidence for genetic transmission comes from family and twin studies. A complex mode of inheritance has been suggested, likely involving contributions of different effect size from several genes. In such complex disorders the co-occurrence of chromosomal aberrations with the phenotype often proves helpful in identifying etiologically significant genes.
We have identified two unrelated families wherein balanced t(6;8) chromosomal translocations occur in individuals diagnosed with TS. In one of these families, there appears to be co-transmission of the translocation with learning and behavioral problems, the translocation in the case in the other family appears to occur *de novo*. The breakpoint on chromosome 8 is common to both families, and takes place in the same q13 band suggesting that a gene or genes in this region may contribute to the TS phenotype. In support of this hypothesis are the previously published reports of linkage to chromosome 8 in families with TS affected individuals, as well as cytogenetic reports that described chromosomal anomalies involving 8q in patients with TS.
We have undertaken positional cloning of the chromosome 8 breakpoint region, and have identified YAC and BAC clones that span the breakpoint, further reducing the region of interest to 120 Kb. Genes and EST's in this region are being screened for disruption in these families, and also for association with TS in a larger patient population.

P763. Monoamine related polymorphisms and haplotypes as risk factors in child psychiatric disorders

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One of the most common childhood onset psychiatric disorders is Attention Deficit Hyperactivity Disorder (ADHD), affecting 1-3% of school-age children. Family and twin studies indicate that genetic factors contribute significantly to the etiology of ADHD. Concerning the pathophysiology of ADHD the dopaminergic hypothesis is the prevalent, but the role of other monoamine systems (serotonin and noradrenaline) is also accepted. The highly heritable Tourette syndrome, which is characterized by chronic motor and vocal tics, is also a dopamine-related psychiatric disorder. A defect in the dopamine system - presumably in the frontal cortex and basal ganglia - is hypothesized in the etiological background of this syndrome. Searching for genetic risk factors in these child psychiatric disorders, as well as in other complex inheritance disorders, the allelic variants of polymorphic candidate genes are examined in association studies. Most of the candidate genes are from the dopamine system, but studies examining the components of the serotonin and noradrenaline systems are also emerging.
In family-based association studies we have examined the gene variants of the highly polymorphic dopamine D4 receptor (DRD4) gene. Besides genotyping the widely studied 48 bp VNTR of the DRD4 gene, direct haplotype analysis of SNPs and a 120 bp duplication polymorphism in the 5' non-coding region of the gene was also carried out. In addition, we have investigated the functional polymorphisms in the genes of the catabolizing enzymes (monoamine oxidase type A and catechol-O-methyltransferase) and VNTRs of the genes encoding the dopamine and the serotonin transporters.

P764. Screening SH3BGR gene for association with manic depressive disorder

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In a project examining the functional significance of promoter polymorphisms on chromosome 21q, we found a significant association between a SNP in the *SH3BGR* gene and Bipolar Affective Disorder 1 (BP) in a trios sample from the UK. The result was in the same direction in a case-control sample.
We screened *SH3BGR* for sequence variants in 16 BP individuals using DHPLC followed by sequencing. We identified a total of 12 polymorphisms - 3 amino acid changes, a deletion of one codon, 1 silent coding SNP, 6 intronic SNPs, and one 5 bp intronic deletion. We selected three more SNPs from public databases taking into account the haplotype blocks structure within the region, in order to also cover the region centromeric to *SH3BGR*.
Individual genotyping was performed with primer extension using fluorescently labelled primers on 6 non-redundant SNPs: 2 in the promoter, 2 amino acid changes, and 2 SNPs from databases (rs2837020 and rs2837012). The rest of the polymorphisms were redundant for genotyping, as they were in high r^2 (nearly identical) with some of the above markers.
Results: The 6 markers were typed in 122 BP trios from the UK, 147 BP trios form Bulgaria, 157 BP cases and 183 controls from the UK. The original promoter SNP (-402A/T) remained the most significant marker: the common allele A was transmitted 103 times and not transmitted 72 times, p=0.02 in the combined set of 257 trios, and its frequency in case/controls was 83.4% v. 78.7% respectively, p=0.16.

P765. The analysis of polymorphisms of 5HTT, 5HT2A, COMT genes in Tatar and Russian suicide attempters

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Suicidal behavior is an important public-health problem. The etiology of suicidal behavior is certainly complex, genetic factors likely playing an important role. The aim of this study was to test contribution of some candidate genes in suicidal behavior: serotonin transporter (5HTT) (VNTR and 5-HTTLPR polymorphisms), serotonin receptor 2A (5HT2A) (Msp1-restricted polymorphism) , catehol-O-methyltransferase (COMT) (Hsp-92II-restricted polymorphism). 65 Russians and 64 Tatar suicide attempters were included in the study. The control group consisted of 300 voluntaries. All subjects were typed for the above-mentioned gene variants using PCR technique. Significant differences were found in 5HT2A genotype (chi2= 5.84, P = 0.05) and allele (chi2 = 4.03, P = 0.05) frequencies between Tatar suicide attempters and controls, connected with decrease of GG genotype (chi2 = 5.81, P = 0.02) in suicidal group. There were significant differences in COMT genotype frequencies between Tatar suicide attempters and controls (chi2= 12.96, P < 0.01) connected with decrease of HL genotype (chi2= 10.7, P < 0.01) and increase of HH genotype (chi2= 10.9, P < 0.01, OR = 3.3) in Tatar suicide attempters group. We found significant differences in 5HTT allele frequencies (I/D polymorphism) (chi2= 6.19, P = 0.01) and in COMT allele frequencies (chi2= 3.77, P = 0.05) between Russian suicide attempters and controls. There was increase of 12/10 (5HTT VNTR polymorphism) (chi2 = 7.52, P = 0.04, OR = 2.5) in Russian suicidal group.

P766. The analysis of a catechol-o-methyltransferase gene polymorphism of different ethnic origins from Bashkortostan with paranoid schizophrenia

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The Val/Met polymorphism of the catechol-o-methyltransferase (*COMT*) gene in schizophrenia was analyzed using PCR. *COMT* is a major component of the metabolic pathways of neurotransmitters such as dopamine, adrenaline and noradrenaline. 344 patients with paranoid schizophrenia from Bashkortostan (127 Russians,

109 Tatars and 108 Bashkirs) aged 15 - 74 were included in the analysis. The control group consisted of 423 persons (115 Russians, 168 Tatars and 140 Bashkirs). The genotypes H/H, H/L, L/L in the *COMT* gene are detected. The results of the investigation are shown in the table 1. Our data suggest that the *COMT* gene is likely to play a major role in the aetiology of paranoid schizophrenia in the Bashkortostan population.

Genotypes and alleles **(P < 0.05, OR- odds ratio)**		
Bashkirs	**Tatars**	**Russians**
H/H (*P*=0.023, OR=**2.11**) H/L (*P*=0.042, OR=**0.53**)	H/H (*P*=0.002, OR=**2.83**) H/L (*P*=0.001, OR=**0.39**)	H/H (*P*=0.004, OR=**2.38**) H (*P*=0.009, OR=**1.70**) L/L (*P*=0.046, OR=**0.50**)
Patients with good heritable anamnesis (N = 168)		
H/H (*P*=0.028,OR=**2.30**), H (*P*=0.046, OR=**1.73**)	H/L (*P*=0.021, OR=**0.41**)	H/H (*P*=0.007, OR=**2.98**), H (*P*=0.002, OR=**2.10**) L/L (*P*=0.035, OR=**0.38**)
Patients with family history of schizophrenia (N = 175)		
--	H/H (*P*=0.003, OR=**4.20**), H (*P*=0.001, OR=**2.07**), H/L (*P*=0.001, OR=**0.34**)	--
Patients with continual type of paranoid schizophrenia (N = 190)		
--	H/H (*P*=0.011, OR=**2.59**) H/L (*P*=0.004, OR=**0.33**)	H/H (*P*=0.011, OR=**2.07**), H (*P* = 0.008, OR=**1.59**)
Patients with episodic type of paranoid schizophrenia (N = 153)		
H/H (*P*=0.006, OR=**2.74**), H (*P*=0.049, OR=**1.79**), H/L (*P*=0.025, OR=**0.42**)	H/H (*P*=0.001, OR=**3.15**), H (*P*=0.036, OR=**1.70**), H/L (*P*=0.018, OR=**0.43**)	--

P767. Gene expression analysis in autism using cDNA microarrays

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There is strong evidence from twin and family studies for the importance of genetic factors in idiopathic autism. The results from independent reports suggest that the disease is caused by the action of several genes, possibly acting epistatically. We have used cDNA microarray technology to clarify the molecular mechanism behind autism. Blood samples were obtained from affected sib-pairs co-segregating for chromosome 7q31, previously shown to be a candidate region for autism. The RNA was obtained from EBV-transformed B-lymphocytes as the only RNA source available. mRNA levels for approximately 7,700 genes were assessed by in-house produced cDNA microarrays. The microarray data was analysed using dedicated computer software in order to identify significant up or down regulation of specific genes. Several genes with small but significant down regulation were identified in the samples derived from individuals with autism. One of these genes encodes SEMA5A, which is involved in axonal guidance.

P768. Association of angiotensin converting enzyme insertion-deletion polymorphism with juvenile rheumatoid arthritis in Kuwaiti Arabs

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Juvenile rheumatoid arthritis (JRA) is a heterogenous chronic disease with autoimmune pathology. Angiotensin converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism influence the plasma and tissue levels of ACE and has an involvement in the inflammatory mechanisms. The incidence of ACE gene polymorphism genotypes has been determined in 82 children with JRA from Kuwait and compared to that in 48 ethnically matched normal healthy controls using a polymerase chain reaction method. A considerably higher incidence of II genotype was observed in the JRA patients compared to controls ($P < 0.008$). In contrast, no statistically significant difference was detected in the incidence of DD and ID genotypes in JRA patients and that in the controls ($P = 0.275$ and 0.598 respectively). The incidence of ACE gene polymorphism genotypes was also studied in clinical subtypes of JRA patients and controls. There was no significant difference in the incidence of DD and ID genotypes in either of the three JRA subtypes (oligoarticular, polyarticular and systemic) when compared to controls. However, the incidence of II genotype was found to be significantly higher in all three JRA subtypes compared to controls. The strongest association between II genotype and JRA subclasses was detected in systemic JRA ($P = 0.005$), followed by polyarticular JRA ($P = 0.03$) and oligoarticular JRA ($P = 0.012$). Our data suggests an association of the I-allele of ACE gene polymorphism with the three clinical subtypes of JRA in Kuwaiti Arabs.

P769. Confirmatory evidence for linkage of relative hand skill to 2p12-q11

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We previously performed the first genomewide linkage screen for a measure related to handedness in humans, in which we found evidence for a quantitative trait locus (QTL) influencing relative hand skill on chromosome 2p12-q11 (pointwise P=0.00007). Now, we have found further evidence for the 2p12-q11 QTL in a new sample of 105 pairs of adult brothers who wrote with their left hands. As before, we used Annett's peg moving task to derive a continuous measure of relative hand skill. We typed 7 microsatellite markers spanning 2p16-q14, and employed basic DeFries-Fulker linkage analysis. The peak multipoint linkage t score was -3.51 (empirical pointwise P=0.0009), thus greatly exceeding significance guidelines for confirmation of linkage. Most twin studies of handedness have dichotomised the phenotype, and have found weak or non-significant genetic effects on the trait. Our replication of the 2p12-q11 QTL may illustrate the greater power that is inherent in quantitative descriptions of etiologically complex phenotypes.

Roughly 90% of individuals perform complex manual tasks preferentially with their right hands, while slightly less than 10% are left-handed, and a small proportion are ambidextrous. No other primates show a population-level bias in handedness, and individual differences in human handedness are correlated with cerebral hemispheric asymmetries that underlie much complex human cognition, including language. We predict that the gene containing variants underlying the 2p12-q11 QTL has an important role in the development of cerebral lateralisation, and may have been involved in the evolution of complex human cognition. Studies aimed at identifying the gene(s) are underway.

P770. RET proto-oncogene polymorphisms define haplotypes associated with Hirschsprung Disease

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Hirschsprung Disease (HSCR) is a congenital malformation characterized by the absence of the enteric ganglia along variable lengths of the intestine.

It shows a complex pattern of inheritance with high proportion of sporadic cases, variable expressivity and incomplete sex-dependent penetrance. The RET proto-oncogene is a major gene, with mutations reported in less than 10-20% of sporadic cases, while other genes, overall, account for less than 5% of HSCR patients.

Common polymorphisms of RET have been reported in association

with modified risk of HSCR development and, in particular, specific haplotypes have been demonstrated to display either protective or predisposing effects, and/or to modulate the severity of the resulting phenotype.
To focus on the role of common variants of RET in HSCR susceptibility, in the present study we have genotyped 98 Italian sporadic cases and 85 population matched controls for 9 single nucleotide polymorphisms (SNPs) distributed throughout the entire gene (50Kb).
Six of these markers showed allele frequencies differently distribuited in cases vs controls: four SNPs (at -5 bp in the promoter, in exons 2 and 13 and in introne 19) resulted significantly overrepresented, while other two (at -1 bp in the promoter and in exon 14) were underrepresented.
Reconstruction of haplotypes for all these variants in the complete cohort of cases and controls is actually in progress using an informatic approach.
Results will enable us to start a search for functionally active, low penetrant RET variants expected in linkage disequilibrium with the RET genotypes demonstrated to affect the HSCR risk.

P771. Human FRDA YAC transgenic mice containing GAA repeat expansions

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Establishing an accurate GAA repeat expansion animal model of Friedreich's ataxia (FRDA) is considered to be essential for investigating potential therapeutic strategies for this disorder. In our initial studies towards this goal, we have demonstrated functional rescue of frataxin knockout mice with wild-type human genomic YAC and BAC FRDA transgenes. Three YAC transgenes and two BAC trangenes, ranging from 140kb to 370kb, all produced successful rescue of frataxin knockout mice. More recently, we have generated human genomic YAC FRDA transgenic mice that also contain human GAA repeat expansion mutations. In particular, we have established a 200 GAA repeat–containing transgenic line that shows intergenerational instability, including both increases and decreases in GAA repeat size. Initial molecular studies of this line detected high levels of human frataxin expression and we are currently performing histological and neurobehavioural analyses on these mice to determine a possible phenotype. We also intend to perform biochemical analysis and more detailed frataxin mRNA and protein expression analysis on tissues from different sized GAA repeat FRDA transgenic mice. A second line that contains two human FRDA YAC transgenes with approximately 100 and 200 GAA repeats is also under preliminary investigation. These transgenic mice may be of considerable use for future investigations of in vivo GAA mutation correction therapies and therapies aimed at alleviating GAA blockage of frataxin transcription, as well as other FRDA gene and drug therapies.

P772. Modeling HLA DRB1 in rheumatoid arthritis

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Objective. The only genetic factor known with certainty in rheumatoid arthritis (RA) is HLA DRB1. Susceptibility to RA is classically studied looking for the shared epitope (SE) hypothesis described by Gregersen et al (H1). To refine this hypothesis, Reviron et al. suggested that, in addition to the predisposing effect of SE positive alleles, the charge of the P4 pocket (P4+, P4-) might influence susceptibility to RA (H2). We proposed to study, on a French sample of cases, these two hypotheses.
Methods. One hundred thirty two affected sib pair (ASP) were recruited as part as a European Consortium (ECRAF). One randomly selected sib of ASP was chosen as index. Genotype Relative Risk (GRR) were computed for the index taking into account for ascertainment scheme, with SE/SE genotype as reference. Conditionally to the genotype, the number of HLA haplotypes shared by the two sibs were computed (IBD distributions). These distributions were compared to what expected under GRR estimates.
Results. Under H1, GRR are 0.42 for SE/X genotype and 0.21 X/X genotype. Under H2, if P= P4+ and SE - alleles, GRR are 0.29 for SE/P, 0.20 for P/P, 0.58 for SE/P4-, 0.19 for P/P4- and 0.31 for P4-/P4- genotypes. The GRR in H2 are not ordered as predicted by Reviron et al. In addition, under both H1 and H2, IBD distributions do not fit what expected.
Conclusion. None of the two hypotheses correctly explain the HLA observations in our sample. An alternative explanation will be presented.

P773. Polymorphism of *NRAMP1* and *IL12B* genes in Russians with tuberculosis and salmonellosis

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A number of investigations are devoted to genetic predisposition to infectious diseases, but the results are controversial. We evaluated the common polymorphisms of the *NRAMP1* (469+14 G/C, 1465-85 G/A, C274T replacements) and *IL12B* genes (A1188C replacement) in Russian tuberculosis (TB, n=58) and salmonellosis (n=55) patients and unaffected controls (n=127).
In TB patients a trend to significant deviation of 469+14 G/C and C274T genotypes from that expected under Hardy-Weinberg equilibrium was shown (p=0.099 and p=0.048, respectively), indirectly suggesting an association of the variants with the disease. A comparison of distributions of genotypes and frequencies of alleles between the TB patients and controls did not confirm this assumption. A comparison of distributions of genotypes and allele frequencies in salmonellosis patients and controls did not reveal a difference for *NRAMP1* polymorphisms, whereas there was a significant discrepancy in the distribution of genotypes of the *IL12B* polymorphism A1188C (table, p=0.034).
Overall, the data obtained suggest an absence of association between the commom polymorphisms in *NRAMP1* and TB or salmonellosis. At the same time, the A1188C polymorphism in *IL12B* seems to be associated with salmonellosis in Russians and this suggestion has to be confirmed in an expanded sample and in other ethnic groups.

Sample	*IL12B* genotypes, %		
	AA	AC	CC
Salmonellosis, n=55	46.9	36.7	16.4
Controls, n=127	60.0	35.6	4.4

P774. Genetic polymorphisms of cytochrome P450 (CYP2E1, CYP1A1) and susceptibility to alcoholic liver disease in male Russian patients

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Alcoholism is an important cause of chronic liver disease, but alcoholics cirrhosis develops only in 10% - 15% of individuals. Alcohol is oxidized initially to acetaldehyde, principally by alcohol dehydrogenase (ADH) and cytochrome P450 (CYP2E1, CYP1A1), and then to acetate by aldehyde dehydrogenase (ALDH). CYP2E1 and CYP1A1 are key microsomal enzymes that metabolize alcohol in the non-alcohol dehydrogenase pathway. Polymorphisms of these ethanol-metabolizing enzymes may by associated with inter-individual difference in alcohol metabolism and susceptibility to alcoholic liver disease (ALD). We determined genotypes and alleles frequencies of CYP2E1 and CYP1A1 genes in male Russian patients with ALD (n=36) and healthy control group (n=105) using PCR-PFLP method. The c1c2 genotype of CYP2E1 gene in ALD group was 11,4% versus 5,5% among control group. The distribution of Val/Ile-genotype of CYP1A1 gene was 11,4% and 5,1% respectively. Although we have not observed significant differences between studied groups, we found higher frequencies of heterozygous genotypes of CYP2E1 and CYP1A1 genes in male Russian patients with ALD.

P775. Glutathion S-transferase M1 gene polymorphism and susceptibility to endometriosis in Bashkortostan population

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Polymorphisms at the glutathion S-transferase M1(GSTM1) gene locus have attracted much interest because the homozygous GSTM1 deletion (GSTM1 0/0) seems to modify the risk for different types of cancer, allergy and other disease. Endometriosis is a multifactorial disease with possible genetic predisposition and involvement of environmental factors in its pathogenesis.
Polymorphism at the GSTM1 gene locus in 66 patients with endometriosis from Bashkortostan and in 90 healthy individuals from control group was studied by PCR method. The frequencies of the GSTM1 0/0 genotypes were 31,8% in patients with endometriosis and 54% in control group. The data of this study demonstrate than the differences in distribution of GSTM1 allele frequencies between patients with endometriosis and control individual were significantly (x2=9,874; p=0,002). We purpose that these results may be a effect of different individual resistance to therapy.

P776. SNP genotyping in candidate genes predisposing to sporadic Creutzfeldt-Jakob disease

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Sporadic Creutzfeldt-Jakob disease (sCJD) is the most common form of prion disease worldwide. The cause of this disease is unclear. One genetic factor predisposing to sCJD is known being a common polymorphism in the coding region of the PRNP gene at the codon 129 (M129V). However, the major predisposing factor(s) for sCJD are still unknown. Genotyping the genomic DNA of 584 sCJD cases and 749 healthy controls by means of MALDI-TOF MS (MassArray, Sequenom, San Diego), we observed significant association between sCJD and the allele frequency of the PRNP-SNP 129[A/G] confirming the predisposing role of methionine and homozygosity at codon 129 in the CJD cases. Furthermore, we detected a highly significant association between the SNP PRNP_117 and sCJD which might act as an additional risk factor to the SNP PRNP_129.
Currently we are genotyping a set of another 10 SNPs in the PRNP locus. Analysing these polymorphic positions in the PRNP locus will be useful to evaluate published data on some of these positions which were derived from much smaller cohorts and produced conflicting data. The role of the PRNP polymorphisms in sCJD aside from the codon 129 polymorphism will be much clearer defined. In addition, we are studying 2 SNPs within the PrP-like protein gene (PRND) as well as 10 polymorphisms in three further genes which are known to be differentially expressed during prion infection or to interact with prion proteins. Genotyping of these candidate genes could result in the identification of novel informative polymorphisms associated with sporadic CJD.

P777. Real-time PCR technology using SYBR Green I dye: A method for the relative quantification of mitochondrial gene expression in Alzheimer´s disease

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Reverse transcription followed by real-time PCR using the SYBR Green I dye is a powerful tool for the detection and quantification of mRNA. The sequence-independent detection of DNA with SYBR Green I means that it can be used to quantify the amplification of any cDNA using gene-specific primers. Generally, two quantification strategies can be performed: An absolute and a relative quantification. The relative quantification is based on the expression ratio of a target gene versus a reference gene and is adequate for most purposes to investigate physiological changes in gene expression levels. In this work we used a relative quantification strategy to determine the relative expression of four mitochondrial genes (cytochrome b, 12S, ND4 and COX II) and one reference gene (cyclophilin gene) in blood and in three brain areas (cerebellum, hippocampus and frontal cortex) from two groups of controls and Alzheimer's disease patients. We used the model developed by Dr. Pfaffl's group to manage our data, this model allows comparisons between groups for the reference gene and for the target genes and tests the group differences for significance with a randomisation test. The expression of the target genes are normalised by the non-regulated reference gene expression. The relative expression ratio is calculated from the real-time PCR efficiencies and the crossing point deviation of an unknown sample versus a control. This rapid and flexible real-time PCR method is suited for researchers wishing to quantify mRNAs from many different genes because it does not require investment in gene-specific hybridisation probes.

P778. 'Contribution of polymorphisms of NOS3,GSTT1, GSTM1, CYP2E1 genes and haplogroups mtDNA to human radiosensitivity'.

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Human radiosensitivity is a trait with complex inheritance determined by multiple genes, but many of which are not yet defined. We studied 82 Siberian Chemical Plant workers from Seversk with cumulative dose of exposition to radiation from 0.02 to 1.37 Zv. They were divided in two groups: control group (n=46) with 0.33-2.45 % of chromosomal aberrations in 100 cells or absence mutations in minisatellite loci B6.7, CEB1, CEB15, MS1, MS31, MS32; case group (n=36) with 5.5-39.37 % of aberrations in 100 cells or presence mutations in minisatellite loci. In these groups we studied polymorphisms of NOS3 gene (VNTR, C774T, G894T, A924T), GSTT1 and GSTM1 (null-alleles), CYP2E1 (DraI-RFLP), and frequency of mitochondrial DNA haplogroups H, U, and T. All of these genes are considered to be candidates for several complex diseases, and it is believed that they could influence individual radiosensitivity of human. It was detected, that Seversk inhabitants are characterized by similar allels and genotypes frequency in comparison with other Caucasoid world populations by polymorphic variants of NOS3 gene (VNTR allel "a"- 26%; C774T allel "c"- 75%; G894T "g"-41%; A924T "a"- 40%); GSTT1 0/0 - 10.4%; CYP2E1 allel "c"- 86.6%; H - 57.1%, U- 19.1%, T - 14.3% mitochondrial DNA haplogroups and higher frequency of GSTM1 0/0 -75.7%. There were not discovered any differences of allels and genotypes frequency among control and case groups. It was detected, that the combination of GSTT1; GSTM1 genes probably contribute to human radiosensitivity (p=0.1).

P779. Genetic Association of Psoriasis and Psoriasis Arthritis to the Candidate Gene SLC12A8 at PSORS5 on Chromosome 3q

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Recently, SLC12A8 on chromosome 3q was proposed as a candidate gene for psoriasis arthritis based on association studies in a Swedish cohort (Hewett et al, 2002). Stratification for joint affection resulted in positive association for five intragenic SNPs and the microsatellite D3S1551. We now analysed these markers in a cohort of 210 Falk-Rubinstein-trios with psoriasis and could not confirm this association as only one SNP (EC2) gave marginally significant evidence for association (p=0.0478). One haplotype (B1551S4/ B1551S3/ EC2) also showed weak association (p=0.0321). As this psoriasis study group contains only few patients with arthritis, we conclude that this gene is not involved in skin type of psoriasis, at least in our sample. Recently we started a new study on psoriasis arthritis aiming at recruiting 300 psoriasis patients with joint inflammation, both in case-control and trio designs. So far, we were able to analyse the

5 SNPs in the SLC12A8 gene for the first 140 patients and 228 healthy controls. In contrast to the skin psoriasis study group, we could confirm association of psoriasis arthritis to SLC12A8 for the SNP B1551S3 (χ^2=5.96, p=0.015). We will now attempt to confirm this finding in the larger cohort. In summary, this report is the first to confirm association to SLC12A8. We conclude that this locus is an interesting candidate for psoriasis arthritis but not psoriasis and that psoriasis arthritis may represent a clinically and genetically divergent entity.

P780. Confirmation of a novel locus for familial hip osteoarthritis on chromosome 6p

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Background – Osteoarthritis (OA) is the commonest musculoskeletal disorder to afflict people in the developed world. Recently a female specific locus on chromosome 6p with a maximum multipoint LOD score (MLS) of 4.6 for marker D6S1573 has been described by Loughlin *et al* in sib pairs with hip OA.
Objective – To determine the significance of this region which encompasses two potential candidates, COL9A1 and bone morphogenetic protein 5 (BMP5), in a cohort of Northern Irish sibling pairs with primary hip OA
Methods – We examined 288 sibling pairs (109 pedigrees) who had undergone total hip replacement for primary OA. Microsatellite markers (D6S1573, BMP5, D6S1276, D6S1557 and COL9A1) were amplified using the multiplex PCR kit (Qiagen®) and the subsequent alleles typed using Genescan and Genotyper software (Applied Biosystems). Multipoint linkage analysis was performed using GENEHUNTER – PLUS .
Results – Table 1 shows the MLS for the markers examined. Marker D6S1573 had the highest MLS of 2.9. No significant linkage was demonstrated to BMP5 and COL9A1. Female sib pairings comprised 80% of the overall maximum MLS. Pedigrees with more than two affected siblings had a maximum MLS of 0.89 for D6S1573.
Conclusion – We support linkage to the region on chromosome 6p mapped by D6S1573 but did not find the locus to be female specific. No significant linkage existed to two potential candidates BMP5 and COL9A1 downstream of D6S1573. Large family pedigrees made only a modest contribution to the overall MLS.

Table 1

Marker	MLS (All pedigrees)	MLS (Female sib pairs only)	MLS (Pedigrees with >2 pairs)
D6S1573 BMP5	2.90 1.57	2.32 1.55	0.89 0.10
D6S1276 D6S1557	1.45 0.53	1.54 0.42	-0.96 -2.99
COL9A1	0.52	0.41	-0.31

P781. Should Sporadic Hirschsprung's Disease be screened for MEN2A associated mutations in the RET proto-oncogene?

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We report the case of three half-siblings who presented with Hirschsprung's disease (congenital aganglionic megacolon, HSCR). There was no family history of HSCR or carcinoma. Sequencing of the RET proto-oncogene revealed a C620R mutation in all the affected children. This mutation is predominantly associated with Multiple Endocrine Neoplasia 2A (MEN2A), though HSCR is found at low penetrance in these families.
The children's mother was referred for endocrinological investigation, and is now being treated for Medullary Thyroid Carcinoma (MTC). Subsequent analysis confirmed that she carries the mutation.
The oldest child is approaching the age at which prophylactic thyroidectomy is advised, and members of the more extended family are undergoing predictive testing.
MEN2A is a dominantly inherited cancer syndrome predisposing gene carriers to medullary thyroid carcinoma (MTC), phaeochromocytoma and hyperparathyroidism. MTC is an aggressive tumour which metastasises early and has a poor prognosis. Up to 50% of MTC cases present with cervical lymph node metastases. MEN2A gene carriers develop MTC in teenage life. Identification of gene carriers, screening and prophylactic thyroidectomy are therefore an important part of management of this condition.
Early diagnosis of MEN2A is vital. HSCR is common, affecting 1 in 5000 live births. We therefore discuss whether ALL cases of HSCR, whether sporadic or familial should routinely be offered molecular analysis for all 3 Cysteine replacement mutations known to be associated with both diseases, in order to identify those families with potential future problems.

P782. A genetic study of autoimmune thyroid diseases in a tunisian isolate.

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The autoimmune thyroid diseases (AITDs), which include Grave's Disease (GD) and Hashimoto's Thyroiditis (HT) are multifactorial diseases with a significant genetic and environmental component. In order to determine genetic factors involved in development of AITDs, we have performed a clinical follow-up, a genealogical study and candidate gene analysis in a large Tunisian isolate. All family members were subjected to a regular clinical follow up. The number of affected individuals has reached 65 in 2002. Genealogical study showed that rates of endogamy are 82.9% and 95.19% in the isolate and in the same region respectively. The mean coefficient of consanguinity, estimated according to the method of Malecot (1948), is 0.02748 in the Akr family and 0.01661 in the region where the family members live. Transmission mode of AITD in the studied isolate was determined using the maximum likelihood method implemented in the Pointer program. We found that it is due to a major recessive mode. Given the role of MHC in the emergence of AITD, we have tested polymorphisms of genes inside the MHC region: HLA class I and II, *HSP-70* gene, *TNF*α and β gene. Significant values were found for *TNF*α and β, *HSP70*-02 and hom and HLADQ loci using the Sibling Transmission Disequilibrium Test (S-TDT) program (z= 7.959; 7.640; 7.988; 9.618; 7.615 respectively ; with p values $<10^{-10}$). This finding suggests that these genes play a role in the development of AITDs in this family. We are currently performing an analysis of the MHC haplotype.

P783. Osteopenia of prematurity (OOP) and genetic polimorphisms: a pilot study

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OOP occurs in more than 30 percent of very low birth weight (VLBW) infants (<1500 gm) and in 50 percent of those weighing less than 1000 gm. In adults an association was found between certain genetic polymorphism (vitamin D receptor (VDR), oestrogen receptor (OER), collagen 1α1 (COL1A1) gene) and the occurence of osteoporosis. The **purpose** of our study was to evaluate the hypothesis, whether the genotypes of these three polymorphic loci are associated with osteopenia in VLBW infants.
We performed a pilot study in order to diagnose osteopenia by collecting serum and urine samples from 20 VLBW infants (7 female-13 male, mean gestational age: 29 ± 2.3 weeks, birthweight 1150 ± 227 gm). OOP was diagnosed radiologically; and by measuring serum ion and hormone concentrations; and by bone resorption assessment (urinary pyridinium cross links). Polymorphisms of the

VDR, OER and COL1A1 genes were identified by generally accepted PCR-based methods. **Results**: Five infants had OOP at the age of 6 months according to radiological signs, high levels of alkaline phosphatase and osteocalcin, and high urinary pyridinium cross-links excretion. Twelve infants had Tt, 6 TT, 2 tt genotype of the VDR gene; we found 10 infants with COL1α1 Cc, 8 infants with CC and 2 infants with cc genotype. There were 9 different oestrogen receptor gene polimorphisms. We did not find any difference in the genotypes examined between infants with and without OOP. **Conclusion:** In our pilot study no association was found between VDR, OER, COL1α1 polymorphisms and OOP.

P784. Mutations of *FOG-2* gene in sporadic cases of tetralogy of Fallot

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FOG-2, a protein acting as a co-regulator of the transcription factor GATA-4, maps on chromosome 8q22. *Fog-2* gene mutations in mice can cause congenital heart defects including tetralogy of Fallot (ToF). Although the multifactorial model was considered a likely mechanism for ToF, a number of familial cases were described. Recently *NKX2.5* and *JAG1* gene mutations were reported in some individuals with isolated ToF. However, single gene mutations seem to account for a minority of ToF. We screened FOG-2 gene mutations in 47 patients with non syndromic ToF with SSCP analysis and sequencing. Unique heterozygous missense mutations were found in two unrelated ToF patients. Both were A◊ G transitions and changed conserved aminoacids, located in GATA factors binding domains . Variations were detected in one unaffected parents in both families, but not among 200 control chromosomes . To better understand the underlying mechanisms of the detected FOG-2 mutations, as cause of some sporadic cases of ToF, functional studies are in progress. Preliminary data has not shown significant reduction in the affinity of FOG-2 for GATA-4 *in vitro* but, because of the presence of four independent zinc finger domains interacting with GATA-4 factor, a single mutation would not be expected to eliminate FOG-2 binding site activity. FOG-2 gene mutations could be considered as a cause of some sporadic cases of ToF, with low penetrance, even if the precise molecular mechanism by which the mutations alter its activity, is not clear.

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P785. Familial aortic dissection/aneurysm with patent ductus arteriosus. A new entity.

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Familial thoracic aortic aneurysms and aortic dissections (TAA and AD) may account for around 20% of total TAA or AD cases, a frequent cause of sudden death especially in young adults. A genetic heterogeneity with two identified genes (*FBN1*and *COL3A1*) and two loci (5q13-q14 and 11q23.2-24) has been shown previously.
We report a large pedigree composed of 179 members from which forty subjects belonging to three generations were investigated and screened (standardized clinical and cardiovascular examination, transthoracic echocardiography and thoracic MRI). There were four cases of AD and four cases of TAA. Six cases of patent ductus arteriosus (PDA) were observed and five were detected in asymptomatic children or adults. Three subjects have both PDA and TAA or AD. There were also five cases of stroke and three cases of sudden death. The distribution of these vascular abnormalities in the family was compatible with an autosomal dominant pattern of inheritance. No subjects have signs of Marfan, Elhers-Danlos vascular type or Char syndromes. Genetic linkage analysis was performed for six genes or loci implicated in familial TAA/AD disease (*COL3A1*, *FBN1*, *MFS2*, 5q13-q14 and 11q23.2-q24) or in Char syndrome (*TFAP2B*). Under the assumption of a rare autosomal allele responsible of TAA/AD (frequency:0.0001; age dependant penetrance, phenocopy rate:4/100000, MLINK program from Linkage package), all the six loci were excluded as responsible for the pathology.
Our findings are consistent for a novel vascular mendelian form of familial TAA/AD, as reported twice in the literature. A genome-wide search will be performed.

P786. Analysis of metobolic genes polymorphisms in endometriosis patients.

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Endometriosis is one of the most common gynaecological diseases. The aetiology and pathogenesis of endometriosis remain obscure so far. It is usually treated as a complex multifactorial disease with obvious genetic predisposition, immunological failure and possible involvement of noxious environmental factors. Polymorphisms of metabolic genes (*NAT2*, *GSTM1*, *GSTT1*) responsible for xenobiotic conjugating enzymes of the Phase II detoxification system were studied by PCR-RFLP in women with and without endometriosis. The study group consisted of 120 women with clinically, endoscopically and histologically proved endometriosis, the control group – 90 women without endometriosis. The frequencies of *GSTT1*0/0 and GSTM10/0 genotypes were significantly higher in the group of endometriosis patients compared to controls (32% versus 18% and 55% versus 41%, respectively). The frequencies deletions in *GSTT1* and *GSTM1* genes increased in the group of patients with severe endometriosis (III-IV stage). The genotype distributions of *NAT2* alleles were identical in controls and in the group of endometriosis patients. Concordance of both *GSTT1* 0/0 and *NAT2* S/S genotypes was found in 28% of endometriosis patients and was almost 6 time more compared to only 5% of these subject in the control (OR 5,92 (CI95% 2,43-14,61)). The 46% of patients had at least two functionally impaired genotypes for studied genes. Screening of *GSTM1*, *GSTT1*, and *NAT2* gene polymorphisms as a feasible predictive genetic test for early identification of women at high risk of endometriosis has been suggested.

P787. FISH-mapping of translocation breakpoints associated with autism.

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Autism is considered to be a neurodevelopmental disorder with major but so far unknown genetic factors involved. Genome scans have implied the existence of candidate loci in many chromosomal regions, including: 1p13.2; 1p21.1; 2q21; 2q31.1; 3p25.3; 4p16.3; 5p13.1; 5p15.33; 6q16.3; 7p15.2; 7q21.2; 7q31.33-36.2; 10p12.1; 10q22.3; 13q12.3; 13q22.1; 15q12; 15q21.1; 16p13.12-13.2; 17p13.2; 18q22.1; 18q21.33; 19p13.12; 19q13.41; 22q11.21; 22q11.23; Xq21.33; Xq26.1, where 7q31-34 and 16p13.1-13.2 are the most consistent findings.
Mendelian Cytogenetics Network (MCN) is a collaborative study to identify disease associated balanced chromosomal rearrangements. MCN database (MCNdb, http://www.mcndb.org) at present contains data on 51 patients with autism and a constitutional balanced chromosomal rearrangement. Approximately twenty of these patients have breakpoints coinciding with regions highlighted by the genome scans. We are currently FISH-mapping the breakpoints of

three patients from MCNdb in our search for genes associated with autism: A: 46,XY, t(4;16)(q24;p13.3); B: 46,XY, t(9;18)(p22;q21.1)pat, inv(10)(p11.2q21.2)mat and C: 46,XX, t(5;18)(q34;q12.2)de novo. The initial mapping of the 16p13.3 breakpoint of patient A has shown that the breakpoint is outside of, and distal to the autism-susceptibility region indicated by the genome scans, but the candidate genes identified at the 16p13-breakpoint as well as at the other breakpoints will be analyzed in patients with autism.

P788. Involvement of detoxification system enzymes in pathogenesis of some common multifactorial diseases. Predictive genetic testing.

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This report summarizes our data concerning involvement of some genes participating in Phase 1 and Phase 2 of detoxification systems in the origin, progression and treatment efficiency of such common multifactorial diseases as endometriosis, bronchial asthma, alcoholic cirrhosis, habitual miscarriages, gestosis, lung cancer, chronic bronchitis etc. A special theoretical and practical value of these studies is the identification of highly nonrandom associations of particular functionally inferior alleles of the genes belonging to the glutathione-S- transferase family (*GSTM1* (0), *GSTT1* (0), *GSTPi* (s) as well as N-acetyl transferase *NAT-2* (s) with these diseases. Thus molecular testing of these alleles might be recommended as a feasible predictive test for evaluation of personal predisposition, for estimation of ultimate prognosis and for the treatment strategy in at least some of these diseases. About 5-7% of all population in the North-West of Russia possess the most unfavorable combinations of GST genotypes (*GSTM1*0/0; *GSTT1*0/0, *GSTPi* s/s). Early identification of these individuals by means of genetic screening supplemented with subsequent adequate social and medical care should be considered as a feasible social program for efficient prevention of these diseases at the national level. Based on these data as well as on the polymorphism studies and common mutation identification of other genes, the idea of a Genetic Form for pregnant women is suggested and briefly discussed . Implementation of new sophisticated and highly productive molecular techniques suitable for widespread population gene polymorphism screening becomes highly advisable.

P789. No evidence for a role of C7orf10 in the aetiology of Silver-Russell syndrome

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Silver-Russell syndrome (SRS) describes a uniform malformation syndrome characterised by intrauterine and postnatal growth retardation (IUGR/PGR), asymmetry of head and limbs, a small triangular face, and other less constant features. About7–10% of SRS patients show maternal uniparental disomy of chromosome 7. Additionally, five SRS patients have been described carrying rearrangements in 7p. Therefore, a central role of chromosome 7in the etiology of SRS can be delineated. Recently, Nakabayashi and coworkers (Genomics 20002, 79:186-196) have shown in two of these patients that the breakpoints on 7p14 were localised within the same gene, C7orf10.We therefore screened our SRS patients (n=45) for mutations of the coding region as well as for rearrangements of C7orf10: By SSCP, we detected two abnormal patterns in exons 8 and 15 of the C7orf10 coding sequence. In case of exon 8, a G>A transition at cDNA position 652 was detected, representing a silent mutation (L243L). In the first SSCP fragment of exon 15, we detected a C>G transversion at cDNA position 1217, corresponding to the published SNP rs1053953. Both variants were detected in similar frequencies in SRS patients and controls. Using a Real-Time-PCR approach based on TaqMan technology, we excluded duplications or deletions of exon 14 of the C7orf10 gene. In total, our results do not indicate a relevant role of C7orf10 in the aetiology of SRS. With C7orf10, a strong SRS candidate has been excluded; however, further studies on (imprinted) genes on chromosome 7 are needed to elucidate the contribution of this chromosome to the SRS phenotype.

P790. Identification of genes involved in atrioventricular septal defects: microarray analysis of fetal heart gene expression profiles.

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Atrioventricular septal defects (AVSD) are a broad spectrum of congenital heart malformations that arise from abnormal fusion of the endocardial cushions and are most common in individuals affected by Down syndrome. To identify genes involved in AVSD we have analyzed the heart gene expression profile in fetuses affected by Down syndrome, with or without AVSD.
Fetal hearts were obtained from therapeutic abortions less than 8 hours after death assessment. Half of the heart, representative of all four chambers, was used to extract total RNA to be used in the microarray experiments while the other half was fixed, paraffin embedded and used to confirm and characterize the heart disease. Ten heart samples, derived from male fetuses at 21 weeks of gestation, were selected: 3 were from Down fetuses with AVSD, 3 were from Down fetuses without AVSD and 4 were from non-Down fetuses, without heart disease, to be used as controls. The Affymetrix oligo–microarrays technique was used; the data obtained by the hybridization of the microarrays were analyzed with the D-Chip and the GeneSpring software. It was found that 74 chromosome 21 genes, out of 134 analyzed, were expressed in fetal hearts. Thirty genes, out of these 74, were upregulated in fetuses affected by Down syndrome. Twenty-two genes resulted to be differentially expressed, with a fold change greater than 1.5, in the comparison between Down fetuses with or without AVSD. We are now validating these data by quantitative PCR, Northern blot and in situ hybridization.

P791. Thrombophilic gene variants in preeclampsia

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Preeclampsia is a pregnancy-specific syndrome that usually occurs after 20 weeks of gestation, with vasoconstriction and hypercoagulability as major physiopathological events. It is considered a multifactorial disease, with both genetic and acquired factors playing a role. Several studies indicate an association between thrombophilic gene variants and severe preeclampsia. In the present investigation we have evaluated the frequency of several thrombophilic gene variants in subjects with mild preeclampsia. The frequency of FV Leiden, PT G20210A, MTHFR C677T and PAI-1 4G/5G gene variants was evaluated. We compared 52 women with preeclampsia to 80 women with normal pregnancy. None of the preeclamptic women suffered of the severe form of preeclampsia.
Results. The FV Leiden, PT G20210A and MTHFR C677T gene variants were not associated to preeclampsia. In the case of the -675 4G/5G polymorphism of the PAI-1 gene, the genotype 4G/4G was more prevalent in the preeclamptic group, while the 5G/5G was more prevalent in the control group (χ^2 test for trend, P = 0.0141). Frequencies of the 4G and 5G alleles of the -675 gene polymorphism were significantly different between preeclamptic and normal women (P = 0.032).
Conclusions. The hypofibrynolitic genotype 4G/4G at position -675 of the PAI-1 gene is associated with the occurrence of mild preeclampsia independently from thrombophilic mutations of the FV, PT and MTHFR genes.

P792. Paraoxonase genes (PON1, PON2 and PON3) polymorphism and lipid profile in a Scottish population

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Introduction:
Paraoxonase is associated with HDL and protects against lipid oxidation. All three Paraoxonase genes (PON1, PON2 and PON3) map to chromosome 7q21-22.
Functional polymorphisms of PON1 and PON2 genes, which modify enzyme activity, could be a marker for atherosclerosis. In some

populations PON1 variants have been associated with higher LDL or Triglyceride levels. Recently, Paraoxonase 3 has been reported to be closely associated with HDL and to have the same protective functions.
Methods:
A cohort of 181 healthy Scottish individuals with lipid profile data (adjusted for age, sex, BMI and smoking, drinking habits) was recruited to determine the effects of different PON genotypes on lipid profile; two common polymorphisms in PON1 [Gln (Q)/Arg (R)] at codon 192 and [Met (M)/Leu (L)] at codon 55; two in the PON2 gene at codon 311 [Cys (C)/Ser (S)] and codon 148 [Ala (A)/Gly (G)]; and two Single Nucleotide Polymorphisms [rs2074353 (PON3-1) & rs1053275 (PON3-3)] (both "A" to "G") in PON3 gene.
Genotyping assays were based on Dynamic Allele Specific Hybridisation (DASH).
Results & Conclusion:
No differences were found in VLDL/Cholesterol, IDL, LDL, HDL2, HDL3, total Cholesterol levels in females or males between different genotypes of PON1-192, PON1-55, PON2-148, PON3-1.
However, VLDL/TG in females with different PON3-3 genotypes was significantly higher in "GG" genotype (Table 1).There was also a weak significant difference in HDL2 levels in females with different PON 2-311 genotype (Table 2). Only PON3-3 "GG" and PON2-311 "CC" genotypes may influence VLDL/TG and HDL2 levels in our female population, respectively.

Table-1: VLDL/TG in different pon3-3 genotypes (females); P=0.036

Genotype	Number	VLDL/TG	Log (± SD)Mean
11 (AA)	24	69.78	1.84 (± 0.28)
12(AG)	39	77.82	1.89 (± 0.29)
22 (GG)	20	115.98	2.06 (± 0.31)

Table-2: HDL2 in different pon2-311 genotypes (females); P=0.049

Genotype	Number	HDL2(mg/100ml)	Log (± SD)Mean
11 (SS)	52	31.50	1.50 (± 0.14)
12 (SC)	32	32.89	1.52 (± 0.16)
22 (CC)	4	21.33	1.33 (± 0.06)

P793. Genetic analysis of GSTM1 and GSTT1 polymorphisms and HLA antigens class II in couples with recurrent early pregnancy miscarriage

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Polymorphisms of glutathione-S-transferase M1 (*GSTM1*), glutathione-S-transferase T1 (*GSTT1*) and HLA antigens class II were studied in relation to recurrent early pregnancy miscarriage (REPM) by PCR-RFLP in 21 couples with histories of REPM in anamnesis. The control group consisted of 20 fertile couples from the central region of Russia. The null homozygotes frequencies were 59.5% (*GSTM1*) and 14.3% (*GSTT1*) in couples with REPM and 47.5% (*GSTM1*) and 12.5% (*GSTT1*) in the control group. The combination of *GSTM1* 0/0 and *GSTT1* 0/0 genotypes were detected in 9% of couples with REPM and in 5% of control couples. The observed differences among these frequencies and the ones from the control group were not statistically significant. Also there were no significant differences in frequencies of *GSTM1* 0/0 and *GSTT1* 0/0 between our control group and an American white control group [Chen *et al*., 1996, *Pharmacogenetics*, **6**, 187-191], but the frequency of *GSTT1* 0/0 was significantly higher for the control group from St. Petersburg 23.3% (χ^2= 5.91, p < 0.02) [Bespalova O.N., 2001, *Obstetrics and women's diseases*, Vol.2]. On the other hand we identified significant differences in the frequency of 2 or 3 shared HLA antigens in couples with the same REPM (66.7%) and control group (45%, χ^2= 3.93, p < 0.05). Our findings indicate that immunologycal factor makes a greater contribution to the development of REPM than *GSTM1* or *GSTT1* polymorphisms.

P795. Search for late onset diseases among carriers of balanced chromosomal rearrangement.

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The aim is to re-examine all individuals with a balanced chromosomal rearrangement in Denmark. If disease-associated breakpoints are found it may lead to the discovery of candidate genes for late onset diseases. In addition, a nationwide population study will be established with the purpose of observing disease incidences and structural patterns of rearrangements.
Material: All individuals with a balanced chromosomal rearrangement in Denmark.
Methods: The clinical data are obtained by questionnaires, registers and medical files. Children and individuals with mental retardation are excluded from the questionnaire study. The questionnaires contain questions about all kinds of late onset disorders: cardiovascular, cancer, endocrinology, fertility, etc.
Results: to date: Of 185 questionnaires sent out to carriers with balanced reciprocal translocation, 152 wanted to participate (compliance 82 %).
1) A number of cases were associated with common diseases like myopia, allergy, asthma, arthritis, hypertension etc. 2) We observed carriers suffering from diseases with breakpoints in chromosomal regions known to harbour loci for the corresponding diseases. 3) In some families we observed co-segregation between the translocation and the clinical phenotype. 4) Among the carriers we observed clinical entities with unknown genetic aetiology and this might provide the first hint for the localisation of a candidate locus.
Conclusion: By this systematic approach we expect to find breakpoints associated with a variety of late onset diseases, and expansion of these investigations to the larger German population is underway.

P796. VDR, Col1a1 and BGLAP genes polymorphism in development of postmenopausal osteoporosis

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Osteoporosis is a common multifactorial disease with a strong genetic component. The frequencies of *VDR*, *COL1A1* and *BGLAP* alleles in 174 non-related individuals Northwest Russian population and in 124 postmenopausal women with osteoporosis (PW) were studied by PCR-RFLP method. The latter group included 70 women with surgical menopause (SM) and 54 women with normal menopause (NM).
Significant differences were revealed only in the frequencies of the functionally abnormal H allele of the *BGLAP* gene (p<0,01). The frequencies of the H allele were 23,7%; 10,0%; 18,1% in the SM, NM groups and in the population sample respectively.
After 12 months survey two groups of the postmenopausal women were defined by the rate of loss of BMD (bone mineral density). Group 1 consisted of women with slow BMD decrease (less than 3% per year); group 2 consisted of women with fast BMD decrease (more than 6% per year).
Significant differences were revealed in frequencies of functionally abnormal alleles of *VDR* and of *COL1A1* (p<0,01). The frequencies of the t allele (*VDR* gene) were 51,3%; 12,2%; 32,6% in group 2, 1 and in the population respectively. The frequencies of the s allele (*COL1A1* gene) were 33,3%; 2,0%; 17,5% in group 2, 1 and in the population respectively.
Significant association between the H allele of *BGLAP* gene and the severity of osteoporosis as well as between the t allele of *VDR3* and the s allele of *COL1A1* genes with a high rate of loss of BMD was shown, and their clinical implication briefly discussed.

P797. Allelic Variation of the Lrp5 Gene Contributes to Stature and Vertebral Bone Mass and Size in the General Population

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Stature, bone size and bone mass are interrelated traits with high heritability, but the major genes that regulate them are unknown. Mutations in the LDL receptor-related protein 5 gene (LRP5) located at 11q12-13 have been shown to cause osteoporosis pseudoglioma and „high bone mass" syndromes. In two independent studies a QTL for bone mineral density (BMD) has also been reported at this chromosomal position. We therefore investigated whether the LRP5 gene contributes to the variability in these bone traits by analysing SNPs in the general population. We found significant associations between LRP5 genotypes (and haplotypes) and stature (P=0.002) and vertebral bone parameters (P=0.001) in 877 healthy individuals. Moreover, through a longitudinal study, stature and vertebral bone growth over two years were shown to be associated with two missense substitutions in the LRP5 gene in 332 children (P= 0.025 and 0.005 respectively). Altogether, LRP5 polymorphisms accounted for up to 4% of the traits variance in adults, and up to 15% in men alone. These results strongly suggest that LRP5 plays an important role in height and vertebral bone parameter determination during growth, and might contribute to oesteoporosis predisposition in the general population.

P798. Association between polymorphism of microsomal epoxide hydrolase and development of preeclampsia

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Preeclampsia is a frequently occurring complication of pregnancy. A great number of polymorphisms are associated with this syndrome. Genetic predisposition to preeclampsia is widely connected with the detoxification system. Lipid peroxides damage endothelium, thus increasing blood pressure. Microsomal epoxide hydrolase is one of the enzymes that inactivates negative effects of these agents. Polymorphisms of the microsomal epoxide hydrolase gene (*EPHX*) correlate with enzyme activity: Tyr113His (exon 3) and His139Arg (exon 4) alleles are responsible for low or high activity, respectively. The genotypes of 121 women with a history of preeclampsia and of 77 healthy female controls were studied by a PCR-RFLP assay. A statistically significant increase in frequency (28%) of the low activity allele (His113 in exon 3) in the preeclamptic patients as compared to controls (13%, OR 2.5, 95% CI 1.4-4.5) has been noted. No difference between both groups was found for His139Arg polymorphism.
CONCLUSION: The presence of His113 allele of EPHX gene (3 exon), which could influence metabolic activation of endogenous or exogenous toxic compounds, might be considered as a risk factor for development of preeclampsia.

P799. The increase in the risk of vascular diseases is related to the allele combinations at the MTHFR gene locus

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We determined the prevalence of MTHFR C677T and A1298C polymorphisms in 40 patients with the obliteration of iliac arteries (Syndrome Leriche; SL), 66 patients with the abdominal aortic aneurysm (AAA) and 77 patients with arterial hypertension (AH) and compared it to that in 100 subjects of the population sample. We noted the different distribution of MTHFR alleles and genotypes in studied groups. The observations presented in this study indicate that the common MTHFR variants, which lead to a mild elevation of plasma homocysteine, predispose to AAA and AH, but not to SL. The MTHFR 1298CC and 677CT/1298AC, but not 677TT genotypes increase the risk of studied vascular disease (either AAA or AT). No case of MTHFR 677T/1298C allele in cis configuration was noted.
The study was supported by grant received by ALP and ES from KBN and a USMS grant received by JG.

P800. In C57BL ahr bd but not in ahr bb mice mothers the survival of the progeny is enhanced following the cigarette smoke exposure as compared to that in unexposed animals

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The *ahr* gene product, as a ligand-activated transcription factor regulates the expression of a number of enzymes involved in metabolism of polycyclic aromatic hydrocarbons (PAHs). Both genotoxicity and reproductive toxicity of PAHs were strongly influenced by the *ahr* gene activity. The mothers breeding capacity of *ahr bb* and *ahr bd* females was compared in the studied series of pairings by measurements of survival of the progeny up to 21-st day of life. This parameter was shown to be unchanged by cigarette smoke (CS) exposure in the *ahr bb* mothers, whereas in *ahr bd* mothers it was found to be enhanced by CS exposure. The observations presented here demonstrate for the first time that in conditions of subtoxic Ahr ligand (CS) exposure the parameters of reproduction may be improved in the presence of the low activity allele of the *ahr* gene.

P801. A polymorphism within plasminogen activator urokinase (PLAU) is associated with Alzheimer's disease

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Previous studies identified a locus between markers D10S1227 and D10S1225 which showed linkage with Alzheimer's disease (AD). Using a functional candidate gene approach within this region we focused on the urokinase-type plasminogen activator (PLAU) gene. PLAU activates the conversion from plasminogen to plasmin, which is capable of degrading amyloid-ß (Aß). We hypothesized that polymorphisms within PLAU modifies this function.
Using a MALDI-TOF-MS system (Sequenom, San Diego) and RFLP, we analyzed a C/T substitution (rs2227564) causing a proline/leucine exchange within the PLAU gene in 186 AD-patients and 151 controls. Afterwards we enlarged this Munich sample (n=498) and analyzed the SNP in two independent case control samples from Bonn (n=282) and Brescia (n=219) and additonally in a discordant sib-pair-sample consisting of 78 german families (n=184). Statistical analysis of case-control samples was performed using logistic regression. Family sample data were analyzed by S-TDT.
We found a significant overrepresentation of the combined CT/TT genotypes in patients with AD compared to controls for all samples (Munich: p=0.001, odds ratio (OR) =1.93; Bonn: p=0.005, OR=1.88; Brescia: p=0.001, OR=3.6). In both German samples we observed a

T-allele dose dependent increase of the ORs from 1.8 for CT to 4.1 for TT genotype (Munich) and from 1.9 to 3.7 (Bonn), respectively. Also, in the sib-pair-sample we detected a highly significant overrepresentation of the CT/TT genotypes in subjects with AD compared to unaffected siblings ($p<0.001$).
We conclude that disturbances within the plasmin system may contibute to neurotoxic effects of Aß in AD.

P802. OLR1 (LOX-1) gene haplotype analysis in atherosclerosis susceptibility

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Atherosclerosis is the principal process contributing to the pathogenesis of coronary artery disease (CAD), cerebral infarction, and peripheral vascular disease. A large number of risk factors such as hypertension, hypercholesterolemia, diabetes, and smoking have been identified in patients with atherosclerosis. Several biochemical, pathologic and functional studies suggest that a lectin-like receptor for oxidized low-density-lipoprotein (ox-DL), termed LOX-1, may be involved in atherogenesis. LOX-1 is a type-II membrane protein belonging to the C-type lectin family. A recent linkage study performed in a mouse model, identified LOX-1 as candidate susceptibility gene for human atherosclerosis. In order to investigate the role of LOX-1 in human atherosclerosis susceptibility, we screened a group of 192 Italian individuals with angiographic CAD phenotype and 52 Italian controls without any angiographically demonstrable coronary disease. We characterized ten different SNPs at the LOX-1 locus. We observed that SNP5 (C>T) is significantly associated with CAD ($X^2 = 10.307$; 2 df; $p=0.006$; Mantel-Haenszel test: $p=0.0023$). A second SNP (SNP3, G>C) shows a trend of correlation to CAD (M-H test : $p=0.0057$) but the association does not reach the statistical significance ($X^2 = 3.865$; 2df; $p=0.145$). We then analysed the distributions of haplotypes relative to the two SNPs and found a very strong association with CAD ($X^2 = 17.144$; 3df; $p=0.00066$). The present data show that LOX-1 may be a potent candidate gene for atherogenesis and endothelial dysfunction in response to ox-LDL.
Work supported by a grant from MIUR (Fondi COFIN 2002).

P803. Lack of association between leber's hereditary optic neuropathy primary point mutations and multiple sclerosis in Iran

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The hypothesis that mitochondrial genes may be implicated in susceptibility to multiple sclerosis (MS) is supported by an increasing number of case reports on Leber's Hereditary Optic Neuropathy (LHON) associated mitochondrial DNA (mtDNA) point mutations in patients with MS. A number of mtDNA mutations with primary pathogenic significance for LHON, a maternally inherited disease causing severe bilateral visual loss predominantely in young men, have been detected in patients with a MS-like phenotype. To evaluate the link between MS and LHON primary point mutations, we investigated 31 unrelated Iranian clinically definite MS patients (23 females and 8 males) with optic nerve involvement, 3 of them with severe bilateral visual loss, as well as 25 patients (16 females and 9 males) without involvement of optic nerve as controls, for the presence of LHON mitochondrial mutations at nucleotide positions (np) 11778, 3460, and 14484 by mutation-specific polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) and single strand conformation polymorphism (SSCP). Our results suggest that there is no association between Iranian patients with MS and mtDNA point mutations at np 11778, 3460, and 14484.

P804. Candidate gene analysis for Multiple Sclerosis on 12p12 in a large North American pedigree

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We previously identified a pedigree of Pennsylvania Dutch extraction, in which Multiple Sclerosis (MS) segregates with an autosomal dominant inheritance pattern and found evidence for suggestive linkage to markers on 12p12 with a maximum multipoint LOD score of 2.71, conditional on the presence of HLA DR15, DQ6 alleles. By comparing the haplotypes in all affected and nonaffected individuals, markers D12S1715 and GATA63D01 located 18 cM apart were designated as the distal and proximal flanking markers of the candidate locus on 12p12. We started a physical analysis of the 12p12 locus in the attempt to identify a possible candidate gene. Based on their biological function, we have initially selected eight candidate genes in this region for mutation analysis. These are SIAT8A, ARHGDIB, BHLHB3, KRAS2, SURB7, SSPN, CDKN1B and MRPS35. We screened all exons based on the information provided by the European Bioinformatics Institute's Ensemble system (http://www.ensembl.org) and Santa Cruz Genome Browser (http://genome.ucsc.edu) by automated sequencing (ABI 3100) after PCR amplification. We did not detect any alterations in either affected or nonaffected individuals for ARHGDIB, SURB7, MRPS35, SSPN, CDKN1B. We are still analyzing SIAT8A, BHLHB3, and KRAS2. However we found a new genetic variant for SIAT8A associated with the affected individuals located at 29 bp from the exon 4 – intron 5 splice junction. We made a construct containing the alteration using an expression vector TOPO II (Invitrogen) and we are in the process of determining whether or not there is a biological function associated with it.

P805. Mutations in Nod2/CARD15 in Scottish and Irish Crohn's disease patients.

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The Nod2/CARD15 gene has been identified as a determinant of susceptibility and behaviour for Crohn's disease (CD) in a number of populations (Hugot et al, 2001; Ogura et al, 2001). Mutations at positions 702, 908 and 1007 have been identified as important, with up to one third of North European CD patients being reported as having at least 1 copy of these common Nod2 mutations (Cuthbert et al, 2002). Already ethnic and geographical differences in the contribution of these variants have been identified, notably in studies of Japanese and Afro-Carribean populations. We have studied the frequency of these mutations in 500 Scottish patients with inflammatory bowel disease and 250 healthy controls and have found no significant association between these mutations and susceptibility to Crohn's disease. Preliminary data on 96 Irish CD patients closely mirror these results, and we hypothesise that these Celtic populations may share a common ancestry and determinants of disease susceptibility, either within the Nod2 gene or distinct to this. We have identified a number of novel variants in the promoter region of the Nod2 gene and are studying these in more detail. The other exons of the Nod2 gene will be examined for novel mutations in our population. We will present data on these and other mutations in the Nod2 gene in the Scottish and Irish population of Crohn's disease patients.
Cuthbert et al, Gastroenterology 2002 122 867-874.
Hugot et al, Nature 2001 411 599-603.
Ogura et al, Nature 2001 411 603-606.

P806. Pyrosequencing analysis of CARD15 gene mutations in Inflammatory Bowel Disease (IBD)

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Crohn's disease (CD) and Ulcerative colitis (UC), the main types of IBD, have a genetic predisposition. A strong body of evidence shows that CARD15 gene is associated with susceptibility to CD. **Aim**: We used the pyrosequencing approach to detect the single mutations of the 3 more common gene variants: 3020insC, Arg702Trp, and Gly908Arg. **Methods:** Reactions were performed with mixing and contained gel pad (sepharose beads)-bound single-stranded DNA with annealed sequencing primer in addition as enzyme: DNA polymerase (exonuclease-deficient), apyrase, luciferase, ATP sulfurylase; and as substrate, adenosine 5'-phosphosulfate, and luciferin. Reactions consisted of stepwise elongation of the primer strand by one or more nucleotides upon sequential addition of the individual deoxynucleoside triphosphates [dTTP, dGTP, dCTP, or dATPaS (deoxyadenosine a-thiotri-phosphate)] followed by degradation of excess nucleoside triphosphates after each elongation step by apyrase. The approach links a DNA synthesis by sequencing to an enzyme cascade generating light whenever pyrophosphate is released during template-mediated primer elongation. **Results:** A total number of 114 IBD families (with two or more affected individuals) were investigated: 46 CD (141 affected members), 50 UC (163 affected members) and 18 Mixed families (61 affected members with CD or UC). The alleles frequency (%) of mutations is showed on the table (X_2 test). **Conclusions**: In the Italian population we confirm the significant association of CARD15 gene mutations and CD. The pyrosequencing is a feasible, fast and cheap methodology for large scale genotyping of known mutations in these complex trait diseases. (P *<.006; **<.0006; ***<.00001 vs HC)

Allele frequency of 702/908/InsC			
CD (n=141)	0.17	0.16**	0.13*
UC (n=163)	0.10	0.04	0.06
Mixed (n=61)	0.16	0.08	0.23***
HC (n=107)	0.06	0.02	0.01

P807. Identification of a chromosomal breakpoint at 12q14.1-14.2 in a patient with Crohn's Disease

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Chronic inflammatory bowel disease (IBD) is a multifactorial disorder with two major clinical forms, Crohn's disease and ulcerative colitis. Eight different chromosome regions have been suggested as putative susceptibility loci for one or both of these IBD forms, and the susceptibility gene on chromosome 16q12 has already been identified. A susceptibility locus for ulcerative colitis (IBD2) is on 12p13.2-q24.1, as suggested by multiple independent studies. The DNA marker D12S83, which is mapped to 12q14.1, gives the highest LOD score (5.47) for IBD2. In this study we present a patient with IBD, who has a translocation involving the IBD2 susceptibility region. The karyotype of the patient is 46,XX,t(10;12)(q25;q14) and the phenotypically normal father has the same translocation. Phenotypical differences between them can be due to sex differences or other genes/factors involved in the physiopathology of IBD. We have used the Fluorescence *In-Situ* Hybridisation (FISH) technique to map the 12q14 breakpoint and demonstrated that the BACs RP11-318E11 and RP11-221N13 hybridised proximal and distal to the chromosome 12 breakpoint, respectively. The proximal BAC includes the DNA marker D12S83. The future work will include narrowing of the breakpoint by further FISH studies to identify genes that may be effected by the translocation. These candidate genes will then be screened for mutations in patients showing linkage to chromosome 12.

P808. A linkage test for multiple susceptibility genes provides support for IDDM15 in affected sibpairs

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We have developed new software to simultaneously map two susceptibility loci in pairs of informative affected relatives. The program extends the method developed by Farrall (1997) to efficiently take multiple markers into account. The approach uses a multilocus linkage test to compare the likelihood of two-locus models with varying degrees of epistasis to that under a single-locus model. We apply the method to genotype data from insulin dependent diabetes mellitus (IDDM) to dissect the contribution of two linked susceptibility loci to the trait.

Previous studies have identified IDDM15 as a susceptibility gene, which is loosely linked to the major locus in diabetes, IDDM1. However, analysing evidence for the presence of IDDM15 presents a challenge due to its proximity to IDDM1 and the resulting interdependence between the two loci. We examine genotype data from 30 microsatellite markers on chromosome 6 in 356 affected sibpairs presented in a genome scan by Mein et al. (1998). The resulting MLS attributed to IDDM15 was 2.33 at D6S294 and 2.16 at D6S286, which are at the proximal end of the region previously identified as the IDDM15 locus (Delepine et al. 1997). Thus IDDM15 has been independently replicated by linkage analysis. This analysis demonstrates the utility of applying two-locus linkage methods to human affected sibpair data.

P809. Tunisian Parkinson's Disease family study.Possible phenotypic overlap in Parkinson's Disease and Essential Tremor.

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In Tunisia due to some socio-cultural conditions (especially the high rate of consanguineous marriages), the frequency of neurodegenerative disorders including Parkinson's Disease (PD) and Essential Tremor (ET) is much higher than in other countries. Factors such as the large size of the families, the small size of the country and the availability of good neurological disease expertise have created favourable circumstances for the study of these genetic diseases.

We have identified a Berber/Arab family in Tunisia among which different members exhibit typical ET, typical PD, and ET with possible Parkinsonian features (postural/kinetic tremor, cogwheel phenomena and impairment of voluntary distal upper extremity movement). The question of whether a genetic overlap exists between PD and ET has long been debated in the literature. Differing opinions support either a genetic link between ET and PD or dismiss this and perceive both diseases as separate entities.

In this family (5 individuals, consanguineous) we observed a possible phenotypic overlap between PD and ET. Several members were examined including 2 brothers with typical ET, their 2 sisters with ET/PS (Parkinsonian Syndrome) and their first cousin who has classical PD. Detailed phenotypic data was collected on all 5 individuals and DNA samples obtained for genetic analysis following informed consent. Phenotypic analysis and sample collection is ongoing in this family, including 12 offspring of the ET/PS and PD individuals. Clinical data and proposed methods of analysis will be presented.

P810. Mutation analysis of *parkin* (*PARK2*) gene in patients with essential tremor (ET)

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Essential tremor (ET) is the most common movement disorder with a prevalence ranging from 0.4 to 3.9%. ET has long been recognised as an inherited disease, with a positive family history being reported in 17.4 - 96% of cases. Transmission is considered to be autosomal dominant with high penetrance (89% by age 65). Linkage of ET to two different chromosomal locations, 2p22-25 (ETM) and 3q13.1 (FET1), was found in families of different ethnic origin, but, to date, the genes involved in ET pathogenesis are still unknown. Several authors reported the association of ET with Parkinson's disease (PD). To evaluate the possible relationship between point mutations within *parkin* gene and ET we carried out a mutational analysis of the coding region of this gene in 110 unrelated ET patients. We set up theoretical conditions for DHPLC mutational analysis of the 12 exons and spilce sites of the *parkin* gene. No clear disruptive mutation (i.e. nonsense or frameshift), nor mutations previously described in patients with Parkinson's disease were identified in our cohort of ET patients. DHPLC analysis detected two already reported polymorphisms (V380L and D394N), and one novel rare variant (frequency <1%) (H215Q) located within exonic regions. Three new polymorphisms [(973-68)C/G; (973–35)G/A; (1387–117)A/G] and one rare variant (2796A/G) were also found within intronic regions. In conclusion, causal sequence variants in the *parkin* gene have not been identified in our cohort of ET patients, even if H215Q missense mutation functional consequences have not been assessed, and cannot be excluded.

P811. Early-onset Parkinson's disease associated with a new parkin mutation in a Italian patient

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Autosomal recessive juvenile parkinsonism (ARJP) is characterized by early onset of parkinsonian signs, dystonia at onset, diurnal fluctuations, slow disease progression and early levodopa-induced dyskinesias. Pathological studies have shown several differences between ARJP and Parkinson's disease (PD), the most striking being the absence of Lewy bodies in ARJP. ARJP is associated with different mutations in the parkin gene, which is mapped to chromosome 6q24.2-27.

We describe a patient with slowly progressive parkinsonism (PD) with early onset before 25 years, . Molecular genetic analyses included sequence analysis and exon dosage of the parkin gene.The coding sequence of the parkin gene (exons 1-12) has revealed a new heterozygous missense mutation (Lys32Thr) in exon 2 in the ubiquitin-like domain. Moreover we tested for gene dosage alterations by new method for precise measurement of exon copy number using real-time PCR, 7900 HT SDS Applera and TaqMan fluorogenic probes, and we have identified a heterozygous deletion of the exon 2. Our data show an association between early onset Parkinson disease (EOP) and compound heterozygosity for a missense mutation in exon 2 with a deletion of exon 2 in the parkin gene.

P812. Preferential paternal origin of microdeletion caused by prezygotic chromosome or chromatid rearrangements in Sotos syndrome

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Sotos syndrome (SS) is characterized by pre- and postnatal overgrowth with advanced bone age, a peculiar face with macrocephaly and pointed chin, large hands and feet, mental retardation, and susceptibility to tumors. It has been shown that the major cause of SS is haploinsufficiency of the NSD1 gene at 5q35, because the majority of patients had either a common 1.8-Mb microdeletion including NSD1 or a truncate type of point mutations in NSD1. In the present study, we traced the parental origin of the microdeletions in 26 SS patients by the use of 13 microsatellite markers at or flanking the commonly deleted region. Surprisingly, deletions in 18 of the 20 informative cases had occurred in the paternally derived chromosome 5, while those in the maternally derived chromosome were found in only two cases. Haplotyping analysis of the marker loci revealed that the paternal deletion in 5 of 7 informative cases and the maternal deletion in one case arose through an intrachromosomal rearrangement, and two other cases of the paternal deletion involved an interchromosomal event, suggesting that the common microdeletion observed in SS did not occur through a uniform mechanism, but preferentially arose prezygotically.

P813. Spectrum of NSD1 mutations in Sotos and Weaver syndromes

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Sotos syndrome is an overgrowth syndrome characterized by pre- and postnatal overgrowth, macrocephaly, advanced bone age and typical facial features. Weaver syndrome is a closely related condition characterized by a distinctive craniofacial appearance. Haploinsufficiency of the NSD1 gene has been recently reported as the major cause of Sotos syndrome while point mutation accounted for a minority of cases (Kurotaki et al, 2002). We looked for NDS1 deletions or mutations in 39 patients with childhood overgrowth. The series included typical Sotos patients (24/39), Sotos-like patients (lacking one major criteria, 9/39) and Weaver patients (6/39). We identified NSD1 deletions (6/39) and intragenic mutations (16/39) in Sotos syndrome patients. We also identified NSD1 intragenic mutations in 3/6 Weaver patients. We conclude therefore that NSD1 mutations account for most cases of Sotos syndrome and a significant number of Weaver syndrome in our series.

Interestingly, mental retardation was consistently more severe in patients with NSD1 deletions. Macrocephaly and facial gestalt but not overgrowth and advanced bone age were consistently observed in Sotos syndrome patients. We suggest therefore considering macrocephaly and facial gestalt as mandatory criteria for the diagnosis of Sotos syndrome and overgrowth and advanced bone age as minor criteria.

P814. Mutation analysis in Sotos syndrome

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Sotos syndrome is characterized by the association of childhood overgrowth (height and head circumference above the 97th centile), advanced bone age and typical facial features. These are represented by macrocephaly, high forehead with frontal bossing and prominent jaw. Developmental delay is frequently associated. Some overlap exists with other overgrowth conditions, in particular with Weaver syndrome.

The gene responsible for Sotos syndrome was recently identified (Kurotaki et al., 2002) and mutation testing revealed some ethnic difference in the prevalence of different types of mutations. In particular, microdeletions involving the gene NSD1 appear to be very

frequent in Japanese patients with Sotos syndrome, but this data was not confirmed in European series (Douglas et al., 2003). Douglas et al.(2003) reported mutations in NSD1 gene in patients with Weaver syndrome and suggested a phenotypic classification for overgrowth patients and the presence of genotype-phenotype correlation.
We report data on mutation analysis of the NSD1 gene in 35 patients: 30 were sporadic cases of Sotos syndrome, 2 familial and 3 cases had a clinical suspicion of Weaver syndrome. FISH analysis was used to detect microdeletion, which proved to be rare in our series. Molecular analysis was perfomed by DHPLC and authomatic sequencing. Novel mutations and polymorphisms detected will be described and genotype-phenotype correlation of our series discussed.

P815. Hailey-Hailey disease: two novel mutations in the ATP2C1 gene identified in Italian families.

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Hailey-Hailey disease (HHD) or benign chronic pemphigus (OMIM 16960) is an autosomal dominant inherited disorder of keratinocyte cohesion, characterised by recurrent blisters and erosions of the skin. Lesions occurs predominantly on the neck and at intertrigineous areas and first appear after puberty.
It has been recently demonstrated (Hu et al, 2000; Sudbrak et al, 2000) that HHD is caused by mutations in the ATP2C1 gene encoding an ATP powered Ca2 + pump.68 different ATP2C1 mutations (nonsense, insertion/deletion, splice-site) have been described nowadays and no single mutation appears to be related to a substantial number of HHD cases in the population.
We studied two Italian familial cases of HHD and analysed all ATP2C1 coding exons by direct sequencing. In the first family we identified the never previously described nonsense R799X mutation. In the second family the 807delAC novel variant was present. This alteration leads to a shift in the reading frame and introduces a premature termination at codon 818.
Our findings confirm the high allelic heterogeneity of ATP2C1 gene in the Italian population, and support the theory of absence of locus heterogeneity in HHD. In addition, clinical findings inour cases, showing an extensive phenotypic intrafamilial variability, further suggest a role of additional genes, and/or environmental factors, in modulating the pathology.

P816. Variable penetrance of novel heterozygous KRT5 and KRT14 mutations in patients with epidermolysis bullosa simplex Weber-Cockayne

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Epidermolysis bullosa simplex (EBS) is a hereditary skin blistering disorder caused by mutations in the genes for keratin 5 (*KRT5*) or keratin 14 (*KRT14*). Mutations disrupt intermediate filament assembly in epidermal basal cells mostly resulting in autosomal dominant EBS. We describe 10 novel heterozygous mutations in 6 unrelated patients with EBS of hands and feet, type Weber-Cockayne (EBS-WC).
One patient with EBS-WC carried the *KRT14* mutation 411delGlu, affecting the highly conserved helix termination region of the 2B rod domain of keratin 14. This would predict a more severe EBS type instead of the observed mild EBS-WC. In another EBS-WC patient we detected the splice site mutation *KRT14*:IVS4+1G>A, the same mutation we have previously found in an unrelated patient with the more severe EBS-Koebner (Hut et al. 2000). In another EBS-WC patient we detected *KRT5*:Ile467Met, almost similar to the Ile467Thr found in patients with severe EBS Dowling-Meara (Irvine et al. 1997). The *KRT5*:Asp197Glu was found in a patient with very mild EBS-WC and in a patient with late onset EBS-WC. The latter patient also carried two missense mutations in *KRT14*, R449H and V452I, located in the H2 (tail) region, and we considered these non-pathogenic polymorphisms. Finally, one patient with a mild EBS-WC phenotype had an in-frame deletion of Ala-Tyr-Leu at position 247-249 in *KRT14* and two missense mutations in *KRT5*, G543S and S528G. Since most *KRT5* and *KRT14* mutations exert a dominant-negative effect, family members will be screened to discriminate between possibly inherited and *de novo* mutations.

P817. Genotype and phenotype study of Noonan syndrome (NS): the French experience

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Background: Noonan syndrome (NS) is characterized by facial dysmorphism, short stature, mental retardation and heart defects. Almost 50% of patients with NS (sporadic and familial) carry mutations in PTPN11 on 12q24. Most are scattered in exons 3,7,8,13.
Objectives: To screen PTPN11 mutations in a cohort of NS patients; to describe the phenotype, genotype and possible phenotype/genotype correlations
Design: Multicentric collaborative study.
Methods: More than 150 patients with NS diagnosed by a clinical geneticist were referred with informed consent to our institution for PTPN11 analysis. The following data were collected: antenatal ultrasound, feeding difficulties, growth chart, dysmorphism, cardiac status, skeletal-, dermatologic-, ophthalmologic-, auditory-, and hematologic anomalies, learning difficulties and development over time. Direct sequencing of PTPN11 (beginning with exons 3,7,8,13) is continuing.
Results: At time of submission, 32 out of 58 patients with NS (55%) carry a PTPN11 mutation. Four novel mutations were identified (I56V, P491L,P491H and G503R). Among mutated patients, 65% had antenatal anomalies. Dysmorphic features were: ear anomaly (62.5%), triangular shape of face (61%), hypertelorism (47%), micrognathia and ptosis (37.5%) and philtrum anomaly (28%). Eighty-three percent of patients with a mutation had a heart defect (pulmonary stenosis, atrial septal defect, hypertrophic cardiopathy), 50% had skeletal anomalies, 31% had feeding difficulties. Surgery for cryptorchidism was required in 55% of NS males. No phenotype/genotype correlation could be clearly delineated from these first 58 NS patients. We expect to present complete genotype analysis of the cohort for the meeting.

P818. Demonstration of genetic heterogeneity in Multiple-Lentignes/LEOPARD syndrome

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Multiple-Lentigines (ML)/LEOPARD Syndrome (multiple lentigines, electrocardiographic-conduction abnormalities, ocular hypertelorism, pulmonary stenosis, abnormal genitalia, retardation of growth, and sensorineural deafness) is an autosomal dominant condition, principally characterised by multiple lentigines, cardiovascular defects and facial anomalies. Some of these features are shared with Noonan syndrome (NS). Recently mutations in PTPN11, a non-receptor protein tyrosine phosphatase, have been identified in about 50% of Noonan Syndrome cases. Mutations in PTPN11 have also been reported in ML/Leopard cases. Here we report the findings of mutation screening and linkage analysis of the PTPN11 gene in three families with ML. The entire coding region of PTPN11 was screened by direct sequencing in the probands of these families. No sequence variations were found in these patients. In order to exclude the presence of mutations in the promoter and regulatory sequences, we performed linkage analysis using intragenic microsatellite markers for PTPN11. Negative LOD scores excluded linkage to the PTPN11 gene in these families. These results show that ML/LEOPARD syndrome is genetically heterogeneous.

P819. 10 year follow up of a large cohort of patients with Noonan Syndrome.

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A detailed clinical assessment of 151 individuals with Noonan Syndrome (NS) was published in 1992. Long term follow up data is now presented and correlated with genotype.
All patients were assessed by a single observer and those who did not wish to be seen were interviewed by telephone or completed a questionnaire. In 1 patient the diagnosis of NS was retracted and 5 patients were lost to follow up. Of the remaining 145 individuals, 11 have died, 8 (5.5%) from causes attributable to NS, 15 declined to take part, 87 have been assessed to date and results on the remaining 32 are expected by the time of presentation.
Feeding difficulties in infancy are known to be very common in NS, however those with severe problems who required feeding via NG tube for >2 weeks (24%) had marked speech delay and were likely (77%) to require learning disability schooling.
Mean final height in males was 167cm (9th centile) and 153cm in females (2nd to 9th).
Pulmonary stenosis was present in 58%. Of this group 56% have had no cardiac intervention at all, with 17% having had balloon dilatation, 27% open heart surgery and 12% underwent multiple interventions. Mortality attributable to NS in these patients was 12%.
Hypertrophic cardiomyopathy was present in 20% overall, with 20% undergoing heart transplant and 7% myectomy. The mortality attributable to NS in this group was higher at 24%.
PTPN11 mutations associated with NS type 1 were identified in 36%.

P820. PTPN11 gene mutations and congenital heart defects in Noonan and Multiple Lentigines/LEOPARD syndrome patients

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Noonan (NS) and Multiple Lentigines/LEOPARD (ML/LS) syndromes share several clinical features, and are consistently associated with a wide spectrum of congenital heart defects (CHD). Most NS and ML/LS patients harbour mutations in the *PTPN11* gene. We investigated *PTPN11* gene mutations in NS and ML/LS syndrome patients, and analysed genotype-phenotype correlations, with major emphasis on the association between distinct *PTPN11* mutations and different CHD. Seventy NS and 13 ML/LS patients including 72 with CHD were enrolled in this study. *PTPN11* gene mutation screening was performed by SSCP analysis.
Fourteen different *PTPN11* mutations were detected in 22 NS and 11 ML/LS patients. Eighty-six percent of NS and 67% of ML/LS patients with *PTPN11* mutations were affected by different types of CHD. Prevalence of CHD was remarkably different between the two phenotypes. In particular, pulmonary valve stenosis (PVS) was the predominant CHD among NS patients with mutations, with a significant hot spot in exon 8, while hypertrophic cardiomyopathy (HCM) was more frequent among ML/LS patients, with hot spots in exons 7 and 12. Atrial septal defect (ASD) was correlated with mutations in exon 3, while atrioventricular canal defects and mitral valve anomalies were detected in different *PTPN11* gene domains. The present study argues for a causal relationship between *PTPN11* mutations and a wide spectrum of heart defects. PVS, ASD and HCM are likely to occur in association with specific exon mutations, with a different distribution between NS and ML/LS patients.This work was supported by the Italian Ministry of Health and Education

P821. Case Report: A case of Cardio-Facio-Cutaneous Syndrome

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Cardio-Facio-Cutaneous (CFC) syndrome was first descirbed by Reynolds et al. in 1986. As its name implies it is a multiple congenital anomalies / mental retardation syndrome. It shows congenital heart defects with consistent pattern of craniofacial dysmorphisms and signs of ectodermal involvement. Various types of congenital cardiac defects have been repeatedly found; atrial septal defects and/or pulmonic stenosis being the most common.
A girl was referred to our department at 18 month of age by her pediatrician because of multiple anomalies. She had motor and mental retardation with prominent forehead, shallow orbital ridges, strabismus, long philtrum, short upturned nose, depressed nasal bridge, coarse facial features, posteriorly rotated ears, ventricular septal defect, hyperextensible knees, multiple palmar and plantar creases, sparse and slow growing hair and hydrocephalus. In addition she had coarse voice and blue sclerae. Pedigree revealed that this was a sporadic case. Based on these findings we concluded that the patient had CFC syndrome. There is no patient in the literature noted to have ventricular septal defect yet. This patient is also the first CFC syndrome case with coarse voice and blue sclerae.

P822. Costello syndrome - Clinical review of the outpatient clinic at the Institute of Medical Genetics

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Costello syndrome is characterised by postnatal growth deficiency and dysmorphic features, predominantly coarse facies, redundant skin on the neck, palms, soles and fingers with hyperpigmentation and acanthosis nigricans. Papillomata represent the most characteristic manifestation, but appear with age.
Costello syndrome appears to have sporadic autosomal dominant mutations.
We will review the clinical data of the patients diagnosed at our outpatient clinic. Diagnostic problems and follow-up will be addressed.

P823. Determining of alpha Thalassemia mutations in Iranian population

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The Alpha-thalassemia is one of the most common single gene diseases in the world. They are characterized by a reduction or complete absence of α-globin gene expression.
In this study we have tested 61 Iranian individuals from a variety of ethnic origins, randomly chosen from a pool of patients with MCV values below 80.0 fL (mean: 67.4 ± 5.9), low MCH (21.4 ± 2.5pg), normal or slightly reduced Hb (12.6 ± 1.4g/dL), normal HbA2 (3.0 ± 1.1%), and negative results in β-thal genotyping for 22 common Beta thal mutations in Iran.
Alpha-thalassemia mutations were analyzed by using 4 sets of multiplex PCR and reverse dot blot analysis (RDB). The mutations screened include South East Asian, Mediterranean, Thai and Filipino type 2 gene deletions, 20.5kb deletion, 3.7 and 4.2kb single gene deletions and 5 point mutations [Hb Constant Spring, Hb Pakse, Hb Quong Sze, Cd30 (-GAG) and Cd59 (GGC/GAC)]. Mutations were identified in 52 of cases [22 were $-\alpha^{3.7}/\alpha\alpha$ (36.06%), 9 were $-\alpha^{3.7}/-\alpha^{3.7}$ (14.75%), 3 were --Med/αα (4.91%), 2 were $-\alpha^{3.7}/-\alpha^{4.2}$ (3.27%), one was $-\alpha^{3.7}/--^{Med}$ (1.63%), one with point mutation $\alpha^{CS}\alpha/\alpha\alpha$ (1.63%) and 12 were normal (19.67%).] but in remaining 11 patients (18.03%) no deletion or common point mutations using reverse dot blot or

southern blot analysis was observed. These 11 individuals are being investigated for possible point mutation by DNA sequencing.
Our study shows that the 3.7kb single gene deletion is a common cause of microcytic, hypochromic anemia whereas other deletions and point mutations are not that common in Iran.

P824. A rare mutation (codon 22 A>C) in beta-thalassemia and its prenatal diagnosis

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Beta-thalassaemia, which is characterised by reduction of beta-globin chain synthesis, is an autosomal recessive disease. So far, more than 500 different mutations have been shown to be associated with this disease. In this study, beta-globin gene mutation screening was performed for a beta-thalassaemia carrier woman who was 12 weeks pregnant, and for her husband and for the fetus by reverse dot blot hybridisation (RDBH) and DNA sequencing .
The IVSII-1 mutation of the beta-globin gene was detected in the father of the fetus by RDBH kit (Vienna Lab), while same technique revealed no mutation for the mother. Direct sequencing of the gene was performed for the mother, and for her fetus following chorionic villus sampling. Genotype of the proband and fetus were determined as codon 22 A>C/normal and IVSII-1/ codon 22 A>C, respectively. Genetic counselling was given to the family, and at the request of the family the pregnancy was terminated.
The codon 22 A>C mutation, which is very rare, could be the reason for the high HbA2 (47.7%) in the proband and, as a result, this finding could be valuable for hemoglobin tetrameric structure and function.

P825. Haplotype analysis in Beta-thalassemia patients and its association with response to hydroxyurea treatment

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β-thalassaemia is the most frequent monogenic abnormality in Iran. Transfusions are often necessary for patients, which result in iron overload and the need for chelation agents.
A number of chemicals have been shown to raise the HbF level in B-thalassaemia affected children. It seems the polymorphism (*XmnI*) in the promoter of the Gamma G gene may be associated with response to treatment with Hydroxyurea (HU). But our observations indicate that this polymorphic site is not consistent patient to patient, and responses to HU differ significantly. To investigate this differences in response to HU, RFLP analysis including XmnI and mutation identification was performed for 50 affected β-thalassaemia patient candidate for receiving HU.
Our results indicate that 52% of patients were homozygous and 18% heterozygous for IVSII-I. Other observed mutations were: Fr8/9, Fr8/7, IVSI-5, C22, C8, Fr36/37, IVSI-I. Fifty two percent of patients were homozygous for the positive allele of XmnI(+/+). Eighty one percent of homozygous IVSII-I individuals are homozygous XmnI(+/+) , and 96% of XmnI(+/+) patients are linked to haplotype III.

P826. Chaperones increase activity of several cystathionine beta-synthase (CBS) mutants

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Misfolding and aggregation of mutant CBS has been recently proposed as a novel pathogenic mechanism in homocystinuria. As chemical and molecular chaperones may prevent newly synthesized proteins from incorrect folding and/or aggregation we studied their effect on activity and protein assembly of selected CBS mutants (delEx12, I278T, E176K, A114V, G85R).
We expressed the CBS mutants in *E.coli* and CHO cells, measured the catalytic activity and monitored the degree of assembly by native western blotting.
We examined the effect of CBS ligands i.e., heme precursor-hemin and pyridoxal-5´-phosphate precursor-pyridoxine, on mutant CBS assembly and activity. Hemin treatment increased CBS activity of I278T, delEx12 and A114V mutants; the pyridoxine had the same effect to the later mutant.
To determine the effect of chemical chaperones on CBS mutants, various chemical chaperons in a broad concentration range were used. The trimethyl-N-oxide treatment restored delEx12 and A114V activity; the betaine had the same effect on the later mutant.
To examine the effect of molecular chaperones, we co-expressed in *E.coli* CBS mutants with GroES/GroEL chaperones. The overexpressed chaperones positively affected both the activity and assembly of A114V, E176K and delEx12 mutants.
Our results suggest that some CBS mutants may be stabilized in native (active) state already by increased ligand concentration (heme, pyridoxal-5´-phosphate). Chemical chaperones (trimethyl-N-oxide, betaine) increase activity of several CBS mutants.
Some mutant proteins can be rescued only by co-expression with molecular chaperones. Ligands and chaperones should be taken into consideration as possible novel treatment modalities for homocystinuria.
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P827. ARC syndrome is not allelic to PFIC I or II

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Introduction. ARC (arthrogryposis, renal dysfunction, cholestasis) syndrome (OMIM 208085), also known as Nezelof syndrome is a rare autosomal recessive metabolic disease. Fewer than 40 cases have been described in the literature over the last 30 years. The patients are born with variable degree of arthrogryposis, renal dysfunction, manifesting usually as proximal tubular insufficiency, and cholestasis. Liver biopsy displays giant cell hepatitis with lipofuscin deposition or biliary duct hypoplasia. Some aspects of liver disease are similar to those found in progressive familial intrahepatic cholestasis (PFIC) type I and II, in which, as in ARC, serum gamma glutamyl transpeptidase (GGT) activity is low. We collected the largest series of DNA from families affected by ARC and investigated whether ARC is allelic with PFIC I or II by homozygosity mapping.
Methods and Results. DNA from patients and their families were collected with informed consent. Initial linkage studies were performed in four families using four microsatellite markers that are within a genetic distance of 1 cM of the *ABCB11* and *ATP8B1* genes. Mutations in these genes are associated with low GGT cholestasis. Linkage to both genes was excluded in these ARC families. We are now proceeding with a genome-wide linkage search to map the ARC locus.
Conclusions. ARC syndrome is not an allelic condition to PFIC I or II. Further research will help to identify the gene that is mutated in this devastating disease.

P828. DHCR7 gene mutations in patients with Smith-Lemli-Opitz syndrome and preliminary estimation of heterozygote disease carrier frequency in the Polish population

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Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder of cholesterol biosynthesis caused by mutations of the DHCR7 gene. The aim of the study was to determine the frequency of the carrier disease in the Polish population. Among 37 Polish SLOS patients, two mutations, W151X and V326L, account for 60 % of all observed DHCR7 changes in our cohort. Both mutations were observed in 85% of diagnosed families on one or two alleles of the DHCR7 gene and were used as a marker of the disease in the Polish population. To date we have screened DNA samples obtained from among 2000 randomly gathered Guthrie cards. Nine heterozygotes with the W151X mutation among 445 screened individuals and no heterozygotes with the V326L mutation among 316 screened individuals were detected. The carrier frequency of the W151X mutation in Poland was preliminarily estimated as 2%. The frequency of carrier disease seems to be higher than estimated for European and American Caucasians (1-1.4%), but is similar to the carrier frequency in populations in the Czech Republic and Slovakia (2%).
The study was supported in part by KBN, Project No. 1/PBZ/02.

P829. Reverse-hybridization analysis of multiple genetic risk factors for cardiovascular disease

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A number of genetic and environmental risk factors have been found or suspected to predispose to cardiovascular disease (CVD), the term collectively used for disorders of the heart and blood vessels. Among the environmental components associated with CVD are physical activity, diet, alcohol and drug consumption, smoking and stress. Genetic susceptibility may be caused by mutations and polymorphisms in a variety of genes mainly involved in blood coagulation, regulation of blood pressure, and metabolism of lipids, glucose, homocysteine or iron.
We have developed a reverse-hybridization assay (CVD StripAssay) for the rapid and simultaneous detection of the following 12 candidate CVD risk factors: FV R506Q (Leiden), FV H1299R (R2), Prothrombin G20210A, MTHFR C677T, MTHFR A1298C, β-Fibrinogen -455 G-A, PAI-1 4G/5G, Factor XIII V34L, GPIIIa L33P (HPA-1), HFE C282Y, Apo B R3500Q, Apo E2/E3/E4. The test is based on two multiplex PCR reactions and hybridization to a teststrip presenting a parallel array of allele-specific oligonucleotide probes for each mutation. The entire procedure from blood sampling to the identification of mutations requires less than 6 hours, and hybridization/detection may be carried out manually or essentially automated using existing instrumentation (e.g. TECAN profiBlot). The test is simple and convenient, requires very small amounts of samples, and can easily be modified to include additional mutations.
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P830. Mutation Analysis of the CTNS Gene for Cystinosis

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Cystinosis is an autosomal recessive disorder affecting ~1/175,000 live births, caused by failure to transport the amino acid cystine out of the lysosomes. Cystine accumulates forming crystals which cause cell and tissue destruction in all systems of the body.
Three forms of cystinosis have been defined by age of onset and severity of symptoms. The infantile form characterised by renal fanconi syndrome accounts for 95% of cases.
Cystinosis is caused by mutations in the CTNS gene (17p13). The most common a 57kb deletion which has been shown to occur in ~76% of Northern Europeans. The remainder of mutations identified in the CTNS gene are spread throughout the coding regions and no hotspots have yet been identified.
CTNS Mutation analysis has been performed in 116 affected individuals whose diagnosis had been confirmed by biochemical analysis. Screening involved an initial PCR test for the 57kb deletion followed by SSCP and sequencing analysis of the 12 exons of the CTNS gene. In total 19 different mutations have been identified across the gene. The most prevalent of these is the 57kb deletion accounting for 46% of mutations identified. This was only seen in individuals of Northern European origin. Other mutations identified include the L158P (3.4%), W138X (2%), G339R (3%) in the Northern European population, the c.18-21delGACT (5%), G95X (3%), c.771del23bp (2%), c.809delCCT (2%) in the Asian population and the E227E variant (6%) in individuals of Turkish origin.
Identification of these mutations has allowed us to offer carrier testing and rapid prenatal diagnosis.

P831. Does osteopontin protect against kidney stone disease?

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Based largely on *in vitro* studies, osteopontin (OPN) is reported to have multiple functions in the kidney, including propagation of inflammation via macrophage attraction and inhibition of stone formation. Several acute kidney injury models in *Opn* knockout mice have demonstrated short-term attenuation of macrophage infiltration, but the effects of OPN deficiency in chronic renal injury are not known. *Opn*-/- mice show normal renal development and histology. The lack of spontaneous deposition of lithogenic substances in kidneys from these mice suggests that OPN may not have a significant reno-protective role *in vivo* in the absence of additional renal insult. Mice with adenine phosphoribosyltransferase (Aprt) deficiency develop 2,8-dihydroxyadenine (DHA) nephrolithiasis similar to human patients due to the conversion of adenine to DHA by xanthine dehydrogenase. We therefore created *Aprt/Opn* double knockout mice to delineate the role(s) of OPN in chronic renal stone disease. Male and female mice at 6 and 12 weeks of age were characterized by HPLC analysis of urinary purines and immunohistochemistry for macrophages. Urinary purine levels showed no significant differences between *Aprt*-/- *Opn*-/- and *Aprt*-/- *Opn*+/+ mice. Immunostaining with a biotinylated antibody against macrophage marker F4/80 also showed no statistically significant differences between the two genotypes. These studies suggest that OPN may not play a crucial protective role in chronic renal injury, and that the expression of other chemokines may be upregulated to compensate for OPN deficiency. A comparison of DHA crystal burden and renal histopathology in the single and double knockout mice is in progress.

P832. Exploring nuclear gene defects in mitochondrial disorders using comparative proteomics

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Leigh Syndrome (LS) is a neurodegenerative disorder mostly based on neuroradiological findings of bilateral symmetric lesions in basal ganglia and in the brainstem, reflecting focal necrotic areas on pathological examination. However, the genetic causes of LS are extremely heterogeneous. The syndrome has been mainly attributed to deficiencies of the mitochondrial energy metabolism such as respiratory chain (RC) deficiencies, ATPase or pyruvate dehydrogenase deficiency. Among these, cytochrome c oxidase deficiency (RC complex IV; COX) is relatively frequent. RC deficiencies are not only caused by mutations in genes coding for the structural subunits of the respiratory chain themselves, but also in genes coding for proteins involved in assembly and biogenesis of the complexes. Here, a proteomic approach was used to investigate whether such mutations would affect the pattern of mitochondrial proteins at a broader level. Purified mitochondria from control cell lines and from patients with known mutations in *SURF1* (COX assembly gene with unknown function) and *SCO2* (encodes

a copper transporter to COX) were analysed by two-dimensional electrophoresis for up- and downregulated proteins. We present a comparison of pathologic and normal results and assignments by mass spectrometry. This approach opens new insights into potential protein-protein interactions, as well as into genotype-phenotype correlation of mitochondrial disorders.

P833. An assay panel for identification of cytochrome P450 alleles with the Pyrosequencing™ technology

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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 impaired catalytic activity of the gene product. The aim of this study was to establish rapid and reliable assays for identification of a number of alleles in seven Cytochrome P450 genes.
The Pyrosequencing™ technology, or real-time sequencing, is a fast and accurate technology for various kinds of genetic analyses. Pyrosequencing AB (Sweden) manufactures the PSQ™ 96MA and PSQ™HS 96A Systems for parallel analysis of up to 96 assays. We here present a panel of Pyrosequencing assays for determination of single nucleotide polymorphisms (SNPs) and deletion polymorphisms in CYP1A2, CYP2D6, CYP2A6, CYP2C19, CYP2C9, CYP3A4 and CYP3A5, representing many of the most important alleles. Multiplex genotyping was used for D6, C19 and C9, and this procedure enabled simultaneous scoring of two or three polymorphisms in the same assay. When patient samples with known genotype were analysed, the Pyrosequencing assays correctly identified 100% of the alleles.
One major feature with the Pyrosequencing technology is the sequence context obtained around the variable positions, which serves as an internal quality control for each assay. The unique combination of sequence context, accuracy and speed makes the Pyrosequencing technology highly suitable for Cytochrome P450 genotyping.

P834. Inhibition of human fibroblasts prolidase

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Prolidase Deficiency (PD) is a recessive connective tissue disorder characterized mainly by skin lesions and caused by mutations in the gene coding for prolidase, an ubiquitous and specific peptidase, cleaving the dipeptides with a C-terminal prolyl and hydroxyprolyl residue. Prolidase is involved in the final stage of protein catabolism. The relation between prolidase functions and the disease is still largely unknown.
We investigate the effect of a potent in vitro inhibitor of porcine kidney and human erythrocytes prolidase, the N-benzyloxycarbonyl-L-proline (CBZ-Pro), on human prolidase, obtained from cultured fibroblasts. A 91 % inhibition was detected incubating cellular extracts with 1:1 ratio of Gly-Pro substrate: CBZ-Pro inhibitor (20mM) at pH 7.8. Pulse experiments performed incubating human fibroblasts with 6 mM CBZ-Pro from 1 min to 1 hour and up to 10 days revealed that the inhibitor permeated quickly the membrane. After 1 min the intracellular amount of inhibitor, calculated by capillary electrophoresis, was 282.5±15.92 nmol/ mg. The uptake of CBZ-Pro tended to saturate as its concentration increased over 8 mM. Acidic pH of the medium (pH=6.0) stimulated the uptake of CBZ-Pro. Long term incubation of fibroblasts with CBZ-Pro caused mitochondria depolarization and increased cellular death as reported for long term culture of fibroblasts obtained from PD patients, which lack prolidase activity. In conclusion we demonstrated that CBZ-Pro is a potent inhibitor of human fibroblasts prolidase with high permeability to the membrane and it could potentially be used in vivo to better characterize the prolidase enzyme and further investigate PD physiopathology.

P835. Maternally-inherited hepatocyte nuclear factor 3β (HNF-3β) mutations in congenital hyperinsulinism

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Congenital hyperinsulinism (CHI) is characterised by profound hypoglycaemia related to inappropriate insulin secretion. Underlying lesions include focal and diffuse forms sharing similar clinical presentations, but differing in molecular mechanisms. Focal CHI has been ascribed to loss of heterozygosity for a paternally-inherited mutation in the ATP-sensitive potassium channel (K_{ATP}) genes SUR1 or Kir6.2 due to a maternal aneusomy (11p15.1) in the pancreatic lesion. Indeed, the loss of the maternal chromosome 11p15 in the focal lesion disrupts the balanced expression of several imprinted tumour-suppressor genes mapped to this region. Diffuse CHI undergoes distinct mechanisms involving autosomal recessive or dominant inheritance. No more than 50% of diffuse and approximately 30% of focal CHI have known molecular bases. Recently, the role of hepatocyte nuclear factor HNF-3β (Foxa2) in glucose sensing and pancreatic β-cells homeostasis has been described and the absence of HNF-3β has been shown to lead to altered expression of the K_{ATP} channel subunits SUR1 and Kir6.2 in transgenic mice and insulin-secreting cell lines. Moreover, targeted HNF-3β gene deletions in pancreatic β-cells have been shown to cause hyperinsulinaemic hypoglycaemia in mice. We regarded therefore this gene as a strong candidate gene in CHI.
We report here the first evidence for HNF3-β gene mutations (A100E, A210V, G101E) in three cases of CHI and their implication in glucose sensing and insulin secretion. All the three patients had maternally-inherited HNF-3β mutations. Electrophysiological studies on pancreatic ß-cells isolated from a CHI patient showed markedly reduced K_{ATP} channels expression. This study, suggests a novel mechanism for focal CHI.

P836. Respiratory chain deficiency presenting as congenital nephrotic syndrome.

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Congenital nephrotic syndrome (NS) in infancy includes NS of Finnish type (*nephrin*), diffuse mesangial sclerosis, idiopathic NS, most often steroid-resistant and nephrotic syndrome related to viral infections. Later in life NS have been described in association with neuro-muscular symptoms, deafness and diabetes in children and adults with respiratory chain (RC) disorders. Hitherto however, NS have never been observed in neonates with RC disorders. Here, we report on RC deficiency in three infants with congenital NS in two unrelated families. Clinical and histopathological presentations were similar to congenital NS of Finnish type and mitochondrial RC complex II+V deficiency was identified in all cases. Based on these observations, we suggest giving consideration to RC disorders in patients with congenital forms mimicking Finnish type NS.

P837. Association of the D2 dopamine receptor gene with opium-addiction in Iran

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Dysfunction of central Dopaminergic neurotransmission has been suggested to play an important role in the etiology of certain neuropsychiatric disorders such as drug abuse. It has been shown that the Dopamine D2 Receptor (DRD2) gene dysfunction is associated with multi-drug addiction. Addiction to opium is the most common form of drug abuse in Iran. We studied the allelic association between DRD2 Taq I A polymorphism in 100 opium-dependent

patients and 80 unrelated controls. A 310 bp region surrounding Taq I site at the DRD2 locus was amplified by polymerase chain reaction (PCR) and the PCR product was incubated with Taq I restriction enzyme. The A1 allele remained intact while the A2 allele was cut. Significant association was observed between A1 allele and addiction in the patients group (P<0.0001). Moreover,the frequency of A1A1 genotype was significantly higher in opium users than controls (P<0.0001). Our result further indicates that DRD2 may be involved in the pathophysiology of opium addiction.

P838. Polymorphism of the CCR-5 gene in Kazakh population of Middle Asia

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Molecular genetic methods give objective criteria to study the origin, migration and ethnogenesis of human populations. Mutation for the 32 base-pair deletion in the CCR-5 gene makes its holders nearly immune to AIDS, since this gene has no receptor for AIDS-related viruses. The mutant allele of CCR-5 is present at high frequency in the Caucasian population (9-13%), but is absent in the Japanese and black populations. The aim of this study was to assess the frequency of the truncated allele of CCR-5 gene in the Kazakh population of Central Asia, representative of Turkic-speaking ethnic groups. A total of 224 unrelated healthy Kazakh people were studied. A portion of CCR-5 gene from genomic DNA was amplified by PCR and analyzed on a 6% polyacrylamide gel. The frequency of the delta CCR-5 allele was 4.91%, significantly lower than reported in the Caucasian population, but more frequent than in other Turkic-speaking ethnic groups (1-3%) and African, East-Asian or Indian populations (0%). 8.04% of Kazakhs had one defective gene, and only 0.89% had both genes defective. Thus our results indicate that the CCR-5-delta-32 mutation is not only immense in its distribution among the population, but it also, according O'Brien's theory, first occurred in northern Europe and then spread south slowly in the course of migrations.

P839. PDS is a new susceptibility gene to autoimmune thyroid diseases : association and linkage study

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Autoimmune thyroid disease (AITD), including Graves' disease (GD) and Hashimoto thyroiditis (HT), is caused by multiple genetic and environmental factors. The *PDS* gene (7q31), responsible for Pendred syndrome (congenital sensorineural hearing loss and goitre), encodes a transmembrane protein known as pendrin. Pendrin is an apical porter of iodide in the thyroid. To evaluate the contribution of the *PDS* gene in susceptibility to AITD, we examined four microsatellite markers in the chromosomal region. One hundred ninety five unrelated patients (GD,141; HT, 54), fifteen multiplex AITD families (104 individuals/46 patients) and 154 normal controls were genotyped. Analysis of case-control data showed a significant association of *D7S496* and *D7S2459* with GD (p= 10^{-3}) and HT (p= $1.07x10^{-2}$) respectively. A family based association test (FBAT) showed significant association and linkage between AITDs and alleles 121bp of *D7S496* and 173bp of *D7S501*. Results obtained by TDT are in good agreement with those obtained by FBAT; linkage and association of the 121 bp allele of *D7S496* with AITD was confirmed (p=0.0114). Multipoint non parametric linkage analysis using MERLIN showed intriguing evidence for linkage with marker *D7S496* in families with only GD patients (Z=2.12, LOD=0.81, p=0.02). Single point and Multipoint parametric LOD score linkage analysis was also performed. The highest multipoint parametric LOD score was found for marker *D7S496* (LOD=1.23; p=0.0086) in families segregating for GD under a dominant model. This work suggest that the *PDS* gene should be considered a new susceptibility gene to AITDs with varying contribution in each pathology.

P840. Analysis of mitochondrial DNA and Y chromosome diversity in Lithuanian population

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There are conflicting anthropological findings regarding the origins of Indo-Europeans and formation of Baltic tribes in Eastern Baltics. The Lithuanians and the Latvians are the only Baltic cultures that survived until today, therefore molecular genetic characterisation of these populations may help to reconstruct the prehistory and ethnogenesis of the people in this area. We have analysed mitochondrial DNA and Y chromosome diversity in six ethnolinguistic groups of Lithuanians. Analysis of molecular variance shows that all variation in Lithuania is due to variation within groups, and no variation was detected among groups neither in mtDNA nor Y-chromosome markers. Comparisons of mtDNA sequences with other European populations have not revealed significant differences, however, more lineages are shared with populations of northern Europe. Y-chromosome analysis shows Lithuanian population to be closest to Latvians and Finno-Ugric speaking Estonians, all three populations being indistinguishable in frequencies of biallelic markers. Interestingly, Lithuanians have a high frequency (~37%) of TatC allele, which is frequent in Uralic speakers of northern Eurasia. However, the analysis of Y chromosome microsatellite markers (DYS19, DYS389, DYS390, DYS391, DYS392, DYS393, DYS385) on TatC allele background in Lithuanians and Estonians revealed significant differences, thus indicating different histories of these populations. Lower gene diversity and nearly star-like median joining network of Lithuanian TatC chromosomes indicates a recent population bottleneck, however major Lithuanian haplotypes are rare in Estonians, suggesting that these populations have different origins or have differentiated before Indo-Europeazation took place in Eastern Baltics.

P841. LD Blocks and SNP based haplotypes in the Fragile X region

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Unstable germline transmission of the FRAXA CGG triplet repeat at Xq27.3 results in transcriptional silencing of the adjacent FMR1 gene when the repeat tract exceeds 200 units. The resulting Fragile X phenotype is the most common form of inherited mental retardation affecting ~1/5500 males. Purity and length of the CGG array influence stability but other *cis*-acting factors are postulated to exist. Over the last decade, three microsatellite repeats (DXS548, FRAXAC1 and FRAXAC2), have been commonly applied to characterise the haplotypic background upon which a FRAXA allele occurs. Although 'high-risk' haplotypes have been identified using these markers, causal determinants of expansion remain elusive.
We have typed 11 single nucleotide polymorphisms (SNPs) across 650 kb of the FRAX region on a panel of 797 independent male chromosomes that span the range of FRAXA repeat sizes. The resultant haplotypes confirm and extend the information gleaned from microsatellite haplotypes. A linkage disequilibrium map of the region was created to identify discrete regions of elevated recombination. Dendrograms showing the relationships between SNP-haplotypes were established by giving most weight to SNPs which occurred within the same LD block as the FRAXA mutation and least weight to SNPs separated from FRAXA by a region of low LD (increased recombination). Haplotypes within distinct branches of the resultant dendrograms show high correlation with regard to FRAXA repeat size, microsatellite marker allele composition and interspersion pattern. The phylogenetic information is further advanced by primate haplotypes and provides new clues on the nature and behaviour of this clinically significant dynamic mutation.

P842. Identification of novel polymorphisms and analysis of haplotypes in the human methionine synthase reductase gene (MTRR)

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Methionine synthase reductase (MTRR) is an enzyme required for the reductive activation of methionine synthase in the remethylation

pathway of homocysteine metabolism. MTRR deficiency causes a rare autosomal recessive disorder, the cblE type of homocystinuria. Several putatively pathogenic mutations and two common polymorphisms were previously found in the gene encoding the human MTRR. The aims if this study were to identify novel polymorphisms in the MTRR gene, to determine their frequencies in the Czech population, and to analyse the MTRR haplotypes.
By sequencing of RT-PCR products we found 12 polymorphisms - 2 known and 10 novel. Three polymorphisms are missense mutations, 7 changes are synonymous mutations and 2 mutations are located in the 3´-untranslated region of the MTRR cDNA. Using PCR-RFLP analysis of 100 Czech control alleles we observed frequencies of these polymorphisms in the range from 0.02 to 0.53. Results from these studies were used for haplotype analysis using statistical program HAPMAX. This program predicted 8 most probable haplotypes with frequencies 0.02 to 0.31 (see Table).

Polymorphism (cDNA)	66 G>A	524 C>T	537 T>C	1049 A>G	1464 A>G	1536 C>T	1653 G>A	1761 T>C	1875 A>G	1911 G>A	2146 A>G	2248 A>G
Protein change	M22I	S175L	L179L	K350R	V488V	S512S	P551P	Y587Y	V625V	A637A	3´UTR	3´UTR
Polymorphism frequency (n=100)	0.47	0.36	0.15	0.15	0.02	0.02	0.02	0.02	0.15	n.a.	0.02	0.02
HAPLOTYPE (frequency)												
1 (0.21)	A	C	T	A	A	C	G	T	A	n.a.	A	A
2 (0.09)	A	T	T	A	A	C	G	T	A	n.a.	A	A
3 (0.10)	A	C	C	G	A	C	G	T	G	n.a.	A	A
4 (0.02)	A	T	T	A	A	C	A	T	A	n.a.	A	A
5 (0.31)	G	C	T	A	A	C	G	T	A	n.a.	A	A
6 (0.21)	G	T	T	A	A	C	G	T	A	n.a.	A	A
7 (0.05)	A	T	C	G	A	C	G	T	G	n.a.	A	A
8 (0.02)	G	C	T	A	G	T	G	C	A	n.a.	G	G

Our data show that the MTRR gene is highly polymorphic, nevertheless, there is a high degree of linkage disequilibrium among these polymorphisms as relatively few haplotypes were found. Due to location of some of these polymorphisms in CpG dinucleotide it was also possible to deduce a cladogram of MTRR alleles.
This study was supported by the GA UK grant 36/00 and in part by the research project VZ111100003.

P843. APOE distribution in World populations with new data from the Indian sub-continent and the British populations.

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Human apolipoprotein E (apo E) is a plasma glycoprotein that plays a major role in lipoprotein metabolism. Three common variants (*E2, *E3, & *E4) of APOE show interesting population genetic variation. Epidemiological studies have found that the *E4 allele is associated with longevity, increased plasma cholesterol levels and increased prevalence for cardiovascular and Alzheimer diseases. In this study we present new data from Indian (10 Indian caste and tribal groups; Punjabi Sikh, Punjabi Hindu, Scheduled Castes, Madhya Pradesh (MP) Brahmins, MP Khatris castes; MP-Maria Gond1, MP-Maria Gond2, MP-Maria Gond3, MP-Kamar, and Koch tribal populations) and 6 regionally divided populations of the UK. We also examine the level and extent of genetic variation at APOE locus in world populations (276 population/studies) and its utility as a population genetic/disease marker using multivariate analyses. The interesting feature of this analysis is low incidence or absence of E4 allele in many caste and tribal populationsof India, even though cardiovascular diseases are relatively common. Longitudinal west to east decreasing cline of APOE*E4 was observed in Indian subcontinent. The UK populations showed higher allele frequency of *E4 allele that is compatible with the observed European North-South cline. Latitudinal clinal variation is also observed in Africa and Asia. A comprehensive statistical analysis of world populations showed that APOE is a useful genetic and evolutionary marker for anthropological studies. The data presented also suggests that authochthon groups (like tribes) in India may throw better insight on the role of apolipoproteins in disease.

P844. Polymorphism of D1S80 in some populations of North Caucasus.

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Polymorphism at the minisatellite locus *D1S80* has been explored in Adygei Highlanders, Adygei Shapsugs, Cherkesians, Abkhazians and Russians of the Krasnodar region (Kuban Kazaks). Abkhazians and Shapsugs live on the Black Sea coast, others inhabit Northwestern areas of the Caucasus. High individual discrimination power of this marker was used to discover phylogenetic relationships both between the ethnic groups studied and reference populations around the world. Allele typing was performed using PCR and electrophoresis followed by silver staining. Twenty-four alleles of *D1S80* were noted in the populations studied. The level of observed heterozygosity is high and varied from 75 to 83 per cent. Observed allele frequency distributions are bimodal with modes at alleles 18 and 24. These distributions in all populations studied were similar to those in European populations. An analysis of *D1S80* variability was made with multidimensional scaling treatment of Nei's genetic distance matrix. The plot obtained reveals close affinity between Cherkesians and Adygei Highlanders. Preliminary single-locus analysis has shown that populations of the North Caucasus are most closely related with East Slav peoples – Russians, Ukrainians and Byelorussians. The study was supported by the Russian Foundation for Basic Research.

P845. Alu –insertion polymorphisms in population of Middle Asia.

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Unravelling the structure and history of populations is one of the important problems of human population genetics. People of Central Asia are very attractive populations for this investigation, because they were formed as a result of consolidation of different ethnic components: Turkic and Iranian-speaking. Alu-repeats are very useful markers, because they reflect both maternal and paternal history of populations; there is no parallel gain of Alu-elements at a particular chromosomal location and thus all chromosomes that carry a polymorphic Alu-insert are identical by descent. We studied polymorphic Alu- insertions at 5 loci: *ACE*, *TPA25*, *PV92*, *APOA1*, and *NBC27* in Kazakhs (n=224), Uzbeks (n=107) and Uigurs (n=69). We observed frequencies of insertion for *ACE* , *APOA1*, *PV92*, *TPA25* and NBC27 of 51.3 %, 85 %, 52.7 %, 48.4 % and 28.3 % in Kazakhs; 52.8%, 88.3 %, 45.8 %, 44.6 % and 28 % in Uzbeks; 58 %, 55.8 %, 50.7 %, 47.8% and 18.8 % in Uigurs. We have found differences between the studied populations (Fst =2.68%). The greatest genetic differentiation between populations was for *APOA1* (11.8%) and the least for *TPA25* (0%) (for *ACE* 0.3%, for *PV92* 0.3%, for *NBC27* 1%). The highest mean theoretical heterozygosity was defined in Kazakhs for *ACE* and *TPA25* (50%) and in Uigurs for *PV92* (50%); the lowest was in Uzbeks for *APOA1* (20.6%). Data from our investigations indicates a similar degree of genetic relations and common origin of Uzbeks and Kazakhs.

P846. Variations in the frequency of Alzheimer disease risk genes in three consanguineous Pakistani communities

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There is a long tradition of intra-community marriage and a preference for consanguineous unions in many Asian and African populations. Previous studies in Pakistan, where marriages are usually intra-community and approximately 50% are between first cousins, showed significant autosomal and Y-chromosome differences between co-resident indigenous communities. In the present investigation, APOE genotypes and the Glu31Gly presenilin-1 polymorphism of the same three communities, the Awan, Khattar and Rajpoot, were studied for evidence of predisposition to Alzheimer disease. The APOe2 allele was absent in all three communities; the APOEe3 frequency was 91% in the Kathar, 97% in the Rajpoot and 72% in the Awan; and the APOEe4 frequency was 9% in the Kathar, 3% in the Rajpoot and 28% in the Awan, the latter including 4/60 individuals homozygous for e4. By comparison, a very high frequency (36%) of the Glu31Gly presenilin-1 polymorphism was found only in the Rajpoot. Further, this polymorphism was restricted to a specific Rajpoot sub-community previously defined by Y-haplotype analysis. As life expectancy increases and age-related disorders become more common, the influence of genetic risk factors will predictably be reflected in the relative prevalence of Alzheimer disease. To date, few studies have been conducted into possible relationships between consanguineous marriage and major adult onset diseases. Fewer still have considered the influence of inter-community differences, and community genetic substructure, on disease profiles. The results have major implications both for the populations of developing countries, and for many migrant communities now resident in Europe, North America and Australasia.

P847. VNTR - Polymorphism of Genes eNOS and PAH in Populations of Northern Caucasus

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One aspect of studying genetics of a population is to investigate polymorphic DNA loci, in particular variable number of tandem repeats (VNTR), and the comparative analysis of allele frequencies of DNA markers in populations. We investigated polymorphic tandem 27 bp repeats in intron 4 of the gene encoding endothelial nitric oxide synthase (eNOS, NOS3) and a 30 bp tandem sequence in the phenylalanine hydrozylase (PAH) gene in populations of Northern Caucasus: Kuban Nogays (n = 92), Caranogays (n = 153), Kymuks (n = 112), Carachaevs (n = 107) and Adygi (n = 163). Due to their unique geographical position and great linguistic diversity, populations of the Caucasus are interesting for studying the influence of geographical and language barriers on the genetic structure of populations. The frequency of allele B (5 tandem repeats) in the eNOS gene ranged from 0.807 in Carachaevs up to 0.891 in Nogays. In all populations the maximal value of theoretical heterozygosity is seen in the VNTR locus (from 0.678 in Kymuks to 0.725 in Carachaevs). The most frequent allele is 500 bp among Adygi (0.364), 380 bp among Kymuks, Nogays and Caranogays (0.398 - 0.449), and 530 bp among Carachaevs (0.371). The allele distributions differ significantly between Caranogays and Carachaevs ($p < 0.001$) and between Caranogays and Kymuks ($p < 0.001$).

P848. Preliminary study of four SNPs in genes involved in folate metabolism to analyze their association with risk of Down Syndrome in Spain

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Low blood folate levels and elevated homocysteinemia produced by abnormal homocysteine/folate metabolism has been associated with Down syndrome (DS). Therefore, genetic variants of enzymes involved in folate metabolism are expected to contribute to the risk of DS. The goal of this work was to start a pilot study to determine the genetic background in our population, analysing several SNPs identified in genes involved in the folate pathway, to subsequently demonstrate if genetic-nutrient effects are involved in risk to DS. Up to now, we have analyzed 85 control mothers and 44 mothers with a child affected by karyotypically confirmed full trisomy 21. We have analysed the frequency of four non-synonymous SNPs located in two proteins involved in the folate and methyl groups metabolism (MTHFR and MTRR) and in one involved in the intestinal absorption of dietary folate, glutamate carboxypeptidase II (GCPII) .We have analysed two SNPs previously studied by other groups, 677C>T in the MTHFR gene and 66A>G in the MTRR gene. We have also included 1298A>C in the MTHFR gene and 1561C>T in GCPII. The assay for the two MTHFR and the MTRR SNPs was performed by real-time PCR analysis in a Light Cycler and simultaneous detection by allelic discrimination using melting-temperature analysis and fluorescence-resonance energy transfer (FRET). The analysis of the GCPII polymorphism 1561C>T was done by restriction analysis using AccI. Allele frequencies were calculated in the sample analyzed and the distribution of the different genotypes is reported.
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P849. Epidemiological surveillance of oral clefts in North - Western Croatia during 1983-2000 period

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We present data on the registration of oral clefts in four regions of North - Western Croatia during the 1983-2000 period. The prevalence rates of oral clefts (cleft lip with or without palate - 9.1/10000; cleft palate - 4.6/10000) are consistent with the average prevalence rate of the EUROCAT registries (cleft lip with or without palate - 9.1/10000; cleft palate - 5.8/10000). We have found no significant changes in prevalence of both cleft lip with or without palate and cleft palate during the studied period. The exception is an unusually high number of cases with cleft palate that occurred in the region of Koprivnica in the year 1995. Five cases were registered, four of which were born within the period of 50 days, with closely situated places of residence. Among the total of 96 cases of the cleft lip with or without palate we have found 78 (81.2 %) isolated malformations, 17 (17.8%) cases with at least one other major malformation present, 1 syndrome, and 6 cases with chromosomal anomaly (trisomy 13). Among 52 cases of cleft palate, there were 36 (69.2%) isolated cases, 13 (25%) in association with other malformations, 3 (5.8 %) syndrome cases, and 5 cases of Pierre Robin sequence. Although the presented prevalence rates of oral clefts have shown to be stable over a long time period, we consider the observation of a cluster in Koprivnica to be an important clue which led us to analyse the influence of possible teratogenic factors.

P850. Population genetic study of N-acetyltransferase 2 genotypes and alleles in Volga-Ural people of Russia

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The arylamine N-acetyltransferase (*NAT2*) gene catalyzes acetylation of aromatic amines, hydrocarbons and hydrazine. The polymorphic status of N-acetyltransferase varies widely between individuals and ethnic groups.
The study population consisted of 250 individuals from different ethnic groups: Bashkirs, Tatars, Chuvashes, Udmurts and Russians. Subjects were genotyped for three polymorphic sites NAT2*5A (C481T), NAT2*6A (G590A) and NAT2*7A (G857A). Major genotypes and alleles among Tatars, Chuvashes and Udmurts were NAT2*5A/*6A, among Bashkirs - NAT2*4A/*5A, among Russians - NAT2*4A/*5A and NAT2*5A/*5A. There were no significant differences in the frequency distribution of genotypes and alleles between Tatars, Chuvashes, Udmurts and Russians. The genotype distribution of *NAT2* differed significantly between Chuvashes and Bashkirs ($\chi 2 = 5.7$; $p=0.02$).
We compared our results with literature data and no significant

differences were observed between Caucasians and the population of Volga-Ural region (χ2= 14.55; p=0.12).

P851. The Avon Longitudinal Study of Parents and Children (ALSPAC) phenome scan; A novel means of hypothesis generation for genetic association studies.

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The Avon Longitudinal Study of Parents and Children (www.alspac.bris.ac.uk) was designed to investigate the interplay between genes and environment. Approximately 14,000 children have been followed since the early stages of pregnancy for over 10 years. Information about the health, development and life style of the children has been collected by means of regular questionnaires and research clinic visits. A wealth of phenotypic and environmental exposure information has been collected about the children and their parents. Genetic studies currently use DNA from blood samples from approximately 10,000 children and 10,000 mothers, but cell lines are being created (target 10,000 children, 15,000 parents) to ensure optimum DNA supplies for collaborative studies. To exploit the huge amount of phenotypic data available we are developing „phenome scanning“ as a hypothesis generator. This reverses the usual method of analysis applied to population studies as it identifies the different phenotypes associated with each variant of a given gene. The result is a description of the full range of consequences of a particular genetic variant and by implication, the range of consequences of a therapeutic measure acting at the same step of the biological process. Once phenotypes have been shown to be associated with particular genotypes, environmental exposure information can be added to explore gene-environment interactions. We envisage offering phenome scans to European gene-discovery teams to investigate the influence of specific gene variants on common traits, varying from signs, symptoms and diagnoses to features of behaviour, cognitive development and achievement. Collaborations are welcome.

P852. Detection of the Founder Effect in Finnish CADASIL Families

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a systemic vascular disorder causing recurrent brain infarcts and subcortical dementia. Symptoms often begin in early adulthood with migraine with aura followed by cerebral infarcts leading to a stepwise progression of cognitive decline and finally to dementia. The disease is caused by missense mutations or small deletions in the *Notch3* gene at 19p13 encoding transmembrane receptor Notch3. In 19 of the 22 Finnish CADASIL families the disease is caused by R133C mutation. We conducted a haplotype analysis of these 19 families to determine whether this overrepresentation of a single mutation is due to a founder effect in Finnish CADASIL families. Six polymorphic microsatellite markers around *Notch3* locus were selected, D19S885, D19S411, D19S930, D19S593, D19S410 and D19S215, and marker regions were amplified by radioactively labelled PCR. The lengths of repetitive sequences were determined by polyacrylamide gel electrophoresis and autoradiography. In all 19 families we found similar marker allele construction linked to the R133C mutation, though in some families marker uninformativity prevented the confirmation of the mutation linked allele. At least ten families share an identical haplotype for markers D19S411–D19S593. In sex-averaged linkage map this region is 3.2 KcM long. The shared haplotype is strong evidence for ancestral mutation in Finnish CADASIL families. The high polymorphism values of microsatellite markers hindered the determination of the age of the mutation with the computer programs available, however, on the basis of genealogy we know the mutation is older than 250 years.

P853. Origins of the common DHCR7 mutations: IVS8-1G>C and W151X causing the Smith-Lemli-Opitz Syndrome

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Smith-Lemli-Opitz syndrome (SLOS [MIM 270400]) is an autosomal recessive multiple congenital anomaly/mental retardation snydrome caused by mutations in the D7-sterol reductase (DHCR7, E.C.1.3.1.21) gene. The incidence of SLOS has been estimated to range from approximately 1:20,000 to 1:60,000 in populations of European origin.
We are analysing the frequencies, origins, and ages of DHCR7 mutations in Europe. Mutational spectra analysed in 204 SLOS patients were significantly different across populations with frequency maxima of common mutations in East-Europe (W151X, V326L), North-West-Europe (IVS8-1G>C), and South-Europe (T93M). Carrier frequency analysis for the common mutations confirmed an east to west gradient for the W151X mutation and a west to east gradient for the IVS8-1G>C and revealed much higher frequencies for the common null mutation than expected from the reported prevalence of SLOS.
Using eight cSNPs in the DHCR7 gene haplotypes were constructed for SLOS chromosomes. All chromosomes carrying the IVS8-1G>C mutation shared the same haplotype. Analysis of flanking microsatellites together with the frequency distribution in Europe suggest a founder for the IVS8-1G>C mutation around 2000 years ago in England or Northern France. The W151X mutation was present on different related haplotypes and the ancestors of carriers of all W151X haplotypes could be traced back to the same area in Poland. Hence the mutation is probably older than the SNPs used for haplotype construction indicating that W151X may be the oldest common SLOS mutation in Europeans. The data suggest an intriguing heterogeneity of the ages and origins of common DHCR7 mutations in Europe.

P854. *MYBPC3* gene shows high level of sequence variability with ethnic/geographic differentiation with most of the changes unrelated to the disease

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MYBPC3 is a common candidate gene for hypertrophic cardiomyopathy (HCM). Both, neutral and functional mutations are frequent in this gene. We have investigated this gene in 25 unrelated HCM patients from Tomsk (Russia) by SSCP screening and sequencing. 8.3 kb (about one third of the gene) were analysed. Three (presumed) functional mutations (one nonsense mutation and two splicing variants) and 15 polymorphisms were identified. Three additional polymorphisms were found in one HCM patient and two asymptomatic probands of different ethnic origin. The SNP occurence (~1/500 bp) did not differ between exons and introns. For 13 SNPs the frequencies of the rare alleles were determined in caucasoid Russians (N=118), in Tuvinians (N=66) and Buriats (N=59) (mongoloid peoples from Siberia). Six SNPs, among them three missense SNPs identified in Russians (frequencies up to 10%) were absent in Tuvinians and Buriats. Comparison with available online and published data showed that only 8 SNPs from our set of 18 were reported earlier. Three of these were previously qualified as disease causing mutations. The results show considerable variation of *MYBPC3* in normal populations, as well as distinct ethnic and geographic distributions of the variants. Thus, the evaluation of supposed disease-related mutations should be based on carefully selected control samples. It seems that the mutation rate of *MYBPC3* is high and mutations even in coding sequences are not under strong selection pressure. Data on SNPs in this gene bear on gene function studies as well as on population and evolutionary genetics.

P855. Mitochondrial DNA polymorphism in the Kazakh population of Middle Asia

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The hypervariable segment 1 sequences of mitochondrial DNA with RFLP sites was analyzed among the Kazakhs to determine the haplogroup's (hg) structure. We have analyzed 246 Kazakh mtDNA samples and found that more than 58% of mtDNA lineages belong to Asian-specific hg D, C, G, A, M, F. The supercluster D was found with high frequency 17,89% that was more frequent than in the populations of the Volga-Ural region. The hgs A, M and F were found with frequencies 3,25%; 2,85%; 2,44% in Kazakhs, respectively. Hg F was less frequent in Tatars, B was less hg in Kazakhs 0,41%, in Bashkirs 0,9% and was absent in Tatars.
West-Eurasian specific hgs H, T, J, K, U2, U5, HV were observed in Kazakhs with frequency 41,46%. Hg H was found with frequency 13% in Kazakhs and was the most variable, it was less frequent than in Tatars – 31%. The frequency of T, J, K comprised around 4%. Hg I was a rare hg in Kazakhs 0,41%, hg U5 3,25% and T 4,07% were more frequent in Kazakhs than in other Turkic-speaking ethnic groups of Siberia 0%, but less frequent than in populations of the Volga-Ural region -T 6,9% and U 25,9%. The frequencies of the V 0,81% and W 1,63% were the same in Kazakhs and Turkic-speaking populations of the Volga-Ural region.
The obtained data allowed us to construct a phylogenetic tree for Kazakhs on female lineage and to detect their position between ethnic groups in Europe and Asia.

P856. Consanguinity in the Azores islands (Portugal): A retrospective study from 1931 to 2000

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The Azores islands are located about 1500 Km from mainland Portugal and 900 Km from Madeira island. They are inhabited by 242,072 individuals, who live mainly in small rural localities. The major economic activities are agriculture and farming.
The aim of this study was to evaluate consanguinity in the Azorean population, using marriage records obtained at the National Institute of Statistics. The data were grouped in 10–year interval, from 1931 to 2000. In Azores the total number of marriages occurring in this period was 157,256, of which 954 are consanguineous (0.61%). Within consanguineous marriages first-cousin marriages are much more frequent (91.7%) than uncle-niece/aunt-nephew (8.3%).
Here, we determined the mean inbreeding coefficient (α) of the population of Azores, Madeira and mainland Portugal, using Wright's formula. To investigate the trend of consanguineous marriages we plotted α over time, observing a similar pattern of decrease for the three regions. Only during the last decade there was a sharp decline of α in Madeira island to levels below that found in Azores. Also, both Azores and Madeira islands have higher α than mainland. Presently, we are comparing α to the number of inhabitants in Azorean localities, to investigate whether mate choice restriction is determined by geographical isolation. This research is a starting point for further studies regarding the role of consanguinity in the incidence of hereditary disorders in Azores islands. (pacheco_pc@hotmail.com)

P857. Why was there a peak in the prevalence of Down syndrome (DS) diagnoses in 1997?

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Trends in the numbers of diagnoses of trisomy 21 have been monitored using data from the National Down Syndrome Cytogenetic Register from 1989-2000. The expected number of DS births in the absence of prenatal diagnoses and selective termination were calculated and found to be exceptionally high in 1997. These were expressed as rates/1000 total births in England and Wales, adjusted for maternal age using indirect standardisation. The standardised rate in 1997 was 7% higher than the average for 1990-2000 (95%CI: 1%-13%).
We hypothesised that this could be related to the 'pill scare' in December 1995, which followed three reports of a twofold increased risk of venous thromboembolism in users of the third generation combined oral contraceptives. A subsequent short lived increase of all pregnancy terminations and births in England and Wales occurred. However the rise in DS was limited to the last two quarters of 1997, as much as 18 months later. On the other hand, in women aged under 35 years, who were more likely to have been affected by the pill scare, the increase was 11% while in older women it was only 4%. In conclusion, the peak in DS diagnoses in 1997 cannot be explained by changes in maternal age distribution, the total number of births or the rate of prenatal diagnoses. A connection with the effects of the pill scare in 1995 cannot be ruled out, but must remain pure speculation.

P858. A normal polymorphism of CCR5delta32 mutation in North Caucasus populations.

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The chemokine receptor gene CCR5 bearing a 32-basepair deletion is known to prevent cell invasion by the primary transmitting strain of HIV-1 and display delayed progression to acquired immunodeficiency syndrome (AIDS). The frequency of the CCR5delta32 allele among healthy unrelated natives from North Caucasus populations has been estimated. Genotyping of the deletion was performed using PCR amplification of mutation flanking regions with polyacrylamide electrophoresis separation of the product. The study encompasses indigenous populations inhabit Northwestern region of Caucasus, namely: Adygeis (Shapsugs), Abkhasians, Cherkesians (all of which belong to Adygei-Abkhazian linguistic family), and Kuban Kazaks (Russians of Krasnodar region, Indo-European linguistic family). The data obtained show that the frequency of the CCR5 deletion allele in Kuban Kazaks does not considerably differ from the frequencies revealed in Europeans (most closely to Ukrainians, The French, Danes, Belgians, Norwegians, The Spanish). The populations of Adygei-Abkhazian linguistic family, which shows possible linguistic affinity to Basque, are characterized with different values of the CCR5delta32 frequency. The minimal frequency of CCRdelta32 was noted in Cherkesians and similar with those for Saudi Arabia, Pakistan, Greece, and Indian populations. The values of deletion frequency in Shapsugs and Abkhazians are closest to both other Caucasus populations (Daghestan, Azerbaijan, but Georgians) and Turks, Italians, Cyprians, Catalans, Portugueses, Basques. The data obtained are in agreement with ethno-historic and linguistic knowledge about the North Caucasus region and may contribute in evolutionary study of genetic relations between European and Asian populations. The study was supported by Russian Foundation for Basic Research.

P859. Sex-specific, sperm-mediated transgenerational effects of prepubertal paternal smoking on gestation length, and food supply of paternal grandparents on longevity.

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Emerging evidence that sperm carry information about the ancestral environment suggests multigenerational designs are needed in genetic epidemiology. Published Swedish data showing that paternal and grandparental food supply affects longevity identified the slow growth period (SGP) just before puberty as the exposure sensitive

period. Using the Avon Longitudinal Study of Parents and Children (ALSPAC) that can take account of relevant biases and confounding variables, we show that onset of paternal smoking in the SGP (but not later) is associated with a shorter gestation of his future sons (but not daughters). This sex specificity for transgenerational effects was confirmed in data from the Overkalix community in northern Sweden using the longevity of cohorts born in 1890, 1905 and 1920. Food abundance in the paternal grandfather's SGP reduced the lifespan of his grandsons (but not granddaughters), whilst food abundance in the paternal grandmother's SGP reduced the lifespan of her granddaughters (but not grandsons). Our joint data suggest that the X and Y chromosome may be mediating these transgenerational environmental effects, a notion that fits with evolutionary theory and should be testable. Conditions for the initial evolution and subsequent spread of any transgenerational epigenetic sensing mechanism (that enhances male reproductive fitness) would be most ‚relaxed' for the non–recombining part of the Y chromosome. Also of note is the X chromosome's enrichment for genes expressed in spermatogonia.

P860. Birth defects on data of the Tomsk Genetics Registry from 1979 to 2001

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The Tomsk Birth Defects Monitoring Program is a population based surveillance system. At present, the program covers approximately 10,000 birth annually in the Tomsk region, including almost 100% of births in Tomsk and about 50% of all births in the rest of the province. Stillbirths at 22 weeks or more gestation are registered. The prevelence is estimated for all diagnostic categories.
The overall frequency of all forms of congenital malformation of development (CMD) in Tomsk for the 23-year period of observation is 23.95 per 1000 births (‰). For monitoring secular changes a basic level for 102,789 new-borns during 1979-1992 has been fixed. A significant increase in the frequency of overall CMD spectrum has been seen (from 22.8‰ to 26.17‰. P<0.001). For forms of CMD registered according to the ICBD reference the overall frequency has increased from 12.79‰ to 14.09‰, P < 0,05). Among them, the frequencies of anencephaly (12.79‰ to 14.09‰), encephalocele (from 0.09‰ to 0.29‰), congenital heart defects (2.47‰ to 3.81‰), anal atresia (0.11‰ to 0.25‰), hypospadias (0.72‰ to 1.46‰), renal agenesis/dysgenesis (0.1‰ to 0.48‰) have increased (p < 0.05). Statistically significant decreases in the frequency of multiple CMD were observed (3.61‰ to 1.56‰, P<0.05). The frequency of Down syndrome has not changed, although significant increases over the time-average (1.62‰) were registered in 1987 (2.93‰) and 1988 (2.74‰). The frequency of multiple CMD during this period was 2.9‰ (range from 1.13‰ to 5.74‰).

P861. Linkage disequilibrium mapping: population differences and positional cloning

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Localization of genetic determinants of disease (positional cloning) by linkage has been impressively successful for parametric analysis of major loci, but nonparametric analysis for oligogenes in complex inheritance has been less triumphant. Recent years have seen use of linkage disequilibrium (LD) among single nucleotide polymorphisms (SNPs) to increase resolution of positional cloning. The Malecot model with association ρ fits pairwise LD better than other metrics when information weights are used. This has led to maps in LD units (LDU) that are additive, with 1 LDU corresponding to the distance in which LD useful for positional cloning is preserved. LD maps reflect haplotype blocks with uniform LD. Graphical representation is useful but secondary to map distance. Differences in LD map length among major ethnic groups and between regions and isolates have been estimated as a guide to informed choice for positional cloning. Scaling a cosmopolitan LD map determined for two or more populations by the Malecot parameters of a local population extracts 95 per cent of the information in a local map at the same resolution. A general theory for haplotype mapping on either a kb or LDU map will be presented, with preliminary comparison of errors in positional cloning when haplotypes of n SNPs are used. LD maps closely parallel linkage maps, but at much greater resolution. They will be as useful for positional cloning of oligogenes as linkage maps have been for major loci, and therefore are being integrated with physical and linkage maps in the same database.

P862. MTHFR 677 polymorphism in an elderly patient cohort

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Methylenetetrahydrofolate reductase (MTHFR) is one of several enzymes that utilise 5,10-methylene tetrahydrofolate. The activity of MTHFR is thus a determinant of the pathway down which folate is directed. Low MTHFR activity such as that associated with the 677T polymorphism reduces the folate available for homocysteine methylation: accumulated homocysteine may then cause artery disease. Higher MTHFR activity such as that associated with the 'normal' 677C allele may reduce the folate available for other pathways, in particular thymine synthesis. Impaired thymine synthesis may result in abnormal DNA synthesis, mutation and cancer. Numerous small studies have examined the MTHFR 677T polymorphism in elderly cohorts but have generally failed to reach statistical significance. We have therefore determined the MTHFR genotype in 1690 mostly male elderly people and 435 younger controls. The observed genotypes in the elderly group were CC, 725; CT, 787 and TT, 178. This group is significantly out of Hardy-Weinberg equilibrium. The effect is most marked in the oldest (>85) men (p<0.01). Comparison with younger controls suggests both TT and CC homozygotes are missing from this elderly group. We estimate that more than 2% of the population may fail to reach old age because of MTHFR genotype. This effect might be prevented by dietary folate supplementation.

P863. New perspective approaches in diagnostics of predisposition to cardiovascular diseases on the example of mutation C677T in the MTHFR gene

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Diagnosing predisposition to cardiovascular diseases is now an important tool in realization of different programmes of preventive medicine. Already associations of many polymorphic alleles with different diseases have been shown. The most precise associations have been found for mutations in genes encoding proteins such as ApoB, ApoE, ApoCIII, LPL, PON 1, MTHFR, MTRR, FV, ACE and AGT.
The method of PCR amplification on a microarray of gel-immobilized primers (microchip), developed in EIMB, has been applied to analysis of the C677T mutation in the MTHFR gene. The amplification was carried out simultaneously both in solution covering the microchip array and inside gel pads. Each gel pad contained the immobilized forward primers, which were allele-specific, while the fluorescently labeled reverse primers, as well as all components of the amplification reaction, diffused into the gel pads from the solution. The effectiveness of amplification was estimated by measuring the intensity of fluorescent signals from the corresponding gel pads using a fluorescent microscope with CCD camera. Using this approach, we could correctly identified homo- and heterozygote genotypes.
We consider this approach as a prospective tool for SNP identification in genomic DNA. We suggest that the use of multiplex allele-specific on-chip PCR will result in essential simplification of the analysis of SNP sets, specific for a disease, allowing the creation of diagnostic microchips for the detection of predisposition to multifactor diseases, like cardiovascular pathology.

P864. Estimation of genetical risk for the urban population of large chemical center

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The population of Sterlitamak is monitored genetically. Analysis of polymorphisms of five loci of systems of blood for Bashkirs and Russians established a baseline for the gene pool. The accumulation of alleles TF*C3 and HP*2 and reallocating of density of alternative alleles is observed. A reduction of frequency TF*C1, increase of TF*C2, and decrease of allele HP*1 were found. The same relation was watched for two generation of the townspeople. A bank of DNA of the townspeople is being created, which now contains 397 samples. The experimental group includes members of 53 families (both parents and children), selected with allowance for professional contact with genotoxicants of at least one of the parents during not less than half a year up to conception of the child. The control group comprises 30 families, where the parents by virtue of the professional work had no similar contact. We fingerprinted members 10 experimental and 5 control families with DNA of bacteriophage M13. Bands were scored as identical if in an electrophoretic profile they differed by less than 1 mm. The number of non-parental bands seen in children was unexpectedly higher than reported elsewhere: 4.87 new bands for the child in experimental group and 8.14 in monitoring. This may be the result of methodological features of this research, or an actual effect on gametes of high concentrations of environmental mutagens.

P865. *KLOTHO* F352V polymorphism in an elderly population.

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Examining an elderly population for depletion of individuals with a particular genotype is a simple test for association of genotype with fatal disease. Moreover it is not necessary to know what disease or diseases are involved. In the mouse, a loss of function mutation of the *klotho* gene results in morbidity and premature death. No similar syndrome has been identified in humans, however homozygosity for the common F352V polymorphism of the *KLOTHO* gene has recently been linked to reduced life span. We therefore selected 1020 elderly people and 435 younger controls and determined F352V status using a PCR based method. Results were analysed by comparison of the elderly group to both the younger group and to Hardy-Weinberg expectations. The F352V homozygote frequency in the elderly group was lower than expected by both methods however neither was statistically significant. This does not exclude F352V as a significant risk factor for death but indicates that despite a fairly large sample, a more powerful experimental design is needed. Our data indicates that the penetrance of the homozygous genotype may be lower than previously suggested.

P866. Origin and evolution of Y-chromosome lineages in populations of Altaic language family

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Paternally inhereted Y-chromosomal lineages in twenty population samples from ten ethnic groups belonging to Altaic language family were studied. Using 18 biallelic markers, each Y-chromosome was assigned to a particular lineage according to Y chromosome consortium classification (YCC, 2002). Most frequent lineages in Altaic populations were R1a1 (27.1%), N3 (23.0%), and C (12.7%). Phylogenetic and principal component analyses of haplogroup frequencies demonstrates that Altaic populations form three different groups: Eastern Siberian populations, Altaic populations of Southern Siberia and Caucasoid Altaic populations of Central Asia. Substantial degree of genetic differentiation within Altaic language family was demonstrated using both biallelic haplogroup frequency and molecular variance of microsatellite haplotypes. Between-population genetic differences within Altaic language family account for 19.4% of total genetic variability for biallelic HGs, and for 18.4% of variance of microsatellite haplotypes. Hierarchical analysis of genetic diversity reveals that geographic distances are more important factor in genetic differentiation of Altaic populations than linguistic affiliation. Using the molecular variance of microsatellite haplotypes, coalescent age of ancestral haplotypes for „binary“ haplogroups was estimated. These data show that most of the gene pool of ancestral Altaic population was formed during re-colonisation of North Eurasia after LGM rather than by admixture with pre-glacial population. Y-chromosomal data reflect recent language replacement in Yakuts and Uzbeks. In case of Uzbek population language replacement took place on the background of partial gene replacement visible at least in paternal lineages. East Siberian Yakuts represent an example of language replacement within the Altaic language family.

P867. VDR, TNFRII, ALPHA2 -MACROGLOBULIN and ACE gene polymorphism in rheumatoid arthritis in South Asians patients in the East Midlands UK.

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Rheumatoid Arthritis (RA) is a polygenic disease in which genetic polymorphisms of the vitamin D receptor (VDR) gene has been described as a significant determinant of bone turnover which may have a pathogenic role in joint destruction. Angiotension-converting enzyme (ACE) is pro-inflammatory and is expressed in the synovial membranes, and TNF alpha plays a significant role in joint inflammation. The polymorphism in TNFRII has been associated with RA in many studies. Alpha-2-macroglobulin (A2M) is a serum pan protease inhibitor, that binds some cytokines and growth factors. A2M gene may modulate the susceptibility to develop RA or the severity of the disease. We have analysed VDR, TNFRII, A2M and ACE gene polymorphisms and evaluated their associations with South Asian RA. The blood samples from 120 South Asians with RA attending Rheumatology referral Clinics and 130 random controls were collected. Extracted DNA was amplified and analysed for VDR, TNFRII, A2M and ACE loci. The association analysis was performed by obtaining Odds Ratio and chi-square. Preliminary results indicate an increased frequency of BB and Bb genotypes of VDR gene in Asian RA patients leading to highly significant association (Odds ratio 2.99, Chi-square = 8.81, DF 2, $P<0.05$). The ACE (I/D) polymorphism was not associated in the present sample (Chi- square = 0.181, DF 2, $P>0.05$). These results as well as the results of other loci will be presented, with specific reference to their role in the pathogenesis of RA in South Asians.

P868. Role of prothrombotic mutations/polymorphisms in children with thrombosis during congenital heart surgery

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Prothrombotic mutations/polymorphisms in 17 children (10 female and 7 male;median age 3.5 years) who had thrombosis during perioperative period of congenital heart surgery were sampled. We studied Factor V Leiden (FVL), prothrombin G20210A (Pt20210), methylentetrahydrofolate reductase C677T (MTHFR), endothelial nitric oxide synthase intron 4 VNTR (EcNOS), alpha-fibrinogen Thr312Ala (AF), Factor XIII Val34Leu (F-XIII), and angiotensin I converting enzyme I/D (ACE) gene mutations/polymorhisms in this group. Their congenital heart disorders were complex defects in 7 patients, atrial and/or ventricular septal defects in 6, outflow tractus defects in 4 patients. The site of thrombosis were lower extremities in 9 patients (6 arterial, 2 venous, 1 arterial and venous), intracardiac thrombosis in 3 patients, pulmonary thromboemboli in 3 patients, inferior sagital sinus thrombosis in 1 patient, and disseminated thrombosis in 1 patient. Molecular analysis revealed that one patient (1/17) was heterozygous for FVL, one patient (1/17) was heterozygous for Pt20210and another patient was heterozygous for both mutations (1/17). The distribution of alleles for EcNOS in 15 patients were a/a in 1, a/b in 4, and b/b in 10; for AF in 17 patients Ala/Ala in 2, Ala/Thr in 13, and Thr/Thr in 2; for F-XIII in 14 patients Val/Val in 8, Val/Leu in 5, and Leu/Leu in 1; for ACE in 17 patients D/D in 12, I/D in 4, and I/I in 1 patient. None of the patients beared MTHFR gene mutation. Our results may indicate that genetic mutations causing thrombosis should be screened in patients with thrombosis during perioperative congenital heart surgery.

P869. Male contribution in the formation of three *quilombo* remnants in the São Francisco Valley (northeast Brazil) analyzed by Y-specific markers

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During the XVII, XVIII and XIX centuries, several communities founded mainly by fugitives slaves (Africans and/or African-derived people) arose in the São Francisco Valley (northeast of Brazil) as well as all over the country. Nowadays we can find some of these communities (~750 noted communities until this moment) that are denominated „*quilombo* remnants". Despite the extensive knowledge about their history and ethnography, there are several unclear questions about the foundation and evolution of these populations. To evaluate the male contribution in the formation of three *quilombo* remnants located in São Francisco Valley (Mocambo, Riacho de Sacutiaba and Rio das Rãs), we analyzed five Y-specific STRs (DYS19, DYS390, DYS391, DYS392 e DYS393) and *Alu* YAP insertion and estimated allelic and haplotypic frequencies, shared haplotypes, haplotype diversity index, population differentiation and AMOVA. Results indicate that there are statistically significant differences among all populations, supporting previous historic information about São Francisco Valley occupation. The lowest haplotypic diversity value was observed in Mocambo, probably due to founder effect and/or genetic drift. The high frequency of -/14/24/11/13/13 haplotype in Mocambo and its presence in Rio das Rãs can be explained by interbreeding with European and European-derived people, even though before the foundation of theses communities. We can conclude: 1) genetic flux among the three population may be very small or absent and 2) haplotype variability can be explained by three factors: foundation number, immigration and STR mutational events.

P870. *PON1* gene polymorphisms and diabetic neuropathy in type 1 diabetes: associations study

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Vascular complications of diabetes are a major cause of morbidity and mortality, but the mechanisms for their development remain elusive. The development and progression of diabetic nephropathy, retinopathy, and neuropathy in type 1 diabetes mellitus are closely related. Neuropathy usually results 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. Serum paraoxonase (PON1) is an HDL-associated esterase that hydrolyzes products of lipid peroxidation and prevents the oxidation of LDL. Oxidized low-density lipoproteins (oxLDL) have been reported to be capable of eliciting neurocytotoxicity. The *PON1* gene has two common coding region polymorphisms *Leu55Met* and *Gln192Arg*. Both polymorphisms have been identified as independent risk factors for cardiovascular disease in diabetic and non diabetic patients. The aim of this study was to examine the phenotype-genotype association between diabetic neuropathy and the two *PON1* gene polymorphisms in subjects with type 1 diabetes. The study consists of 51 patients with diabetic neuropathy and 150 without diabetic neuropathy matched to the patients by age, gender, and diabetes duration. These subjects were characterized for *Leu55Met* and *Gln192Arg PON1* polymorphisms employing standard primers. Distribution of the *PON1* genotypes did not differ between diabetic neuropathy subjects and diabetic patients without complication*: Leu55Met* (chi-squared=1.85, p>0.05) and *Gln192Arg* (chi-squared=1.24, p>0.05). The present study did not findstrong genotype-phenotype associations.

P871. Heart rate heritability scale transformation: mathematical-statistical reasons versus allometrical ones

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Heritability (h^2) of metric characters is important due to its predictive role in population genetics, specially for variables of biological importance. Heart rate (HR), a semi-quantitative trait, should be square root transformed, nevertheless, most biological functions increase with some power of body size (allometry), imposing log transformation. So-called intraspecific allometry was studied in a sample including both sexes and different age groups.HR (beats/min) was digitally monitored during 5 min in 133 nuclear families (random-mating population with no selection of parents). h^2 calculation: transformed and non-transformed data, considering both mathematical and biological concepts. H^2: regression coefficient and correlation in non- transformed (h^2-NT), and log (NL-h^2) and square root (Sq-h^2) transformed data.
HR: Mother, 73; Father 71; Son 71 and Daughter 79 (F=15.01 p=0.0000).
Some h^2 data in table. Refer. ± SE; # significant difference, z=1.85, p=.06.
Conclusions: 1**)** HR is higher in daughters. 2) Intramodel comparisons (NT, NL-h^2, Sq-h^2) show significant difference only between NT-h^2 vs Sq-h^2 in M/D. 3) Differences, though non-significant, are observed whenever female sex is involved. 4) Log-transformation is the most appropriate, and should always be used, since HR is affected through the allometric equation by an empirical exponent (-0.27), therefore, not only to normalize the variable distribution in the population, which otherwise might be done mathematically by square root transformation of a semi-quantitative variable. Allometry is the first step to a non-linear dynamic analysis to be considered when performing both heritability calculation and/or complex segregation analysis (data not shown) of a variable.

Transformed and Non-Transformed Data (*adjusted by sex)			
Model	H2-NT	LN-H2	Sq-H2
Mother/daughter (77)	.08±.28 #	.22±0.22	-.22±.23 #
Mother/son (69)	0.48±0.20*	.50±.22*	.30±.23*
Father/daughter (77)	.11±.50*	.18±.22*	.20±.23*

P872. Rapid analysis of HumTPO, HumFES/FPS, HumvWA31A, PAHVNTR, HumTH01 and D3S1359 polymorphic genetic markers using a tetraplex and duplex PCR. A report from the Iranian population.

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In this study, a tetraplex PCR method for STR loci HumTPO, HumFES/FPS, HumvWA31A and the phenylalanine hydroxylase VNTR (PAHVNTR), and a duplex PCR method for STR loci HumTH01 and D3S1359 was developed. These polymorphic alleles were evaluated for paternity testing and individual identification in the Iranian population. The data demonstrated that the STR marker D3S1359 and HumvWA31A were highly informative. Eleven alleles were found for D3S1359 and nine for HumvWA31A with a highest observed allele frequency of 0.34 and 0.32, respectively. The D3S1359, HumFES/FPS and HumvWA31A had the highest observed heterozygosity of 79.07%, 73.1% and 72.9%, respectively. Evaluation of the PAHVNTR marker indicated the presence of 3, 7, 8, 9 and 13 repeats with different frequency. Among them, the 3 repeat had the most frequency (34%) and the 13 repeat had the lowest frequency (3.8%). This study provides a simplified and rapid method for analysis of multiple genetic markers in population.

P873. Biology and Human Genetics in Birmingham. 1766-1956

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Human Genetics in Birmingham 1766-1956.

Foundations:

1766 The Lunar Society formed. Monthly meetings at full moon, often in Birmingham. Erasmus and Robert Darwin, Samuel Galton, Joseph Priestley, John Small, Josiah Wedgwood, William Withering.

Priestley (1733-1784):

1776 Reversible colour change in blood from absence of either ‚common air' or ‚dephlogisticated air' (oxygen)

1777 Regeneration of ‚phlogisticated air' (nitrogen) by leaves of plants.
1791 Destruction of his laboratory from a ‚conformist' riot.
Samuel Galton (Rich Quaker gunmaker):
1782 Invents colour top. Describes trichromatic perception of colour.
1812 Francis Galton born to Tertius Galton, son Samuel Galton.
The post-war decade 1945 - 1955:
Hogben, Jinks, McKeown, Mather, Medawar, Squires, Woolf, Zuckerman.
Lancelot Hogben returns to first chair of Human Genetics in Britain after leaving chair of Zoology to become statistician to the Army Medical Service.
Thomas McKeown appointed to new chair of Social Medicine. He started the Journal of Social Medicine, with emphasis on clarity of prose and simplicity of analysis, and emphasis on the nature and nurture interface.
Hogben and McKeown exploit the coding systems, based partly on homonyms and dates of birth, later modified in various ways, encoding data on punched cards. This lead to detailed birth registers with special reference to weight, gestation, parity and maternal age, later to be linked with hospital admissions, smoking habits and the ‚11 plus' IQ test.

P874. Genetic polymorphism of the FKBP12 gene in renal patient

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Background: Tacrolimus (FK-506) is a potent immunosuppressant that blocks the early steps of T lymphocyte activation by inactivating the calcineurin pathway in T lymphocytes, when complexed with its specific intracellular receptor, FKBP12. The gene contains 5 exons. **Objective:** To define genetic polymorphisms in the genes encoding key molecules involved in immunosuppressive agents including FK506 absorption, mechanism of action and metabolism. **Methods:** To investigate whether polymorphisms in the FKBP12 gene may influence drug tolerance, two markers (+370 A→ G and +407 A→ G) both within exon 5 were selected and genotyped by using the snapshot technique in 48 patients with renal disease and 48 healthy UK controls**. Results:** The allele frequencies of the FKBP12 (+370 and +407) polymorphism are shown as follows**.**

		Controls	Renal cases
FKBP12 +370		**N (%)**	**N (%)**
Allele	A	57 (59.4)	54 (56.3)
	G	39 (40.6)	42 (43.3)
FKBP12 +407			
Allele	A	69 (71.9)	69 (71.9)
	G	27 (28.1)	27 (28.1)

No significant differences were found between FKBP12 (+370 and +407) polymorphism in controls and cases. **Conclusion:** Several polymorphisms exist in this gene that influence FKBP12 biological activity and this study suggests that at least polymorphism in these two positions might not be associated with severity of renal disease. Alleles of both markers are present at high frequency in our UK renal patient population and may have functional significance.

P875. Spatial mapping of Down syndrome at small-area level in England and Wales

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Study outline
Down syndrome rates (including all cytogenetically diagnosed non-disjunction livebirths, stillbirths and pregnancy terminations obtained from the National Down Syndrome Cytogenetics Register (NDSCR) in England and Wales, 1993-1998), were calculated across deprivation and maternal age strata and corrected for early prenatal diagnosis. Corrected Down syndrome relative risk estimates were then mapped across 9508 wards in England and Wales using Bayesian disease mapping techniques.
Results
After correction for the effects of potential differential over-estimation of Down syndrome rates (resulting from inclusion of cases that would normally have aborted spontaneously without detection) the study found highest Down syndrome risk (compared to the reference category) among mothers who were (i) most deprived and <=28 years of age and (ii) least deprived and >28 years of age. Bayesian mapping analyses confirmed these findings and showed improved model fit with the inclusion of an interaction term between Carstairs quintile and maternal age compared to models without the interaction term.
Discussion
To date no environmental or lifestyle-related factor (apart from maternal age) has been consistently associated with Down syndrome and studies investigating spatial heterogeneity in Down syndrome have tended to report negative findings. The present study detected significant spatial variation of Down syndrome risk associated predominantly with socioeconomic deprivation and maternal age. This may point to factor(s) that act directly on the process of meiotic non-disjunction and/or via differential intrauterine survival of fetuses with Down syndrome.

P876
Analysis Of Alu-insertions In Seven Turkic Speaking Populations Of Eurasia

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Polymorphic Alu-insertions are interesting DNA markers for population genetic investigations. The Alu family of SINEs is one of the most successful mobile genetic elements and they account for 10% of the human genome.
We have analyzed three Alu-insertions: YaNBC182, YaNBC102, YaNBC361 in 857 individuals from seven Turkic speaking Eurasian populations: Tatars, Bashkirs, Cuban-Nogays, Kara-Nogays, Kumyks, Karachais, Kazakhs, Uzbeks and Uygurs. These populations live in the Northern Caucasus, Volga-Ural region of Russia and Central Asia.
The frequencies of YaNBC182 varied from 0.463 in Uzbeks to 0.673 in Tatars; YaNBC102 Alu-insertion from 0.416 in Cuban-Nogays to 0.602 in Bashkirs; YaNBC361 from 0.388 in Kazakhs to 0.590 in Karachays. Average heterozygosity for all loci ranged from 0.349 in Cuban-Nogays to 0.479 in Uzbeks. 7 out of 21 tests for Hardy-Weinberg equilibrium showed a significant departure from equilibrium (YaNBC182 in Tatars and Cuban-Nogays, YaNBC102 in Cuban-Nogays, Kumyks, YaNBC361 in Cuban-Nogays, Kara-Nogays, Kazakhs). The genetic diversity coefficient Fst was observed to be high (from 0.88% to 1.14%).
The populations of Central Asia demonstrated some homogeneity for distributions of Alu-insertion. Kazakhs showed similarities to populations of Central Asia and significant differences from others. Tatars differed from all analyzed populations except Cuban-Nogays. A possible explanation is the presence of mostly European components in Tatars and mostly mongoloid components in the gene pool of Kazakhs. This information can be used in analysis of population relationships and for study of population history and structure.

P877. Mutation Analysis Of The Hfe Gene In Populations Of Volgo-ural Region And Republic Of Yakuts (sakha)

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Two missense mutations of the HFE gene - Cys282Tyr and His63Asp – are associated with hereditary hemochromatosis (HH). HH is an autosomal recessive genetic disorder of iron metabolism. Studies in European populations showed that heterozygous carriers of these mutations occur with frequency of 1 in 10 to 1 in 15 with decreasing frequency in a gradient from north to south. We analyzed Cys282Tyr and His 63Asp mutations of HFE gene in 590 healthy

individuals in 9 populations of Volga-Ural region of Russia and Yakuts from Republic Sakha (Yakutiya). His 63Asp was detected in all populations, heterozygosity for this mutation ranged from 0.08 in Maris to 0.27 in Mordvins. His 63Asp homozygotes were noted in Udmurts (1.64%), North-Western Bashkirs (1.89%), Chuvashes (3.58%) and Mordvins (11.9%). The Cys282Tyr mutation was not found in NorthWestern Bashkirs and Yakuts. The frequency of the Cys282Tyr mutation ranged from 1.79% in individuals of Maris to 1310% in Udmurts. We detected a genotype of homozygosity for His 63Aspand heterozygosity for Cys282Tyr. It is possible that this individual has not yet come of the age of manifestation of disease by selection of healthy donors. These findings correspond to reported data on the high frequency of these mutations in European populations. The distribution these mutations in Eurasian populations of Russia demonstrates the existence of Caucasoid and Mongoloid components in their gene pools, with mostly Mongoloid components in Bashkirs, Maris and Yakuts.

P878. Why say no? Reasons for non-participation in the North Cumbria Community Genetics Project

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The linkage of population based DNA samples with personal medical information has gained much attention as a method of examining the combined effect of genes, environment and lifestyle factors on common multi-factorial diseases. Such biobank collections raise many issues, including: informed consent, confidentiality, and governance. In light of these issues, a central question concerns the public acceptability of biobanks. However, we know little about the views of people who have been asked to donate to a population-based DNA collection.

This paper explores the views of such people by using a local biobank in the north-west of England as a case study. The North Cumbria Community Genetics Project (NCCGP) collects (i) blood and tissue samples from the umbilical cord of newborn babies; (ii) maternal blood samples, and (iii) information about health and lifestyles.
The NCCGP has enjoyed participation rates of over 80% (though only 60% of those approached complete a health and lifestyle questionnaire, as well as donating samples). This paper explores reasons for refusal by using data from interviews with potential donors (both participants and non-participants), as well as NCCGP team members and community groups who opposed the establishment of the biobank. It asks to what extent non-participation is attributable to primarily local factors (such the NCCGP's links to British Nuclear Fuels) or to other, more widely held, ethical concerns cited above. The paper will also examine the extent to which non-participation represents a distinct stance to that of participation. Our research is funded by the Wellcome Trust.

P879. Initial population study of the frequency of C677T polymorphism in the methylenetetrahydrofolate reductase gene (MTHFR) in Bulgaria.

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A common C677T polymorphism in the gene coding methylenetetr ahydrofolate reductase (MTHFR) results in an enzyme variant with lower enzyme activity. Reduced dietary intake of folates, combined with the T/T genotype has been associated with a number of disorders: from birth defects to heart diseases. It is reported that the frequency of C677T mutation differs between different ethnic populations. In our study, a total of 148 DNA samples from unrelated individuals were genotyped for the C677T polymorphism. The investigated population sample consists of 76 Bulgarians and 72 Gypsies, randomly chosen from different geographical regions of Bulgaria. The C677T substitution was detected after restriction of the PCR products with HinfI, followed by agarose gel electrophoresis. The T-allele was detected in 39% of Bulgarians and 22% of Gypsies. The C/C genotype was observed in 37% of Bulgarians and in 60% of Gypsies; C/T genotype – in 47% and 36%, and T/T genotype – in 16% and 4%, respectively. An initial study of women with previous child with Neural Tube Defects or Down syndrome (n = 11 and 12, respectively) was performed as well. The C/C genotype was observed in 9 of women, C/T genotype – in 11, and T/T genotype – in 3 of them. The number of the investigated DNA samples is still small for performing statistical analysis of the results. Further studies will be done, for establishing the association between the frequency of the C677T mutation in Bulgarian population and frequency of NTDs and Down syndrome. Supported by grant No. MU-706/2002.

P880. Genetic diversity of candidate genes for cardiovascular disease in populations of Siberia

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2000 individuals from 21 populations belonging to 8 ethnic groups living on the territory of Siberia Asia were investigated. These include Northern and Southern Altai, Tuvinian, Buryat, Evenk, Yakut and Russian. Seven candidate gene for coronary artery disease (ACE, AGT, NOS3, APOA1, MTHFR, PLAT, F13) have been studied. Population-specific particulars of genotypes were noted and genetic relationships between samples. We detected specific polymorphisms of genes involved in cardiovascular pathology. Buryat, Evenk, Yakut and Northern Altai form a big cluster, seen also in data on linguistics and anthropology. The second cluster includes Tuvinian and Southern Altai. This division of ethnic groups has two causes, geography and particulars of foundation of the gene pools. The gene pools of different populations are very different with respect to candidate genes for cardiovascular disease, and this fact has an effect on structures of hereditary pathology in diverse populations.

P881. Dynamics of molecular genetic diversity in the East Midlands, England.

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Highly polymorphic genetic markers like short tandem repeats (STRs) and Variable number of tandem repeats (VNTRs) have been used successfully in disease, human evolution and genetic diversity studies. However DNA based comprehensive genetic studies of the East Midlands, England are limited. There is strong evidence that the continental European invading migrants have left significant imprints of their genetic heritage in the region. This study was designed to quantify molecular genetic variation in the five regional populations and assess their genetic affinities with European populations. Blood samples, with consent, were collected from donors whose three generations have lived in this region. Seven VNTRs (MS1 (D1S7), MS31 (D7S21), MS43a (D12S11) and YNH24 (D2S44), D1S80, APOB, YNZ22 (D17S5)), Six STRs (HumTHO1, HumVWA31A, HumF13A01, HumFESFPS, HumCSF1PO, HumTPOX) and Six Alu Insertion/deletion polymorphisms (TPA25, ACE, PV92, F13B, APO, D1) were analysed on 500 individuals using standard molecular genetic techniques. Chi-square method and exact tests were used to assess Hardy-Weinberg equilibrium. Using multivariate analyses like genetic distances and correspondence analyses, we assessed genetic affinities of the populations. Overall heterogeneity was observed for five loci, MS43A, MS31, HumF13A01, Hum FESFPS and HumTHO1. 23 of 190 pair-wise population comparisons were also statistically significant. GST values for molecular systems were higher than conventional systems (0.012 vs. 0.005). Genetic distance and correspondence analyses showed distinctive position and significant genetic variation in the five contiguous regional populations. This variation may be due to local geographical barriers, genetic drift and settlement patterns of the European invaders of the past 2000 years.

P882. Analysis of ACE, eNOS and MTHFR genes polymorphisms in the elderly people from the north-west region of Russia.

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The polymorphisms of three genes (I/D-ACE, 4a/4b-eNOS, 677C/T-MTHFR) associated with some multifactorial diseases (cardiovascular diseases, diabetes) were studied by PCR-RFLP in two groups of people from the north-west region of Russia. Group 1 included 65 unrelated individuals of middle age (25-45), Group 2 147 unrelated individuals older than 70 years. The distribution ofACE genotype frequencies was different in both groups. A low frequency of D/D and I/I genotypes and high frequency of I/D genotype were found in the group of old persons compared to middle age group (I/D-50.3% and 37%, respectively). Homozygosity for both alleles of this gene is known to be detrimental for human health: the D/D genotype determines increasedACE level and is associated with cardiovascular diseases, I/I genotype is associated with type 2 diabetes mellitus. The distribution of eNOS genotypes and alleles also differed in both groups. In the group of old persons the frequencies were: 4b, 64.3%; 4a, 35.75%; 4a/4a, 66%, 4a/4b, 31.3%, 4a/4a, 2.7%, compared to 80%, 20%; and 60%, 40%, 0% respectively. The 4b allele was significant decreased in the group of old persons compared to the middle age group (64% and 80% respectively; p<0.01). The frequencies of genotypes and alleles of MTHFR gene were similar in the studied groups. Combined analysis revealed significant prevalence of genotype I/D(ACE), 4a/4b(eNOS), C/C(MTHFR) in old people compared to middle age ones. It might be speculated that this genotype has provided some inherited advantages for their longer survival.

P883. Molecular genetics predisposition to myocardial infarction in young men.

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In 202 men with myocardial infarction before 45 years (mean age 39.7±5.9) and in 186 sex and age-matched controls (mean age 39.7±3.9), we investigated the following gene polymorphisms: Plasminogen activator inhibitor-1 (*PAI-1*) 4G/5G; Paraoxonase (*PON1*) Q191R and L54M; methylenetetrahydrofolate reductase (*MTHFR*) C677T; Factor V (*FVL*) R506Q; Prothrombin (*FII*) G20210A; Fibrinogen (*FGB*) G–455A; angiotensin-converting enzyme (*ACE*) I/D; and Factor VII (*FVII*) -323 insertion.
Only the frequency of *PON1* BB genotype was significantly higher in patients than controls (OR=2.58, 95%CI: 1.14-5.80; p< 0.03). A tendency to higher frequency of *FVL* and A20210 *FII* in MI patients was observed (OR=2.1, 95%CI: 0.72-5.99 and 2.79, 95%CI: 0.3-24.68 respectively). The genotype distributions are summarized in table 1. The frequency of the *ACE* DD genotype among patients with MI and controls was 19.8% and 15.6% respectively. The frequency of the *PAI-1* 4G/4G genotype among patients with MI and controls was 30.7% and 28.5% respectively. The -323 insertion was 2% in patients and 1.6% in controls. However, the *FGB* gene AA genotype was 5.9% and 8.6% respectively in MI patients and controls. Gene-gene interactions were analyzed. The risk of MI development was increased in *MTHFR* T677 and *PON1* BB carriers (4.5% and 1.1% in patients and controls respectively; OR=4.29, 95%CI: 1.03-17.81; p<0.05). This interaction might provide an increase of oxidative stress and lipid peroxidation.

PON1			
Q191R	**AA**	**AB**	**BB**
Patients	52%	37.6%	10.4%*
Control	63.4%	32.3%	4.3%
L54M	**AA**	**LM**	**MM**
Patients	52%	43.1%	10.4%
Control	63.4%	45.2%	9.1%
FVL	**NN**	**NM**	**MM**
Patients	94.6%	5.4%	0
Control	97.3%	2.7%	0
G20210AFII	**NN**	**NM**	**MM**
Patients	98.5%	1.5%	0
Control	99.5%	0.5%	0
C677TMTHFR	**CC**	**CT**	**TT**
Patients	47%	46%	7%
Control	50.5%	40.3%	9.1%

P884. Effects of apoE gene polymorphism on plasma Lp(a) levels in Serbian patients with ischemic heart disease

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Introduction: Polymorphism in apoE gene is associated with variations in plasma levels of LDL cholesterol. Variation in plasma Lp(a) levels was inconsistently associated with variation in apoE. Elevated concentrations of Lp(a) are risk factor for atherosclerotic disease. Therefore, it is important to understand whether genes other than apo(a) gene are involved in regulation of plasma Lp(a) levels. **The aim:** of this study was to investigate association, if any, of apoE alleles and genotypes with plasma Lp(a) levels in patients with ischemic heart disease (IHD) and healthy controls in Serbian population.**Methods:** Lp(a) values were measured by imunonephelometry. ApoE genotypes were determinated by PCR and restriction isotyping, with *Hhal* endonuclease. **Results:** Frequencies of apoE genotypes were: E2E3 14.9 %, E3E3 66.7 %, E3E4 18.4 % in patients with IHD and E2E3 12.9 %, E3E3 70.9 %, E3E4 16.2 %, E4E4 1.0 % in controls. There was no significant difference in distribution of apoE genotypes or alleles between patients with IHD and controls. Although the Lp(a) values in patients was significantly higher than in controls, we found no significant difference in these values regarding apoE genotypes.**Conclusions:** Increased Lp(a) values are risk factor for IHD. Lack of association between Lp(a) plasma levels and apoE genotypes was found in patients with IHD and in healthy controls in Serbian population.

P885. The angiotensin-I converting enzyme gene polymorphism and Alzheimer's disease

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Introduction: Alzheimer disease (AD) is a progressive neurodegenerative disorder characterized by irreversible cognitive and physical deterioration. The levels of angiotensin-I converting enzyme (ACE) in brain influence cognitive processing acquisition and recall of newly learned tasks in animal. The insertion/deletion (I/D) polymorphism influence the variation of ACE level in plasma and brain. In previous studies this polymorphism was associated with AD, but the results were controversial maybe due to ethnic background. **Aim of the study**: To investigate the possible association between I/D polymorphism in the ACE gene and AD in Serbian population. **Methods:** The study sample consisted of 160 persons: 30 late-onset AD patients (85.50 ± 5.47 years old), 30 healthy age-matched non AD controls (84.22 ± 3.99 years old) and 100 healthy young controls (33.8 ± 8.9). The diagnosis of AD was made according to the NINCDS-ADRDA criteria. Genotyping for I/D was performed by polymerase chain reaction (PCR) using a modified three primer method. **Results:** Frequencies for II, ID and DD ACE genotypes in AD patients were 21.43 %, 57.14 %, 21.43 % respectively and I/D allele frequencies were 0.5/0.5. No significant difference in distribution of ACE genotypes between AD patients and healthy age-matched controls or young controls was found. **Conclusion:** Further

study with larger number of AD patients could suggest that the ACE I/D gene polymorphism may constitute a genetic susceptibility factor for dementia in Serbian population.

P886 Association of lipoprotein lipase gene Asn291Ser DNA polymorphism with plasma lipid levels

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Introduction: The enzyme lipoprotein lipase (LPL) has a central role in lipid metabolism. LPL hydrolyses triglyceride (TG) rich core of circulating chylomicrons and very-low density lipoproteins. Asn291Ser substitution is located in the N-terminal end and may influence the catalytic activity of LPL. It was recently reported that this polymorphism was associated with Alzheimer disease, increased risk of pre eclampsia and ischemic heart disease.**The aim**: of this study was to determine genotype and allele frequencies of Asn291Ser polymorphism in 193 healthy and unrelated subjects from Serbia. Relationship between this polymorphism and plasma lipid levels was investigated in our healthy population as a base for future studies on different groups of patients. **Methods**: Genomic DNA was extracted from whole blood by proteinase K/phenol method. LPL genetic variants were detected by ASA method combined with semi-nested PCR. **Results**: Frequencies of Asn291Ser and Asn291Asn genotypes were 1% and 99%, respectively and Ser291/Asn291 allele frequencies were 0.05/0.95. Variation of lipid levels among individuals with different LPL genotype were not statistically significant. We detected a trend of increase in triglyceride levels and both, systolic and diastolic blood pressure in carriers of the 291Ser allele.**Conclusions**: Genotype and allele frequencies in our population are consistent with other Caucasian populations. Data from our study could be important for further research of pathological states considering lipid metabolism.

P887. Mitochondrial DNA variability in Bosnians and Slovenians

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Mitochondrial DNA variability in two Slavonic-speaking populations of the northwestern Balkan peninsula - Bosnians (N = 144) and Slovenians (N = 104), was studied by hypervariable segments I and II (HVS I and II) sequencing and restriction fragment-length polymorphism (RFLP) analysis of the mtDNA coding region. The majority of the mtDNA detected in South Slavonic populations falls into the common West Eurasian mitochondrial haplogroups (e.g., H, pre-V, J, T, U, K, I, W, and X). About 2% of the Bosnian mtDNAs encompass East Eurasian and African lineages (e.g., M and L1b, respectively). The distribution of mtDNA subclusters in Bosnians, Slovenians and the neighboring European populations reveals that the common genetic substratum characteristic for Central and Eastern European populations (such as Germans, Poles, Russians and Finns) penetrates also South European territories as far as the Western Balkans. However, the observed differentiation between Bosnian and Slovenian mtDNAs suggests that at least two different migration waves of the Slavs may have reached the Balkans in the early Middle Ages.

P888. The Microsatellite Polymorphism of Heme Oxygenase-1 is Associated with the Inflammatory Level but not with Restenosis after Coronary In-Stenting.

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Background: Vascular smooth muscle cells (VSMCs) can express Heme-oxygenase (HO), a rate-limiting enzyme in the degradation of heme to bilirubin, ferritin and carbon monoxide (CO). VSCM-derived CO can suppress VSMC proliferation and may serve as an antiproliferation factor. The promoter region of HO-1 shows a functional microsatellite polymorphism with different (GT)n repeats; with shorter alleles associated with higher gene expression and protection from restenosis following in-stent treatment in peripheral arterial disease. The object of this study was to confirm the effect of this variation on the occurrence of restenosis after in-stent treatment in patients with coronary artery diseases.
Methods and Results: 187 patients who underwent successful coronary stent implantation were studied. The genotype for HO-1 promoter microsatellite polymorphism was determined using PCR and automated DNA capillary sequencing. Repeat length ranged from 22-42, with $(GT)_{25}$ and $(GT)_{32}$ being the two most common alleles. The allelic repeats were divided into short class(S) with ≤29(GT)n, the middle class(M) with 30~37 (GT)n and the long class(L) with ≥38 (GT)n according to their distribution and previous studies of promoter activity. There was no difference in the prevalence of angiographic restenosis between the genotype groups or between post operation levels of inflammation markers; although carriers of the S allele (n=120) had 33.3% lower baseline IL-6 compared with non-S carriers (n=67)(p=0.0008).
Conclusion: Our results do not confirm an association between the HO-1 promoter polymorphism and restenosis following in-stent treatment, however the association with plasma IL-6 levels suggests that HO-1 S allele might protect from atherosclerotic inflammatory processes.

P889. Tachyphylaxis to β2-agonists in asthmatic patients is modulated by ADRB2 polymorphisms

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Objectives: The aim of this study was to investigate whether genetic polymorphisms within ADRB2 gene modulate the outcome of the individual response to β_2-agonists and the development of desensitization in patients with asthma.
Methods: Sixty three asthmatic patients were included in the study. Based on the clinical records, patients were classified into two groups: patients with tachyphylaxis and good responders to β_2-agonists. DNA samples were genotyped for the Arg16Gly and Glu27Gln alleles of the ADRB2 gene.
Results: Arg16 allele was slightly more frequent within the group with tachyphylaxis (p=0.05), whereas, Gly16 allele carriers were overrepresented within the group of good responders (p=0.02). On the other hand, the allelic frequency of Gln27 and the proportion of Gln27 carriers was higher within the group with tachyphylaxis (p=0.0015 and p=0.027 respectively) and Glu27 allele carriers were overrepresented within the group of good responders (p=0.005). The Arg16 and Gln27 alleles were in linkage disequilibrium.
Conclusions: The predisposition to develop tachyphylaxis seems to be linked to the Arg16 and Gln27 alleles, although the later isoform plays the main role. The Arg 16 allele is perhaps overrepresented due to the linkage disequilibrium between both polymorphisms. On the other hand, the Glu27 allele seems to be a protector factor.

P890. Polymorphic genes of xenobiotic-metabolizing enzymes associated with predisposition to bronchial asthma in children

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The frequencies of the *CYP1A1* valine allele, homozygous deletions of *GSTM1* and *GSTT1*, and two point mutations of the *NAT2* gene S1(C481T) and S2(G590A), were compared in healthy children and children having bronchial asthma. The S1 mutation was associated with resistance, and all other traits, with predisposition to the disease.

P891. Analysis of the CFTR gene in patients with disseminated bronchiectasis and chronic obstructive pulmonary disease

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Disseminated bronchiectasis of unknown origin (DBE) and chronic

obstructive pulmonary disease (COPD) are conditions belonging to the spectrum of respiratory disorders characterized by airflow limitation. Both genetic and environmental factors may influence the development of these conditions.
This study was aimed at investigating the possible involvement of CFTR gene in the etiology of DBE and COPD in Yugoslav patients. We analyzed 16 patients: 10 with DBE and six with COPD. Of six COPD patients 4 were with pulmonary emphysema and 2 with chronic bronchitis. CFTR gene was screened using the combination of PCR and subsequent DGGE analysis of 16 exons, followed by DNA sequencing.
Among 10 patients with DBE none of the mutations were found. F508del mutation was found in one patient with COPD. One patient with COPD was heterozygous carrier of R74W and one of R75Q mutation.
Frequency of 7T and 9T alleles at the Tn polymorphic site was 81.7% and 18.3%, respectively. 5T allele was not detected in any of the patients in both groups.
Common polymorphisms 2694T/G, 875+40A/G and 1716G/A were frequently detected in both groups of patients. Analysis of M470V polymorphism showed that M470 allele was present with frequency of 37.5%, significantly higher than in general population (33%).
These preliminary findings suggest that CFTR gene may be involved in the ethiology of COPD, but not of DBE. Further studies on larger groups of patients are needed to confirm these results, as well as to reveal the role of CFTR mild mutations and polymorphisms.

P892. "S and Z alleles of *PI* gene among chronic obstructive lung disease patients and their relatives."

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Alpha1-antitrypsin deficiency (AATD) is an autosomal-recessive disease caused by mutation in *PI* gene which leads to decreasing of alpha1-antitrypsin level and declares itself as chronic obstructive pulmonary disease (COPD). Unfortunately, there are no screening programs available in Russia, and data on *PI* gene variants frequency among different populations and risk groups are not sufficient. Totally 212 persons were examined to detect AATD cases, including 66 COPD patients, 53 relatives of the 1st and 2nd relation degree, and 93 persons that constituted the control group. Examination was conducted among Tatars (17 patients, 15 relatives, 43 persons of the control group) and Russians (49 patients, 38 relatives, 50 persons of the control group) residing in Tomsk and Tomsk Region. Mean age of Russian patients is 56.1 14.5 years; Tatar patients– 50.5 12.7 years. Mean age of the control group is 73.6 4.6 years (Russian); Tatars – 55 18.4 years. Among the patients 65 percent had smoking experience, smoke anamnesis "pack/year" is 62.5±37.9; mean FEV_1 is 66.58+30.3. Professional exposure was determined among 62 percent of patients. Decrease of serum AAT level was detected among 46 percent of Russian patients; decrease of AAT was not detected among Tatar patients. Genotyping of two most common deficient alleles of *PI* gene (S and Z) was conducted. Examination detected two MS heterozygote (4.08 percent) among COPD patients (Russian), so, S deficient allele frequency in this group was 0.01. These deficient alleles were not detected among Tatar sampling group.

P893. Detoxifying enzymes genes (mEPHX, CYP1A1 and GSTP1) genotypes and risk of chronic obstructive pulmonary disease

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Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide. COPD is characterised by a slowly progressive irreversible airflow obstruction that is due to a loss of lung elasticity resulting from parenchymal destruction and peripheral airflow obstruction. The genes that contribute to the genetic susceptibility to chronic obstructive pulmonary disease (COPD) remain largely unknown. One possibility to account for a susceptibility to the effects of environmental pollutants may be the genetic variations in the xenobiotic metabolizing enzymes such as microsomal epoxide hydrolase (mEPHX), cytochrome P450 1A1 (CYP1A1) and glutathione-S-transferase P1 (GSTP1) These enzymes plays an important role in metabolising highly reactive intermediates in the lung.
We designed PCR-RFLP -based genotyping assays to determine Tyr113His, His139Arg (mEPHX gene), Ile462Val (CYP1A1 gene) and Ile105Val (GSTP1 gene) polymorphisms on susceptibility for COPD. Blood samples were taken from 127 COPD patients and 164 healthy controls.
The frequencies of the slowest activity mEPHX genotype(His113/His113/His139/His139) were significantly increased in COPD patients than in controls (4.6 % vs 0.6%; OR 7.66, $p < 0.05$). There was also a higher risk of COPD for subjects with slowest mEPHX genotype with combined CYP1A1 Ile-Val variant (OR=8.71; 95% CI, 0.98-202.05).
The proportion of GSTP1/Val105 mutant homozygotes was significantly higher in the patients with COPD than in the control subjects (7.2% vs 3.3%). The odds ratio for GSTP1/Val105 homozygotes versus all other genotypes was 2.23 for COPD.

P894. Allele combination of six genes associates with atopic asthma developing in patients from Northern-Western Russia

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Asthma, as many diseases, results from the interaction between adverse environmental events and constitutional (genetic) resistance or susceptibility. As with other complex diseases, genes contributing to asthma may be found either by examining candidate genes or by genetic linkage. In our study we analysed mutations of CYP1A1 (cytochrome P4501A1), GSTM1, GSTT1, GSTP1 (glutathion S-transferases M1, T1, P1), NAT2 (N-acetyltransferase 2), ACE (angiotensin-converting enzyme) and CFTR (Cystic fibrosis transmembrane regulator) genes in asthmatic patients and control individuals from Northern-Western Russia. Our data suggests that GSTM1 and GSTT1 null-polymorphism and „normal" genotype GSTP1 A/A are susceptibility factors to atopic asthma among the Russian population.
Furthermore, the importance of allele combination of different genes was shown by our study to develop atopic asthma. Theoretically, 288 possible genotypes result in allele combination of six investigated genes. We found 36 genotypes in the control group and 39 genotypes in the group of asthmatics. 21 allele combinations (or 38.9% of all found genotypes) were equal in both groups. The most common genotype in asthmatic patients has significantly different frequency from the general population: GSTM1 0/0, GSTT1 0/0, GSTP1 A/A, ACE D/D, CYP1A1 N/N, CFTR N/N -11.0% vs. 1.3%, subsequently (OR= 9.53, 95%CI: 2.972-25.128).
Thus, our data confirm importance of factors that modulate functions of major genes, predisposing directly to asthma, and therefore disease expression. Investigation of the allele combination of „major" and „minor" genes, predisposing to asthma, will be of interest.

P895. Identification of protocadherin 1 as a candidate asthma susceptibility gene by positional cloning in a collection of Dutch asthma families

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Asthma results from a complex interaction between numerous (unknown) susceptibility genes and environmental triggers. The identification of these susceptibility genes has proved difficult and progress has been slow. Using positional cloning, we have identified the gene for protocadherin 1 (*PCDH1*) as being genetically associated with asthma and bronchial hyperresponsiveness (BHR; a cardinal clinical feature of asthma) in a collection of 200 Dutch asthma families. *PCDH1* is located on 5q31-q33, a region that has been genetically linked to asthma in several different populations.

PCDH1 was one of 6 genes in a 550 kbp stretch of DNA identified by genetic linkage analysis of the Dutch families using microsatellite markers spanning the 5q31-q33 region. SNPs in each of the 6 genes were identified by sequencing overlapping PCR fragments from the exons, promoter, 1st intron and 3'-end in a selection of 16 patients from these families. The 200 families (n=1200) were then screened for the presence or absence of the identified SNPs by allelic discrimination using Taqman™ technology. Genetic association between the individual SNPs, asthma and asthma subphenotypes was examined using the allelic transmission disequilibrium test which revealed that 2 SNPs in *PCDH1* showed significant associations (p<0.0005) with BHR. Northern blotting and RT-PCR show that *PCDH1* is expressed in lung, as well as in human bronchial epithelial cells and lung fibroblasts. *PCDH1* has not previously been linked with asthma either at the genetic or biological level. Replication of the genetic association in other population samples will be necessary.

P896 Analysis of functionally impaired genotypes of metabolic genes in atopic bronchial asthma children.

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Atopic bronchial asthma (ABA) is a complex polygenic disorder. Asthma is characterized by airway inflammation, a critical component of which is oxidative stress. Oxidative stress, with the formation of reactive oxygen species (ROS), is a key component of inflammation. The polymorphisms of metabolic genes (*NAT2*, *GSTM1*, *GSTT1*, *GSTP1*) were studied by PCR-RFLP in children with ABA. Significantly higher levels of *GSTM1*0/0 (79%) and *GSTT1*0/0 (58%) have been found in ABA children. The frequency of "slow" alleles for *NAT2* was higher in the patient group (79% versus 66%). Polymorphism of the *GSTP1* gene was not found to be associated with asthma. Null allele homozygotes for both GST genes (*GST1* 0/0 and *GSTT1* 0/0) occur in 49% of asthmatic patients, whereas these genotypes comprise only 12% in the controls. The presence of both *GSTM1* and *GSTT1* null alleles results in a 7-fold increase of asthma disease risk (OR 7,15; 95% CI = 2,70-18,98). Moreover combinations of functionally impaired genotypes of all three GSTs was rarely encountered in the controls (8%), but it was rather common (35%) in the patient group. 90% of ABA children with atopic dermatitis had *GSTM1*0/0 genotypes against 57% in the ABA children without this complication. Genetic tests for *GSTM1* and *GSTT1* null homozygotes (*GSTM1*0/0; *GSTT1*0/0) identification might be useful for identification of persons at risk of ABA and thus might be of great practical value for predictive medicine service.

P897. Asthma: New defined subphenotypes and traits in an expanded genomewide scan

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As time goes by, nearly every chromosomal spot was shown to be linked or associated with asthma or other atopic traits in human beings. Recently, strong evidence was given for asthma genes both on chromosomes 14 (Harkonarson et al. 2002) and 20 (Van Eerdewegh et al. 2002). The investigation of the genetic etiology of a complex human disease aims at the improvement of preventive strategies, diagnostic tools, and therapies. These final steps are not yet reached or even in sight.
Our first contribution to the field was one of the first genomewide scans in a family study with at least two affected siblings (Wjst et al. 1999). Based on a precise analysis of genomewide scans of complex diseases, in particular the permanent increasing number of genomewide scans in asthma with inconsistent chromosomal findings, we decided not to focus on our four loci with best linkage results, but to expand the sample size and genotype again the same microsatellite markers. The total number of 201 identically pheno- and genotyped families gives us the opportunity to define sub-phenotypes, which seems to be the most promising approach in diseases with etiological heterogeneity like asthma.
Both well-known traits like IgE and new-defined sub-phenotypes of disease severity and etiology showed loci of at least suggestive linkage (Lander and Kruglyak 1995). This might indicate that our detailed splitting into subphenotypes allows not only to find susceptibility loci for asthma, but also to discuss the phenotypic, pathophysiological impact of the gene that lies underneath.

P898. Deletion 4q35/duplication 10p15 associated with allergy and arthritis.

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We present a familiy with a cryptic reciprocal translocation between 4q35 and 10p15. The proband is an 8 years old girl with mild mental retardation, long philtrum, hypotelorism, immunological defect and rheumatism. Her parents are phenotypically normal. G-band analysis indicated a normal karyotype 46,XX, but high resolution CGH revealed reduced material on 4qter and additional material on distal 10p. A balanced reciprocal translocation t(4;10)(q35;p15), which could not be seen by classical banding analysis, was demonstrated in the mother by chromosome painting. The mothers sister is multiallergic, and CGH analysis displayed the same imbalance, dim(4q35),enh(10p15), as observed in the proband. FISH mapping with BAC probes from the two regions indicated that the breakpoints were within distal 10p14 and 4q35.1. The 4q35 deletion involved the facioscapulohumeral muscular dystrophy (FSHD) locus, suggesting that its deletion does not result in FSHD. Among the potential candidate genes involved in the imbalances which may be associated with the phenotypes are protein kinase C-theta (PRKCQ), interleukin 2 receptor, alpha chain precursor (IL2RA) and interleukin 15 receptor, alpha (IL15RA) on 10p15, and toll-like receptor 3 (TLR3) on 4q35.

P899. Genetic susceptibility of COPD: a large family based collection.

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In this study, we have aimed to identify genes and /or loci associated with susceptibility to Chronic Obstructive Pulmonary Disease (COPD). It has previously been shown that COPD clusters within families suggesting that shared genetic factors may predispose some smokers to development of COPD. Affected sibling pairs with severe COPD were ascertained and diagnosed after clinical, physiological and radiographic evaluation. Probands were recruited between 45-65 years with an FEV1 <60% predicted and >5 pack years smoking history, without α1 antitrypsin deficiency. Probands, siblings and parents were asked to complete extensive interviews about general health, family history and risk factors for COPD. A High Resolution Computer Tomography (HRCT) scan was conducted in consenting probands and siblings and the extent and distribution of emphysema was quantified by two independent radiologists. To date, 3135 individuals and 681 sibling pairs have been enrolled. This constitutes the largest published study of its kind. A preliminary data analysis has identified a correlation between family phenotype and extent of disease in the proband, with evidence that affected individuals have a genetic susceptibility to airways disease dependent on pack years smoked. Development of emphysema in genetically susceptible smoking individuals is less dependent on pack years smoked than airways disease. Analysis of the relationship between pulmonary function and HRCT measurements indicated that emphysema and airway wall dimensions independently contribute to airflow obstruction in COPD, suggesting separate genetic control. These serve as independent phenotypes for linkage analysis. The differentiation between phenotypes is currently being conducted and will be presented.

P900. HLA DQ relative risks for coeliac disease in European populations

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Coeliac disease (CD) is an enteropathy due to intolerance of gluten. The association between DQ genes and CD has been clearly demonstrated. The majority of patients carry the same HLA-DQ heterodimer encoded by *DQA1*05 – DQB1*02*, either in cis or in trans. The aim of the study was to estimate the differential risks associated with DQ genotypes.
DQA1 and *DQB1* information was available for 470 trio families (one affected child and both parents) from three countries : France (117), Italy (128) and Norway/Sweden (225).
For each country, we computed the relative risks associated with DQ genotypes taking into account the *DQA1-DQB1* haplotype frequencies, estimated from the set of parental haplotypes untransmitted to the affected child. Homogeneity of relative risks between countries was tested pairwise by maximum likelihood ratio statistics.
The relative risks associated to DQ genotypes are dissimilar between Northern and Southern European countries; neither are they ordered in the same way. Among the heterodimer carriers, the *DQA1*0201-DQB1*02* haplotype (H1) seems to confer greater risk than the *DQA1*05-DQB1*02* haplotype (H2) in Southern Europe, whereas the opposite was seen in the North.
Differences of risks observed in the North *versus* South could be explained by the involvement of another HLA factor, subdividing the haplotypes H1 and/or H2 into several haplotypes with different risks and different frequencies according to geographical areas.
This study was funded by the Commission of the European Communities (QLKT–1999-00037)

P901. Mapping of a coeliac disease locus in a four generation Dutch family to chromosome 9

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Coeliac disease (CD) is an enteropathy of the small intestine, which is caused by ingestion of dietary gluten from wheat, barley and rye. It is a multifactorial disorder, with a strong genetic contribution. The importance of the HLA-region in CD has been well established with the majority of CD patients expressing HLA-DQ2 and almost all remaining patients expressing HLA-DQ8. Non-HLA genes are expected to be involved in CD too. We performed a genomewide screen in a family with an exceptionally large number of CD patients in four generations. The family originates from a small region in the northern part of the Netherlands. The grandmother has CD, and the grandfather has antibodies against gliadin, but normal Ema and tTG antibodies and refused a small-intestinal biopsy. Ten out of 13 participating children have CD. The disease was further transmitted to five children in the third generation and also to one child in the fourth generation. All patients and both grandparents were HLA-DQ2. Parametric linkage analysis was performed using an affecteds-only approach with a dominant and a recessive model. Seven regions with a lod score >1 were detected, including the HLA-region with a lod score of 2.33. The most promising non-HLA region is on chromosome 9, with a lod score of 2.61. Furthermore, a lod score of 1.14 was present at 19p13.1, which was identified as the major CD locus in our affected sibpair study in 82 Dutch families. These results indicate the presence of a novel CD susceptibility locus in this family.

P902. Identification of novel candidate genes in celiac disease pathogenesis using microarrays

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Celiac disease (CD) is a multifactorial, gluten-sensitive enteropathy of unknown pathogenesis, characterized by villous atrophy of the small intestine. The causative molecular pathways underlying CD pathogenesis are poorly understood. To unravel novel aspects of the specific etiological pathways in CD, expression cDNA microarrays were used to determine global changes in expression of duodenum biopsies of CD patients.
RNA was extracted from duodenal biopsies of 15 CD patients showing complete villous atrophy and from 7 normal control biopsies and then hybridized on to cDNA microarrays containing 19,120 genes.
We found 109 genes that show a significant change ($p<0.001$) in the level and pattern of expression between CD and non-CD patients. Several of these genes control proliferation and differentiation pathways of cells in the intestinal crypts, while others regulate the structure of the villi, which may explain the occurrence of villous atrophy. Interestingly, a prolyl-endpopeptidase, which is related to gluten metabolism, also shows a significant change in its level of expression ($p<0.005$) when CD patients following a gluten-free diet (GFD) were compared to CD patients not on a GFD. Recently, a bacterial homolog of this endogenous prolyl-endopeptidase has been proposed as treatment for these patients.
We identified a number of novel genes not previously reported to be implicated in the molecular pathogenesis of CD. These genes may help elucidate the molecular and cellular pathways underlying intestinal atrophy after gluten ingestion in genetically susceptible individuals.

P903. A non-HLA locus for coeliac disease on chromosome 6 may predispose to autoimmunity

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Coeliac disease (CD) is an autoimmune disease of the gut, due to intolerance to proteins of gluten. Besides involvement of HLA-DQ2 and HLA-DQ8 in this complex disorder, non-HLA genes are also contributing to disease aetiology. Previously, we performed a genome screen in 101 affected sib pairs to localize non-HLA genes involved in CD. This genomewide screen indicated the presence of a second CD susceptibility gene on chromosome 6 (6q21). To exclude that our results were due to extended linkage disequilibrium of the HLA locus finemapping was performed between 6p21 (HLA) and 6q21. A total of 41 markers on chromosome 6 were analysed, with an intermarker distance <5 cM in the region of interest. The multipoint maximum lod score (MMLS) increased from 2.50 to 2.95 and narrowed the linkage region to a region of 12 cM. Finemapping showed that this region is independent from HLA. This region contains 97 genes. Microarray study showed differential expression of 8 genes from this region when comparing CD patients to normal controls.
Our results clearly demonstrate the presence of a second CD locus on chromosome 6. Interestingly, this region is also implicated in other autoimmune disease, such as diabetes mellitus type I and rheumatoid arthritis, indicating the presence of a gene involved in autoimmune processes.

P904. Exclusion of interferon gamma as a susceptibility gene in coeliac disease

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Coeliac disease (CD) is a gluten-induced enteropathy with a prevalence of about 1:200 in Western populations. Ingestion of gluten results in the progressive transformation of the intestinal mucosa from intra-epithelial lymphocytosis to complete villous atrophy. Major susceptibility genes are the HLA DQA and DQB loci on chromosome 6p. Recently we identified an additional major risk locus on chromosome 19. Together these loci contribute no more than 60% of the total genetic risk, suggesting additional (minor) risk genes. One

of the first steps in tissue remodeling is the binding of gluten-peptides to HLA DQ2 and the subsequent activation of T helper cells leading to the excretion of interferon gamma (IFNG). This results in tissue damage and an enhanced immune response. Since not all individuals carrying DQ2 develop CD we argued that genetic variations in the IFNG gene could contribute to the disorder. We performed real- time RT-PCR on mucosal biopsies from CD patients and observed an increase in IFNG expression correlating with a progression in tissue damage. To determine the genetic contribution of the IFNG gene, a linkage analysis in 82 affected sib-pairs was performed but revealed no evidence for linkage to this region. Also, TDT of an internal CA-repeat in the IFNG gene in 123 affected parent-offspring trios, and a case-control study in 199 CD patients and controls showed no association. We can conclude from our results that the IFNG gene shows no linkage or association with CD, and that its upregulation is merely a consequence of the disease process.

P905. Hereditary Hyperferritinaemia Cataract Syndrome associated with carrier status for haemochromatosis

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Purpose: To describe genetic and biochemical findings and cataract morphology in a four generation family with hereditary hyperferritinaemia cataract syndrome. (HHCS)
Methods: All family members underwent slit lamp examination to document presence of cataract or lens status. Cataract morphology was documented where present. Biochemical and genetic tests were performed.
Results: During investigation of a 35-year-old female with anaemia an elevated serum ferritin was noted. Genetic analysis of the HFE gene revealed her to be a carrier for haemochromatosis. (C282Y mutation) but after consideration of the dominant family history of early onset cataracts, a putative diagnosis of HHCS was made. This was confirmed by the demonstration of a +32 C to G substitution in the L-ferritin gene (L-ferritin Baltimore I). Two affected children had not yet undergone cataract extraction and cataract morphology was found to be pulverulent or sunflower configuration. Biochemical testing demonstrated high ferritin levels only in affected individuals.
Conclusions: Ferritin levels should be measured in all individuals with early onset cataracts. An individual with a normal ferritin level in a known family can be reassured that they will not develop early onset cataracts. Confusion with haemochromatosis can be avoided by testing for TIBC and transferrin saturation. The effect of co-inheritance of the C282Y allele with an L-ferritin mutation is under further investigation.

P906 Frequency and spectrum of hemochromatosis mutations in Tunisia

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Hereditary Hemochromatosis (HH) is a disorder of the iron metabolism inducing iron overload, which usually manifests during the 3rd decade of life. The gene associated to the disease (HFE) is located on the short arm of chromosome 6. The majority of the patients affected with severe pathology are homozygous for the C282Y mutation. A second (H63D) and a third rare mutation (S65D) usually produce less severe symptoms in compound heterozygotes with C282Y. The average allele frequencies of the two common mutations are 4% (C282Y) and 18% (H63D) in north Europeans in a decreasing gradient from northern to southern Europe. The allele frequencies measured in Italy are 1.65% and 13.3% respectively. In northern Africa, only the H63D mutation was detected with an allele frequency of 13.2% in a mixed Moroccan and Algerian populations living in France. Similarly no C282Y mutations were found in Ethiopian, Algerian and Senegalese. The occurrence of the C282Y and H63D mutations in Tunisia was unknown. We report the screening of 194 chromosomes from 97 randomly collected cord blood samples. The mutations were analyzed by PCR followed by DNA sequencing. The mild H63D and the severe C282Y mutations were found in 17.5% and 0.5% of alleles respectively. Risk for homozygosity for the severe C282Y mutation is present in the Tunisian population at a low theoretical incidence. However, due to the relatively high rate of consanguinity in the country, liver pathology due to HH cannot be discounted.

P907. Spectrum and haplotypes of the HFE hemochromatosis gene in Iran. H63D in β-thalassemia major and the first E277K homozygous case

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We present the molecular analysis of the HFE gene in 400 south-west Iranian individuals. We have studied 43 newborn selected for the presence of HbBart's at birth, 203 normal adult individuals and 154 multi-transfused patients, affected with β-thalassemia major. Mutation analysis consisted of amplification and direct sequencing using two different pairs of forward and reverse primers. The C282Y and S65C mutations were not found. The H63D mutation was present with an allele frequency of 0.10 in newborn, 0.082 in normal adults and 0.080 in the β-thal major populations respectively. No difference was found between normal adults and β-thalassemia major patients indicating that this mutation do not increase the mortality in β-thalassemia. The H63D mutation was associated with haplotype 6 in 41% of the chromosomes. Other haplotypes were found suggesting a multicentric origin rather than a single mutation of European origin. Sequencing exon 4 a G→A mutation was found in proximity of the C282Y mutation. The effect of this single base substitution (E277K) previosly reported in an Asian individual and now found in homozygous form in a young transfused and chelated homozygous β-thal patient is not yet known.

P908. Reverse-hybridization assay for multiple mutations associated with hereditary iron overload

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Inherited iron overload is a heterogenous disorder, including „classical" 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 ferroportin (FPN1/SLC11A3/IREG1) gene 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 (V53M, V59M, H63D, H63H, S65C, Q127H, P160delC, E168Q, E168X, W169X, C282Y, Q283P), TFR2 (E60X, M172K, Y250X, AVAQ594-597del) and FPN1 (N144H, V162del) genes. The test is based on multiplex DNA amplification and hybridization to a teststrip presenting a parallel array of allele-specific oligonucleotide probes for each mutation. The entire procedure from blood sampling to the identification of mutations requires less than 6 hours, and hybridization/detection

may be carried out manually or essentially automated using existing instrumentation (e.g. TECAN profiBlot). The test is simple and convenient, requires very small amounts of samples, and can easily be modified to include additional mutations.
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P909. Genetic analysis of genes involved in mitochondrial iron homeostasis and Fe-S clusters biogenesis in *Caenorhabditis elegans*

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Friedreich ataxia and X-linked sideroblastic anaemia and ataxia are caused by mutations in *FRDA* and *ABC7* genes, respectively. In both disorders iron is accumulated in the mitochondrial matrix of patients' cells. Mutants of orthologue yeast genes, *YFH1* and *ATM1*, accumulate iron in mitochondria. Recent data suggest that both yeast proteins, Yfh1p and Atm1p, participate in the biogenesis of iron-sulphur clusters (ISCs). We are addressing the analysis of both iron homeostasis and ISC metabolism in mitochondria by genetic studies in *C. elegans.*
The *C. elegans* frataxin homologue gene, F59G1.7 (*frh-1*) spans 730 bp within an operon of eight genes. It is constituted by three exons and encodes a 136 amino acid protein. To elucidate the expression pattern of the nematode frataxin we generated transgenic lines by injecting different *frh-1*/*gfp* gene constructs differing in the size of the 5' fragments of genomic DNA. *frh-1* is expressed in specific head neurons, muscles, pharynx and intestine. Transient knock-down experiments by RNAi produce a specific phenotype in worms of the F1 generation. The worms are: thin and short, *egl* (egg laying defective), have slow and arrhythmic pharynx pumping, and a decrease in the rhythm of defecation. We have also demonstrated that Y74C10AM.1 is the nematode orthologue of *ABC7* and *ATM-1* genes. The gene spans 13.4 kb and has 8 exons encoding a protein of 703 amino acids. This gene belongs to a two-gene operon on chromosome I. Preliminary analysis suggests expression in pharynx. RNAi experiments show slow growth and high reduction of the defection rhythm.

P910. The CRMGEN Project: Prototype Reference Materials for Hereditary Haemochromatosis and Haemoglobinopathy Testing

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The use of appropriate Reference Materials (RMs) to validate test equipment or testing methods is an important part of any analytical testing system. Certified reference materials (CRMs) are RMs whose characteristics have been fully documented and validated. Currently, no CRMs are available for genetic testing. The CRMGEN project, funded by the European Commission's Measurement and Testing program (Contract G6RD-CT-2001-00581), aims to develop the methodology to produce CRMs for any given molecular genetic test. The usual formats for positive controls for genetic testing, cell lines or genomic DNA, are expensive to produce and will not be suitable for all applications (e.g. multiplex tests). We are therefore investigating whether PCR products can be used for this purpose.
Using long range PCR, prototype RMs were developed for the common mutations involved in hereditary haemochromatosis (H63D & C282Y). The first round of prototype RMs have been tested by project partners. Based on feedback from this first trial, the RMs were re-optimised and a second round of prototype RMs produced for testing.
Using similar methodology, prototype RMs have been developed for three beta globin mutations, IVS1-nt110 (G>A), codon 39 (C>T), and the sickle cell mutation HbS codon 6 (A>T).
Results so far indicate that PCR products will make useful reference materials.

P911. Hemochromatosis C282Y and H63D Mutations Predispose to Joint Pain, Chondrocalcinosis and Osteoarthritis

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Up to 85% of type 1 hereditary hemochromatosis (HH) cases are explained by the two mutations (H63D and C282Y) in the HFE gene. Joint pain is the most common manifestation in patients with HH. We investigated the association between the HFE mutations and joint complaints in the general population. From a population-based cohort of 7893 people aged 55 years and over, we genotyped 2260 randomly selected subjects for the HFE mutations. We compared the frequencies of joint pain, chondrocalcinosis at X-ray, and clinically verified Heberden's nodes in carriers with non-carriers of the HFE H63D and C282Y mutations adjusting for age and gender using regression analyses.
Overall, the frequency of joint pain at hands was 2.1 times (95%CI 1.0-4.3; P=0.05) increased and pain at multiple joints (polyarticular) was 1.6 (1.0-2.6; P=0.05) times increased in H63D homzygotes. In H63D homozygotes aged 65 years or younger, the frequency of joint pain was 3.1 (95%CI 1.3-7.4; P=0.02) times increased while the frequency of polyarticular pain 2.7 (1.3-5.6; P=0.005), joint pain at hands 4.0 (1.4-11.7; P=0.006), knees 3.5 (1.2-10.1; P=0.02) and hips 3.2 (1.0-10.7; P=0.057) times. Heberden's node (OR 3.1; 1.3-2.8; P=0.02) and chondrocalcinosis (OR 4.7; 1.2-18.5; P=0.02) at hip and knee joints were found significantly more often in H63D homozygotes. Polyarticular joint pain and Heberden's nodes but not chondrocalcinosis was found more often in C282Y homozygotes. Our study suggests that the HFE H63D mutation is a more important determinant of generalised arthropathies than C282Y in the general population.

P912. Analysis of the promoter region of the human HFE gene in hemochromatosis patients

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HFE-related hemochromatosis is a common autosomal recessive disorder of iron metabolism that affects 1 in 300 individuals in Caucasian populations. The C282Y mutation, and two variants, H63D and S65C, are the main cause of the disease; few private mutations have also been described. In most studies, the analysis of HFE coding sequence failed to characterize 5-10% of the hemochromatosis patients. Indeed, the promoter region may also be a site for mutation liable to alter HFE expression. Here we analyzed, by direct sequencing, the -1403/-1 region of the HFE gene in 31 hemochromatosis patients lacking mutation in the coding region of the gene. Thirteen nucleotide variations have been found, those found more than once in patients have been also analyzed in a series of control subjects. Three polymorphisms have been characterized: -467 G/C (C 46.4% and G 53.6% in controls and C 59.5% and G 40.5% in the studied patients) is out of any regulatory element, and the C282Y mutation is in linkage disequilibrium with the C allele; -970 G/T that is in complete linkage disequilibrium with the -467 G/C change, is located within a NF-E2 motif TGTATAGC; -1206 G/C (G 61.4% and C 38.6% in hemochromatosis patients). Ten private variations in the 5' flanking region of the HFE gene have been detected only once in hemochromatosis patients. One of them (-491A/T), identified in a C282Y heterozygous patient, is located in a core GATA sequence. The hypothesis that it could alter the level of the HFE expression remained to be investigated.

P913. Spinocerebellar ataxias (SCA) in Estonia – a relatively high incidence of SCA2.

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Currently available DNA tests can define the genotypes of up to two thirds of patients with dominantly inherited SCAs. SCA3 seems to be the most common form in the United States of America, China and Germany, SCA1 and 2 in the United Kingdom and Italy, SCA2 in India and Cuba, and SCA 3 and 6 in Japan. During the last year, the DNA-based testing of SCA 1, 2, 3 and 6 became feasible in Estonia. This far, we have analysed 15 patients with suspected diagnosis of different forms of SCA. In six cases, no expansions, characteristic to SCA 1,2,3 or 6, were detected. In one case, we found a patient to have SCA1. In eight cases, DNA analysis confirmed the diagnosis of SCA2. The possible mechanisms affecting the relatively high incidence of SCA2 in Estonia will be discussed.