Eur J Hum Genet 1996; 4(suppl 1): 1-188

Spoken presentations

*** signifies that the presenter is eligible for the Young Investigator Award**

Symposium 1: Molecular genetics

S1.001

The locus for a novel form of neuronal intestinal pseudoobstruction maps to Xq28

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Primary chronic idiopathic intestinal pseudoobstruction (CIIP) due to neuronal defects most commonly results from developmental failure of enteric neurons to correctly migrate or differentiate This leads to clinical syndromes with varying anatomo-pathological features characterized by symptoms and signs of bowel obstruction caused by an intestinal motility disorder, in the absence of a mechanical obstacle Most of these conditions are congenital and some are inherited A condition characterized by intestinal pseudoobstruction with morphological abnormalities of the argyrophil neurons in the myenteric plexus, associated with short small bowel, malrotation and pyloric hypertrophy has been previously described (OMIM 243180) We have studied a family affected by this condition in which the disease apppeared to segregate as an X-linked recessive trait In order to map the CIIP locus in this family, we have performed linkage analysis in 26 family members using highly-polymorphic microsatellite markers from the X chromosome One of these markers, DXYS154, located in the distal part of Xq28, shows no recombination with a maximum lod score of 2 32 Multipoint analysis excluded linkage with markers spanning other regions of the chromosome Our results integrated with the current genetic and physical map of Xq28 determine the order of loci as cen-DXS15-(CIIPX)-DXS1108/DXYS154-tel This study establishes for the first time the mapping assignment of a neuropathic form of CIIP other than Hirschsprung disease

S1.002

*Linkage analysis to the α2 laminin locus on chromosome 6q2 in merosin-deficient congenital muscular dystrophy.

Naom, Isam[1], D'Alessandro, M[1], Topaloglu, H[2], Sewry, C[1], Helbing-Leclerc, A[3], Guicheney, P[3], Dubowitz, V[1], Muntoni, F[1]

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Approximately 50% of children with the classical form of congenital muscular dystrophy (CMD) show a deficiency of one component of merosin (α2 laminin) in their skeletal muscle This observation was followed by a significant advance in the understanding of CMD and establishment of the responsible gene location to a 16cM region on chromosome 6q2 We have investigated the location of the a2 laminin locus on chromosome 6q2 using both linkage analysis in informative families and homozygousity mapping in consanguineous families A total of 26 merosin-deficient CMD families (9 of which were consanguineous) were typed with 13 highly polymorphic microsatellite markers spanning an area of 17cM on chromosome 6q2 The results obtained in all the informative families (including families with partial merosin deficiency) were compatible with linkage to chromosome 6q2 Homozygousity mapping in consanguineous families and the recombinants found in informative families suggest that the actual location of the a2 laminin gene is more centromeric than previously thought In particular, various recombinant events suggest that the locus is more centromeric than the D6S457 marker Finally, linkage analysis was used in three cases of prenatal diagnosis in at-risk pregnancies Mutation analysis of the a2 laminin gene is in progress in these patients

S1.003

*Identification of second autosomal locus predisposing to multiple deletions of mitochondrial DNA.

Kaukonen, Jyrki[1], Amati, P[2,3], Suomalainen A[1], Rotig A[3], Piscaglia MG[4], Salvi F[5], Weissenbach J[6], Fratta G[7], Comi G[8], Peltonen L[1] and Zeviani M[2],

National Public Health Instit, Dept of Hum Mol Genet, Helsinki, Finland [1], National Neurological Instit "Carlo Besta", Division of Biochemistry and Genetics, Milan, Italy[2], INSERM U393, Hopital Necker-Enfants Malades, Paris, France[3], Division of Neurology, Public Hospital, Rimini, Italy[4], Hospital Bellaria, Division of Neurology, Bologna, Italy[5], Genethon, Evry, France[6] Division of Neurology, CSS-IRCCS, San Giovanni Rotondo, Foggia, Italy[7], Institute of Clinical Neurology, University of Milano, Italy[8]

Mendelian traits leading to either qualitative or quantitative abnormalities of mtDNA are likely due to mutations in genes controlling the nuclear-mitochondrial cross-talk A locus linked to autosomal dominant progressive external ophthalmoplegia associated with multiple mtDNA deletions (adPEO), has recently been identified on chromosome 10q in a large Finnish family (Suomalainen et al Nature Genet 9 146-151, 1995) However, no linkage with the 10q locus was demonstrated in two Italian adPEO families, indicating heterogeneity We applied random mapping approach to informative non-10q-linked Italian families to assign the second locus for adPEO Since muscle biopsy specimens were not available from asymptomatic subjects and true affection status could thus not be verified, the linkage calculations were carried out as affected only analyses We have now assigned the second disease locus for ADPEO within 14 cM region between markers D3S1581 and D3S1600 to chromosome 3p 14 1-21 2 in three Italian families A maximum two-point lod score obtained was 4 62 at 0 0 0 with marker D3S1300. However, three adPEO families showed clear exclusion for this same region and statistical evidence for heterogeneity was obtained by the HOMOG program These data indicate the existence of at least three adPEO loci Assignment of the third adPEO locus is currently in progress

S1.004

*The mouse homolog of PEX is deleted in Gy mice.

Strom, Tim M[1]; Francis, F[2], Econs, MJ[3]; Lorenz, B[1], Meindl, A[1], Rowe, PSN[4], O'Riordan, JLH[4], Oudet, C [5], Drezner, MK[3], Lehrach, H [2], Meitinger, T [1]

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A gene mutated in patients with X-linked hypophosphatemic rickets has been described recently by the HYP consortium The gene named PEX was identified by a positional cloning effort in Xp22 1 and is predicted to encode a protein with homology to a family of endopeptidases including neutral endopeptidase (CALLA), endothelin converting enzyme (ECE-1) and Kell antigen Gy mice (gyro) show hypophosphatemia, rickets and a characteristic circling behaviour The locus is known to map to a region of the mouse X chromosome syntenic to the human PEX locus An intragenic deletion was detected in the murine homolog using the human PEX cDNA as an hybridisation probe A cDNA clone was obtained by screening a mouse spleen cDNA library Sequences obtained so far show a 90% identity on protein level to the human homolog The Gy mouse represents a model for the human disease hypophosphatemic rickets Mutation analysis of Gy in a second hypophosphatemic mouse strain (Hyp) is currently being performed

S1.005

Mutation analysis of myelin genes in nonduplicated Charcot-Marie-Tooth type 1 (CMT1) patients: comparison of single-strand conformational polymorphism analysis (SSCP) and heteroduplex analysis (HA).

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Charcot-Marie-Tooth type 1 disease (CMT1) is a genetically heterogeneous peripheral neuropathy In the majority of the cases the disease is caused by a 1 5 Mb duplication in chromosome 17p11 2 (CMT1A) In the nonduplicated patients mutations were identified in PMP22 on chromosome 17, in MPZ on chromosome 1q22-q25 (CMT1B) and in Cx32 on chromosome Xq13 (CMTX) Most of the mutations are detected by SSCP and HA To compare the sensitivity of these two mutation detection techniques we analyzed a set of 72 nonduplicated CMT1 patients for mutations in the coding region of PMP22, MPZ and Cx32 In PMP22, 2 sequence variations were detected by both SSCP and HA Sequencing revealed two different missense mutations In MPZ, 13 sequence variations were detected of which 3 were detected only by SSCP Sequence analysis revealed 4 different missense mutations, 1 nonsense mutation and 2 different silent mutations Analysis of Cx32 revealed 5 sequence variations by both SSCP and HA, 1 mutation only by SSCP and 1 only by HA Sequence analysis identified a missense mutation in 2 patients and 1 missense mutation combined with a deletion of 11 bp Three samples still have to be sequenced These results indicate that the majority of the mutations are detected by both techniques, however, HA revealed more false negative results

Symposium 2: Clinical Genetics

S2.001

Macrocephaly-cutis marmorata telangiectatica congenita: Description of twelve patients with this previously undescribed common multiple congenital anomaly syndrome.

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Butterworth Hospital, Grand Rapids, MI [1], Indiana University, Indianapolis, IN[2], and University of Minnesota Health Center, Minneapolis, MN[3], USA

Cutis marmorata telangiectatica congenita (CMTC) is a condition characterized by congenital generalized or segmental marbling of the skin This is a heterogeneous entity, and has been described in association with other anomalies such as hemihypertrophy, macrocephaly, and limb defects in approximately 50% of reported cases We have identified 12 patients since 1991 with what we believe is a previously undelineated multiple congenital anomaly syndrome The phenotype includes in addition to CMTC, congenital macrosomia with subsequent failure to thrive, macrocephaly, postnatal onset hydrocephalus, scaphocephaly, cutaneous facial hemangiomas, with philtral hemangiomas being particularly common, hemihypertrophy, hyperelastic skin, hyperextensible joints, cutaneous syndactyly of toes 2-3 or 2-3-4, and wide space between the great and second toes Although this condition is superficially phenotypically similar to Klippel-Trenaunay-Webber syndrome, we believe this is a distinct entity which should be distinguished from KTW We will discuss differential diagnosis, review the literature with regards to other described cases (usually diagnosed as KTW), and provide natural history on several patients with this disorder

S2.002

Dominance and homozygosity.

Zlotogora Joel

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Because of the high consanguinity rates in many communities in Israel we had the opportunity to study homozygosity for some dominant disorders This experience and the review of the literature confirmed that in most cases in dominant disorders homozygotes are more severely affected than heterozygotes Molecular analysis allowed in some cases to understand the mechanisms involved Heterozygosity for point mutations or deletions of PAX3 lead to similar symptoms (Waardenburg syndrome) while in homozygotes the phenotype is much more severe probably in direct in relation to the loss of function (one personal observation) Charcot Marie Tooth 1A is caused by a duplication of PMP22 and further overexpression lead to a more severe disorder In diseases in which the mutation lead to an abnormal structural protein the homozygote may be affected like the heterozygote (epidermolysis bullosa simplex) or more severely (achondroplasia) The polyglutamine tract is translated in disorders caused by CAG triplet expansions In homozygotes for Machado Joseph disease (6 personal observations), the onset is earlier and the symptoms more severe than in heterozygotes while in Huntington disease homozygotes are affected like heterozygotes

S2.003

The X-linked form of Charcot-Marie-Tooth disease type 1: clinical and electrophysiological features

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The X-linked form of Charcot-Marie-Tooth disease type 1 (CMT1) is caused by mutations in the gap junction protein Connexine 32 (Cx32) It is the second most frequent CMT1 mutation next to the 1 5 Mb tandem duplication in chromosome 17p11 2 that is responsible for the majority of CMT1A cases We identified 7 different Cx32 mutations in 10 unrelated CMTX families Although these mutations (5 missense mutation, 1 nonsense mutation and 1 deletion of 3 base pairs) occurred in different parts of the gene, the phenotype was similar in all families The male patients all developed distal paresis and atrophy in the legs before the age of 20 years Atrophy and some weakness of hand muscles occurred early in the disease course Female gene carriers were often asymptomatic but they all showed some abnormalities on clinical examination or nerve conduction velocity (NCV) testing The motor NCV's of the Median and Ulnar Nerve were slowed in all male patients (Motor Median <40m/s and Motor Ulnar <43m/s) The amplitudes of the compound action potentials were always reduced In female gene carriers the motor NCV's of the Median and Ulnar Nerve were usually slowed but less than in male patients and sometimes results within the normal limits were obtained

S2.004

Multiple congenital anomalies, brain hypomyelination and ocular albinism in a female patient with dup(X)(pter->q24::q21.31->qter) and random X inactivation

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The majority of females with duplication of portions of the X chromosome do not show a distinct phenotype In most of these cases, the X chromosome inactivation is not random, with the duplicated X being inactive We report on an 18 month old female with multiple congenital anomalies (trigonocephaly, fine hair, club foot,

brachydactily) and severe mental retardation The funduscopic examination revealed retinic hypopigmentation The head MRI showed signs suggestive of a diffuse hypomyelination Chromosome analysis showed a 46,X,dir dup(X)(pter->q24 q21 31->qter) karyotype In situ hybridization performed with YAC 122E11 containing the PLP gene, involved in Pelizaeus-Merzbaker disease, showed two sets of signals. Both cytogenetic and molecular sudies on the X chromosome inactivation status indicated a complete random pattern both in lymphocytes and in fibroblasts The patient reported herein appears to be the first case of a female bearing a large duplication of Xq with a random X-inactivation demonstrated both on leukocytes and fibroblasts The severe associated phenotype and the MRI findings suggestive of Pelizaeus-Merzbaker disease are likely to be due to the functional diploidy for the Xq21 31-q24 region, present in a large cell population

S2.005
The Cape Town cleidocranial dysplasia kindred; the gene is on chromosome 6

Beighton, Peter, Greenberg, J, Martin, R, Goliath, R, Bardien, S, Bartmann, L, Ramesar, RS
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Several hundred members of a Cape Town kindred have cleidocranial dysplasia (CCD) This family, which was initially documented by Jackson (1951) is descended from a polygamous person who arrived at the Cape in 1896 The main phenotypical features are persistent fontanelles, hypoplasia of the clavicles, dental anomalies, a wide pubic symphisis and short stature Following reports of rearrangements on chromosomes 6 and 8 in individuals with CCD, we carried out linkage analysis in a branch of this kindred (consisting of 38 individuals, 18 of whom are affected using) highly informative dinucleotide repeat markers from the reported rearranged regions Maximum LOD scores (at theta = 0 00) of 3 51 (for marker TCTE1), 4 988 (D6S452), 6 024 (D6S269) and 4 91 (D6S465) confirms the localisation of the CCD gene to the pericentromeric region of chromosome 6 Our data suggests that the gene lies within a minimal region of approximately 5cM, flanked by markers D6S459 and D6S466 This information is important for the construction of a physical map of this region and for the isolation of the CCD gene

Symposium 3: Advanced technologies

S3.001
DNA Sequencing technology for Genome analysis

Wilhelm Ansorge, Josef Stegemann, Vladimir Benes, Jurgen Zimmermann, Heiko Drzonek, Christian Schwager and Hartmut Voss

One of the key goals of the Human Genome Project - determination of the complete nucleotide sequence - requires high throughout systems with support from automated and robotics devices. Current automated DNA sequencing technology represents a ten to twenty fold improvement over the performance at introduction, allowing to determine up to 80 kilobases odf sequences from a single gel run

Automated DNA sequencing systemes developed recently at EMBL allow simultaneous on-line sequencing of two complementary strands of double stranded template in a single sequencing reaction, with two different flurescent labels Per sequence reaction are obtained 2000 bases or more, improving the cost efficiency The throughou t was inc5reased to 48, 64 and recently to 80 clones run from both strands simutaneously on one gel, with possible extension to 120 clones The novel optical light dection system improves the horizontal sample track resoultion maintaining the high sensitivity Since the sequences of both strands of the template are determinerd simultaneously, the costs of labor, DNA template preparation and sequencing reactions, as well as of gell casting is reduced and improve further the overall cost efficiency The system has been developed with the support from the European Union program and has been partly applied in the routine sequencing of about 500 kilobases in the Yeast Genome Project Model genome projects (C elegans, Yeast) have demonstrated the importance of efficient sequencing strategy, high quality enzymes, labelling, accuracy of the final data and data analysis, all affecting the final cost per base (now below one dollar) Mapping and gene functional analysis are facilitated by high quality consnsus sequence data

S3.002
New approaches to molecular cytogenetics

Lichter, Peter

S3.003
*Sensitive FISH by the deposition of biotinylated tyramine: analysis on metaphase chromosomes and DNA fibers.

Laan, Maris, Palotie, A
Department of Clinical Chemistry, University of Helsinki and the Laboratory Department of Helsinki University Central Hospital, Helsinki, Finland

Rapidly developing FISH techniques are powerful tools in genome mapping Up to now the detection of short unique sequences (<2 kb) has been complicated and often unreliable Recently, a novel sensitive in situ hybridization detection technique based on the deposition of biotinylated tyramine was published (Raap et al , Hum Mol Gen 4 529-534, 1995) We further modified and tested the applicability of the detection method for hybridization of small insert probes, for fiber FISH and for combined two-colour FISH (one probe detected via tyramide-mediation and the other by standard technique) In the hybridization of a 1 1 kb cDNA, ~70% of the metaphase spreads showd a fluorescent signal on at least one of the chromosome homologues in both one and two-colour FISH In fiber-FISH mapping, the signal of large-insert-clones (e g PACs) was more uniform and continous compared to the often spotlike signals of larger clones, achieved by standard detection Thus, the determination of the specific hybridization versus backround is much easier Furthermore, the uniform fluorescent signals allowed the reliable visualization of replication bubbles and forks inside the analyzed genomic fragments

S3.004
*MyoD induced myo-differentiation enhancing myogenesis of fibroblasts and facilitating the diagnosis of Duchenne Muscular Dystrophy.

Roest, Pauline, van der Tuijn, A , Den Dunnen, J T , Van Ommen G J B
MGC-Dept of Human Genetics, Leiden University, The Netherlands

We have used an in vitro myo-differentiation system to study muscle-specific gene defects in non-muscle cells Fibroblasts are forced into myogenesis by transformation with a retroviral vector containing the MyoD-gene Transfected cells are selected for vector derived neomycin resistance, grown to confluency and myogenesis is induced by a transfer to low serum medium Immunohistochemical analysis of transfected fibroblasts showed the appearance of muscle-specific proteins desmin, titin, myosin and dystrophin Besides being a potentialy valuable, non-invasive diagnostic method for genetic muscle pathology, it also fasilitates study of the basic ethiology RNA isolated from Myo-differentiated cells have increased levels of muscle-specific transcripts, facilitating RNA based mutation detection of muscle diseases The technique was used to study dystrophin, the protein defective in patients with Duchenne Muscular Dystrophy (DMD) Currently, we are searching to apply forced myo-differentiation as a tool to assess DMD carrier status using fibroblasts Our results show that in transformed fibroblasts of patients dystrophin is absent indeed However not all differentiated cells of non-carriers are dystrophin positive, indicating the need for a better marker protein which is expressed in exactly the same time frame as dystrophin Furthermore, we are testing adenoviral vectors as an alternative for the retroviral vectors, since these vectors are expected to deliver multiple gene copies and potentially enhance muscle differentiation Results of the application of the system for DMD and other muscle disorders will also be presented at the meeting

S3.005

Development of a very fast fully efficient mutation screening method using solid-phase fluorescent chemical cleavage of mismatch

Green, Peter, Rowley, G, Saad, S, Giannelli, F
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In order to improve by more than an order of magnitude the extent of sequence that can be scanned for mutations per person per day, the fluorescent labelling of DNA and solid-phase chemistry have been combined with the chemical cleavage of mismatch technique The introduction of fluorescent dyes has allowed a 3-4 fold improvement in the speed of this previously laborious method by multiplexing different colour-tagged PCR products, while the addition of biotin primers allows capture on streptavidin magnetic beads greatly simplifying the whole procedure Using the three available fluorescent dUTPs to internally label the DNA during PCR, it is possible to screen up to 432kb (3 colours x 1 5kb x 96 wells) of DNA with hydroxylamine in a microtitre plate in one day on three ABI gels This will ensure detection of about 95% of mutations while a further screen with osmium tetroxide will increase this figure to 100% with considerable redundancy in the detection of mutations Alternatively, using end-labelled primers, it is possible to screen 288kb of sequence with hydroxylamine and osmium tetroxide [(4 colours x 1 5kb x 96 wells)/2 as 48 wells are treated with each chemical] We have used this method successfully to locate mutations in the factor IX gene of all 126 cases examined prospectively and 50 cases examined retrospectively

S3.006

Multiplex, fluorescent minisequencing: a powerful method for detecting single nucleotide polymorphisms

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We have developed a robust and technically simple multiplex minisequencing method, for the simultaneous detection of multiple polymorphisms or point mutations In this method biotinylated PCR products from the DNA areas of interest are captured on avidin coated manifold supports and rendered single stranded Multiple primers, which anneal immediately adjacent to the variable sites in the single stranded immobilized template, and vary in size, are extended with a fluorescent ddNTP by a DNA polymerase The extended primers are separated by size in a sequencing instrument and the incorporated ddNTP is identified The length of the primer defines the site of variation and the incorporated ddNTPs identify the nucleotide(s) at this site The HLA Class II genes served as model systems for evaluating the method A multiplex minisequencing procedure for combined type of HLA-DQA1 and HLA-DRB1 genes was designed Samples of known HLA-genotype were analyzed and the correct genotype was assigned in all samples The procedure, comprising a minimal number of pipetting steps, fast detection and easy interpretation of the results is performed efficiently in the analyses of large number of samples

S3.007

Genetic Bar-Coding: Rapid Detection and Localization of Sequence Changes by Cleavase® Fragment Length Polymorphism (CFLPTM) Analysis

Lance Fors, Mary Ann D Brow, Mary Oldenburg, Victor Lyamichev, Laura Heisler, Jeff Grotelueschen, Natasha Lyamichev, James Dahlberg[1], Lloyd Smith[2], and D Michael Olive
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Rapid and inexpensive methods for the detection of genetic mutations resulting in human diseases are critical to providing cost effective healthcare While some mutations may cause significant changes in their respective genes, many mutations, such as those found associated with the p53 gene, are changes in single nucleotides Methodologies such as single strand conformation polymorphism (SSCP) and denaturing gradient gel electrophoresis (DGGE) have yielded limited success in detecting mutations in short DNA fragments Under the best conditions, these methods are limited to indicating the presence or absence of a base change, some of which may not result in phenotypic change Until now, DNA sequencing has been the only method which can successfully identify phenotypically significant mutations, yet the cost and complexity of DNA sequencing has prevented its general use by the clinical community We report here the use of a thermostable structure-specific nuclease, Cleavase® I, for the rapid detection and localization of mutations in clinically significant human genes including the p53, msh 2, beta-globin, and tyrosinase genes, and the M tuberculosis rpo B and kat G genes that confer resistance to the antibiotics rifampin and isoniahzed, respectively Cleavase® I recognizes the folded structures assumed by DNA strands following denaturation and cleaves these structures, within 1 to 2 minutes, at the junction of the single stranded and duplexed regions The Cleavase® Fragment Length Polymorphism (CFLPTM) technique generates a very high resolution collection of fragments which, when separated by a 5 to 20 minute gel electrophoresis, yields a unique bar code for each DNA sequence analyzed DNA fragments differing by as little as a single base change can be rapidly and reproducibly distinguished We have been able to detect and localize 102 of 103 polymorphisms in a total of 15 genetic systems Using CFLPTM analysis, point mutations in the human p53, msh 2, beta-globin and tyrosinase genes, and the M tuberculosis rpo B and kat G isolates were reproducibly detected and distinguished from phenotypically silent mutations in 30 minutes or less The CFLPTM technique is simple and reproducible, and represents a powerful new mutation scanning method that can facilitate the rapid and cost effective detection and localization of mutations associated with human diseases

Symposium 4: The genetics of skin disorders and of deafness

S4.001

Genes and the Skin: A Clinician's view

Rudolf Happle, Marburg (Germany)

More than 60 genodermatoses have already been regionally assigned within the genome In order to complete the huge task of human gene mapping, a close co-operation between clinician and molecular geneticists is needed Absence of such co-operation has caused mistakes such as the incorrect assignment of incontinentia pigmenti to Xp11 or the erroneous mapping of focal dermal hypoplasia to Xp22 3 By the advances of molecular research, new nosological categories are emerging Mutations within the keratin type I and II clusters are responsible for epidermolytic ichthyosis, epidermolytic palmoplanter keratoderma, several types of epidermolysis bullosa simplex, white sponge hyperplasia of the mucosa, and pachyonychia congenitia

S4.002

Genotype/phenotype correlation in autosomal recessive lamellar ichthyosis (LI) caused by TGM1 gene mutations and evidence for genetic heterogeneity of LI

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Lamellar ichthyosis (LI) is a severe inherited skin disorder characterised by generalised scaling and variable redness of the skin In autosomal recessive LI, linkage has recently been described with microsatellites at the TGM1 gene locus on chromosome 14q11 Mutations in the TGM1 gene have been shown to drastically reduce the activity of the keratinocyte transglutaminase We have now investigated a total of 28 families with LI from Germany, Turkey, Greece, Morocco and Arabia 19 of these were analysed for linkage using microsatellites at TGM1, D14S64, and D14S264 Ten families showed cosegregation with these loci, three were uninformative Six families, however, clearly showed recombination events Mutation analyses in the TGM1 gene with SSCP and direct sequencing in LI patients revealed so far two novel missense mutations, G144R and R315C, and mutation R323Q Two unrelated patients were homozygous for the splice mutation A3447G in intron 5, one further patient was heterozygous for this mutation The splice mutation was always found on the same haplotype for the TGM1 linkage group suggesting a common origin of this mutation No obvious difference in clinical presentation between linked and unlinked families was detectable Moreover, the two patients homozygous for A3447G clearly differed in their clinical picture One of these presents the form of LI characterised by fine, whitish scaling, accompanied with erythema The second shows larger scales but lacks erythema Hence, clinically indistinguishable forms of LI can be caused by mutations in the TGM1 gene as well as in at least one other gene Furthermore, clinical variability in patients within the linked group indicates a lack of a strong genotype/phenotype correlation in LI

S4.003

Genetic linkage of Vohwinkel's syndrome to the epidermal differentiation complex on 1q21 and identification of a mutation in the loricrin gene

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The coordinated expression of an ensemble of over 25 functionally interdependent genes is necessary for keratinocyte differentiation, many of which have been mapped to a 2 Mb region of chromosome 1q21 known as the epidermal differentiation complex (EDC) Keratoderma hereditaria mutilands or Vohwinkel's Syndrome (VS) is an autosomal dominant genodermatosis which includes hyperkeratosis of the palms and soles with a "honeycomb" appearance, starfish-shaped salmon-colored keratotic structures on the hands, and constricting bands encircling the fifth digits of the hands and feet, known as pseudoainhum, which frequently lead to autoamputation A genome wide search for linkage in a large VS family resulted in a LOD score of Z=7 86 at theta=0 with microsatellite markers near the EDC region of chromosome 1q21 Furthermore, a delayed termination codon mutation was found in the loricrin gene, which results in a carboxy-terminal extension of the polypeptide by 22 missense amino acids, and dominant-negative interference These findings provide the first evidence for a role of a mutation in an EDC gene in the pathogenesis of a genodermatosis, and disclose novel insights into perturbations in the cornified envelope proteins involved in the terminal differentiation process

S4.004

Myosin genes and deafness

Steel Karen P, Self, T, Fleming J Mahony M Pearce A, Harvey D, Cable J, Mburu P Walsh J, Gibson, Varcula A, Brown, SDM
MRC Institute of Hearing Research, Nottingham, U K Imperial College of Science Technology & Medicine, London U K

Mouse models can be invaluable for investigating the causes of human genetic disease One example is the gene involved in Usher syndrome type 1B, which was shown to the uncoventional myosin gene, myosin VII, after this was identigfied by poisitional cloning of the mouse homologue shaker-1 (Gibson et al 1995, Nature 374 62-64, Weil et al 1995 Nature 374-60-61) The myosin VII gene is the first to be identified as causing a primary sensory hair cell defect when mutated The gene is expressed in inner ear sensory hair cells from as early as 16 days of gestation in the mouse, and analysis of seven different shaker-1 mutations suggests that the myosin VII protein has at least two roles in hair cells development and function Firstly, it is involved with organisation of the sterocilia bundle at the top of each hair cell Seconldy, it has another role in inner hair cell function, because the mildest of the shaker-1 alleles shows normal sterocilia bundle formation but the inner hair cells never function properly in initiating cochlear nerve action potentials In both Usher syndrome type 1B and shker-1 mouse mutants, snesory hair cells defects lead to deafness and vestibular dysfunction, but in humans, progressive retinitis pigmentosa also occurs We have seen no evidence yet of retinal defects in shaker-1 mutants up to a year old, and we are currently investigating why the mouse myosin VII mutants escape this feature of the syndrome

S4.005

Snell's waltzer encodes an unconventional myosin: A mouse model for human autosomal recessive deafness.

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The mouse represents an excellent model system for the study of genetic deafness in humans Many mouse deafness mutations have been identified and the anatomy of the mouse and human inner ear is similar One of these mutations, Snell's waltzer (sv), is a model for non-syndromic hereditary hearing impairment, the most prevalent form of human genetic deafness Using a positional cloning approach, we recently discovered that sv encodes an unconventional myosin heavy chain, myosin VI Furthermore, myosin VI is expressed within, and appears required for maintaining the structural integrity of, the sensory hair cells of the inner ear These and previous studies showing an important role for another unconventional myosin, myosin VIIa, in mouse and human hearing, suggests an important role for the unconventional myosin supergene family in hearing The requirement for myosin VI in hearing makes this gene an excellent candidate for a human deafness disorder While no human deafness mutations have yet been mapped to the homologous chromosomal region in humans, the cloning of the mouse myosin VI gene makes it possible to determine directly if myosin VI is altered in any human deafness disorder

Research sponsored by the National Cancer Institute, DHHS, under contract with ABL

S4.006

Two new extended families linked to a deafness locus (DFNA2) on chromosome 1p and further refinement of the candidate region .

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Recently, we reported the localization of a gene for progressive autosomal dominant hearing loss starting in the high tones (DFNA2) to chromosome 1p in an Indonesian and an American family In this study we analysed two extended European families showing a similar hearing loss with markers from the DFNA2 region One family originating from Belgium shows lod scores with closely linked flanking markers D1S432 and MYCL1 of 7 0 and 7 7, respectively The second family originates from the Netherlands and gave lod scores of 4 7 and 7 3 for D1S432 and MYCL1, respectively As 4 extended families originating from 3 different continents are linked to DFNA2 , this gene is most likely important for progressive hearing loss The analysis of key recombinants in the 4 linked families enabled us to reduce the DFNA2 candidate region to 1 2 megabase between D1S432 and MYCL1 A YAC contig spanning the candidate region was constructed

and cosmids and P1 clones from this region were isolated We used these cosmids and P1 clones to identify new genes from the DFNA2 region by direct cDNA selection and exon trapping Several transcripts were identified, and the possible involvement of these genes in the disease will be investigated

S4.007
The chromosomal localisation and identification of human genes responsible for sensorineural, non-syndromic autosomal recessive deafness.

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Members of the "groupe méditerranéen de recherche sur les déficits sensoriels héréditaires" (GMRDSH)
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Non-syndromic (NS) inherited deafness is by far the most frequent cause of sensory defect in children The autosomal recessive forms (DFNB) are the more severe and accounts for 75% of the cases Their extreme genetic heterogeneity and the absence of clinical characteristics combined with non-assortive mating in the deaf communities of developed countries presents serious obstacles to the chromosomal localisation and subsequent cloning of the DFNB genes To overcome these problems, a collaborative group (GMRDSH) has undertaken a study on large consanguineous affected families living in geographically isolated regions, around the Mediterranean Sea Using this approach, we have mapped the two first genes responsible for recessive sensorineural deafness, DFNB1 and DFNB2, to chromosomes 13q11 5 and 11q13 5 respectively, in Tunisian families, and recently, a third one, DFNB6 to 2p22-23, in a Libanese family The DFNB1 gene which seems to be frequently defective is also responsible for a dominant form of deafness, DFNA3 We have hypothesised that myosin VIIA gene, the Usher IB gene, could also be the DFNB2 gene Results of the mutations screening in these patients will be discussed

Symposium 5: Psychosocial aspects of predictive testing

S5.001
To predict or not to predict: the psychological reactions one year after predictive testing for Huntington's disease.

Gerry Evers-Kiebooms, Center for Human Genetics, Leuven, Belgium

Almost one decade ago, when predictive testing for HD became technically possible, the fear for suicide and for adverse psychological reactions was at the origin of the initial use of strict research protocols and/or of an intensive multidisciplinary approach Meanwhile the psycholigical complexity of predictive DNA-testing, the pitfalls in pretest counselling as well as the short termpsychosocial consequences have been appreciated and documented by several centres Moreover the experiences with HD have been of considerable value in relation to genetic testing programmes for other late onset disorders

First a critical reflection on the overall experiences with predictive testing for HD, from a psychologist's point of view, is made Thereafter the definite impact of the test result on reproductive decision making during the first post test year is illustrated Finally the results of stepwise multiple regression to identify important "predictors" pf psychological functioningone year after the test are discussed They reveal that a careful evaluation of pretest ego-strength depression level and coping strategies may be helpful in predicting post test reactions

S5.002
Predictive testing for Breast/Ovarian cancer

Craufurd, David
Department of Medical Genetics, University of Manchester

Women identified as carriers of mutations causing inherited breast-ovarian cancer can use this information to plan preventative strategies such as screening or prophylactic surgery However, the efficiency of these preventative measures has not been fully established, and testing has many of the disadvantages of predictive testing for HD because of the potential psychological impact As with HD, several hypothetical surveys suggested a very high level of demand among those at risk, but this has not been born out in practice In a study of 70 at-risk individuals from 5 BRCA1 families the overall uptake was 52 9%, considerably more than for HD prediction but less than for FAP and VHL where there are greater advantages to knowledge of genetic status Uptake was higher in women (61 9%) than men (39 3%), higher in those with a greater subjective perception of risk, but lower in those with an affected mother or sister than those where the risk was transmitted by an unaffected father The most common reasons for testing were to help children and to plan appropriate strategies for the future Attitudes to testing may be influenced by the greater residual uncertainty after BRCA1 testing, by differences between men and women in attitudes to health-related activities generally and by previous traumatic experiences of the disease in their own families

S5.003
Is health care sufficiently equipped for predictive testing for hereditary late-onset disorders?

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The attention for psychological effects of predictive testing for Huntington's disease has prompted comparable protocols in predictive testing for other inherited neurodegenerative diseases (myotonic dystrophy, Alzheimer's disease, hereditary cerebral haemorrhage, cerebellar ataxia, neurofibromatosis) and hereditary forms of cancer (multiple endocrine neoplasia-type 2A, adenomatous polyposis coli, non-polyposis coli, breast- and ovary cancer etc) The far-reaching effects of becoming identified as either a gene carrier or non-gene carrier are even more evident when prophylactic surgery options are becoming considered Still, there is the risk that clinical medicine will regard DNA-diagnosis simply as 'another' laboratory test With the expanding impact of genetics in all fields of medicine, there is a growing need for studies on risk perception, (prophylactic) treatment options, family dynamics, testing children and development of adequate support systems At this moment, only few follow-up studies are available on the impact of testing for 'other disorders' Probably there may be increasing difficulties in funding those programmes, but both the clinical and genetic workers must emphasize the absolute necessity of those studies It will remain essential to study the acceptability and effects of predictive testing An overview will be given of testing programmes and the associated specific medical-psychological and ethical issues

S5.004
Genetic testing: the example of familial adenomatous polyposis

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Implicit in many studies of health screening is an assumption that those receiving low risk results will react positively, and those receiving high risk results will react negatively There is accumulating evidence to show that this assumption is unfounded We have now set up an international multi-centered prospective study (7 UK centres, 2 Australian) to describe and explain the predictors of psychological responses to predictive DNA testing for familial adenomatous polyposis (FAP) The

main dependent measures are mood (six item short form of the Spielberger state-trait anxiety inventory Marteau & Bekker, 1992, and The Hospital Anxiety and Depression Scale Sigmond & Snaith, 1983), and subjective stress (Impact of Events scale Horowitz, Wilner & Alvarez, 1979) The main independent variables are FAP illness cognitions and self-esteem (Rosenberg, 1965) In addition to collecting data from those tested and their parents, we are tape recording some of the consultations when test results are given, to examine the influence of framing of test results upon subsequent responses The study is due to report in December 1996 Preliminary results from this study will be presented

Symposium 6: Model organisms illuminate Human Genetics

S6.001
The lessons from yeast
Carr, Dr Tony

S6.002
From worm to man: the C. elegans paradigm
Hodgkin, Jonathan
Laboratory of Molecular Biology, MRC, Cambridge CB2 2QH

The nematode Caenorhabditis elegans has become established as a major model system for the study of a great variety of problems in biology and medicine Its small size, simplicity and invariance have permitted complete descriptions of cell lineage, development and anatomy In addition, the genome is small 100 million base pairs, 1/30 the size of the human genome As a result, it has been possible to construct a complete physical map, and to embark on the complete sequencing of this genome A consortium based at the Sanger Centre (Cambridge, UK) and the Genome Sequencing Center (St Louis, USA) has so far completed over 30 million base pairs of genomic sequence, and obtained data for over half of the estimated total of 13,900 genes Currently, about 48% of sequenced genes have significant similarities to genes characterized in other organisms Homologs for many human genes can be readily identified and studied, using powerful genetic methods Classical and reverse genetics can be used to identify mutations in any sequenced gene *Screens for enhancers and suppressors of any mutant phenotype can be* carried out, leading to the identification of interacting genes Examples of the relevance of this approach to human genetics will be discussed

S6.003
Drosophila to man: signals conserved
Hafen, Ernst

S6.004
Zebrafish: a clear model
Nusslein Volhard, Prof Christiane

S6.005
Mouse mutants point the way
Abbot, Cathy

S6.006
From Human Disease back to Basics
Veronica van Heyningen,
MRC Human Genetics Unit, Edinburgh EH4 2XU

Human disease causing genes are being identified at an accelerating pace, often by positional cloning Frequently the function of the responsible gene is known only superficially or not at all. Exploring function experimentally in the human situation is often not feasible In many cases this is much more readily accomplished in one or more model system Occasionally sequence homology searches reveal the existence of related genes in other organisms, where mutants are already known and where the detailed role of the gene in development or maintenance of the organism is understood to some extent and can be explored further Examples where this approach has helped to understand gene function and therefore the disease processes that might be triggered by mutation in the gene include many tumour predispositions such as neurofibromatosis, xeroderma pigmentosum and colon cancers Ataxia telangiectasia will be another example with yeast and *Drosophila possessing mutations in homologous genes One recent example* involving a developmental regulator with mutant homologues in a wide spectrum of phyla is the the PAX6 gene which is mutated in human aniridia, mouse Smalleye, Drosophila eyeless and C. elegans chemotactic and male abnormal mutations The gene is highly conserved, particularly at the DNA-binding paired and homeodomains, throughout these phyla Although we are exploring the role of PAX6 in eye and neural development in man and mouse, it is highly likely that our understanding of target and interacting genes will progress much faster through the study of the more amenable fruitfly and nematode Meanwhile, vertebrate developmental manipulations are carried out in accessible chick embryos

Symposium 7: Cytogenetics

S7.001
Chromosomal Structural Loops and Functional Domains: Sars and Boundary Elements
Ulrich k Laemmli, Keji Zhao, Craig Hart and Reiner Strick
Department of Biochemistry and Molecular Biology, University of Geneva, Switzerland

The long-term goal of this laboratory is to approach a strcutural and biochemical understanding of the chromatin loops of mitotic chromsomes and to address the role of chromatin domains in gene expression *The topological loops of metaphase* chromosomes are thought to be anchored by special highly A + T - rich regions of about 1 kb termed SARSs (scaffold associated regions) Recent evidence strongly support this notion, this evidence and the role of SARs in chromosome banding and gene expression will be reveiwed The first part of the presentation will address in some details the question of whether SARs are CIS elements involved in the dynamic structural transitions of chromosomes such as condensation and decondensation The second part of this talk will focus on the DNA boundary elements (BE) which seperate the chromatin fiber into fucntional domains BE's are proposed to insulate one domain from the enhancer or repressor activities of flanking domains The scs and scs' elements, which flank the Drosophila 87A7 hsp70 heat shock locus, were experimentally identified as BE's by Schedl's group We will report about our progress to characterize proteins termed BEAFs (Boundary Element Associated Factors) which bind to these elements and are implicated in the insulation of chromosomal domains

S7.002

*Transfer of the entire DMD-gene to mammalian cells with the use of a putative mammalian artificial chromosome

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To test the feasibility of shuttling the giant (2 4 Mb) Duchenne muscular dystrophy (DMD) gene in mammalian cells and to study its expression, we have set out to construct an autonomous replicon with Mendelian segregation, i e a mammalian artificial chromosome (MAC) This approach would relieve most of the restrictions of current methods to study mammalian gene expression like the limitation to cDNA and small sized constructs or the need for random integration In previous research, a 2 6 Mb YAC containing the complete DMD gene was constructed from a set of overlapping YACs by meiotic recombination Using mitotic recombination we now have joined the 2 6 Mb DMD-YAC with a 200 kb YAC containing pure alphoid DNA (currently the best candidate for a functional centromere) and we added human telomeric sequences and selectable markers, while replication origins were assumed to be present in the DMD gene Two basic constructs, the DMD-YAC and our putative MAC were introduced into human and mouse cells by spheroplast fusion So far, six of the colonies seem to contain the complete DMD gene, on which expression studies are being performed Some cell lines exhibit extrachromosomal structures that are currently tested for mitotic stability

S7.003

*New tools for genome cartography by fluorescent in situ hybridization

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FISH images obtained with conventional epifluorescence microscopes are always blurred by glare and out of focus light emissions In order to restore high contrast images, a procedure based on a modelling of the optical system in the microscope was developed and used for the processing of images acquired with a cooled CCD camera mounted on a fluorescence microscope This procedure was tested on images of both mouse and human chromosomes stained with DAPI and on images of interphase nuclei hybridized with pairs of cosmid probes This method improves the definition and the sharpness of the DAPI G-banding and thus facilitates and speeds up the identification of chromosomes When performed on images of interphase cell nuclei, this procedure allows the discrimination of fluorescent signals which appear partially overlapping on raw images This significant improvement of spatial resolution is of particular interest for ordering sets of probes on DNA fibers Iterative deconvolution was also applied on images of stretched DNA molecules obtained by molecular combing This method which provides a reproducible stretching permits the visualisation of isolated molecules stretched in a linear fashion and an easy detection of nucleic sequences of 1Kb by FISH

S7.004

*The same molecular mechanism at the maternal meiosis I produces mono- and dicentric 8p duplications.

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We studied 16 cases of 8p duplications, with a karyotype 46,XX or XY,dup(8p), associated with mental retardation, facial dysmorphisms and brain defects We demonstrate that these 8p rearrangements can be either dicentric (6 cases) with the second centromere at the tip of the short arm or monocentric (10 cases) The distal 8p23 region, from D8S349 to the telomere, including the defensin1 locus, is deleted in all the cases The region spanning from D8S252 to D8S265, at the proximal 8p23 region, is present in single copy and the remaining part of abnormal 8 short arm is duplicated in the dicentric cases and partially duplicated in the monocentric ones The distal edge of the duplication always spans up to D8S552 (8p23 1) while its proximal edge includes the centromere in the dicentric cases and varies from case to case in the monocentric ones The analysis of DNA polymorphisms indicates that the rearrangement is consistently of maternal origin In the deleted region only paternal alleles were present in the patient In the duplicated region, besides one paternal allele, some loci showed two different maternal alleles, while others, that were duplicated by FISH analysis, showed only one maternal allele We hypothesise that, at maternal meiosis I, there was abnormal pairing of chromosomes 8 followed by anomalous crossover at the regions delimited by D8S552 and D8S35 and by D8S252 and D8S349, which presumably contain inverted repeated sequences The resulting dicentric chromosome, 8qter-8p23 1(D8S552) 8p23 1 (D8S35)-8qter, due to the presence of two centromeres, breaks at anaphase I generating an inverted duplicated 8p, dicentric if the breakage occurs at the centromere or monocentric if it occurs between centromeres

S7.005

Chromosomal abnormalities in human preimplantation embryos

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We have used dual fluorescent in situ hybridisation (FISH) with probes for chromosomes X and Y to sex embryos for preimplantation diagnosis of X-linked disease Detection of chromosome abnormalities has lead to further work on embryos donated for research purposes with probes for chromosomes X and Y or autosomes 1 and 17 Four types of chromosome arrangements have been detected, (i) normal embryos, all nuclei normal for the chromosomes examined (ii) abnormal embryos, all nuclei uniformly abnormal, e g XO, XXX, trisomy 21, etc (iii) diploid mosaics, the majority of nuclei diploid with a few nuclei aneuploid, polyploid or haploid, and (iv) chaotic, where all the nuclei show different chromosome complements We now routinely use triple colour FISH for the preimplantation diagnosis of X-linked disease to reduce the chance of transferring abnormal embryos To examine the level of chromosome abnormalities in embryos from the 2-cell to blastocyst stage, we have embarked on a study using 5 probes,.X, Y, 1, 9 and 18 The age of the patient and the morphology of the embryos generated have been considered The results of this study will be discussed

S7.006

Chorionic villi in prenatal diagnosis : The UK collaborative study update.

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Following the highly successful prospective data collection on diagnostic chorionic villus samples undertaken by the ACC working party on prenatal diagnosis for the years 1987-1989, a further retrospective period of 4 years has been added to the data (1990-1993) thus giving a cumulative experience of 7 consecutive years data A total of 28 laboratories provided data for this study with the majority supplying data for all the years covered From an initial collection of 7,500 diagnostic cases we now report the findings on well over 16,000 cases Data collected included gestational age, reason for referral, karyotype (direct and/or long term culture) cell ratio (for cases of mosaicism), karyotype of any prenatal follow-up, pregnancy outcome, and karyotype outcome This data allows us to determine the predictive value of the various mosaic and non-mosaic chromosome abnormalities encountered The success rates remained high (>98%) and the cumulative data adds support to the conclusions gained from the initial survey, although now the new data includes the finding of false positive diagnoses not encountered in the initial data

Symposium 8: Expressed sequences

S8.001
IMAGE: Integrated Molecular Analysis of the Human Genome and its Expression

Auffray, Charles

S8.002
Construction of a dense "exon" map (1 in every 17 kb) of the Down syndrome critical region of chromosome 21.

Blouin, Jean-Louis, Chen, HM, Duriaux Sail, G, Rossier, C, Guidi, S, Mittaz, L, Scott, HS, Antonarakis SE
Division of Medical Genetics, University of Geneva Medical School and Cantonal Hospital, Geneva, Switzerland

Molecular analyses of partial 21 trisomies have permitted the identification of a Down syndrome critical region (DSCR) in 21q22 2 between markers D21S17 and ETS2 Well developed physical and genetic maps of chromosome 21 provide now the tools for the creation of a genic map Genes that map in the DSCR are important in the study of Down syndrome because dosage imbalance may modify the function of multimeric enzymes and/or other multisubunit proteins An initial map containing 102 exons in the DSCR has been constructed (Lucente et al Hum Mol Genet 4 1305, 95) We continue to use exon trapping from cosmids of the DSCR to identify more coding sequences and saturate the transcription map of this region After elimination of 26% clones with identities to previously reported exons, we found (and map to specific cosmids) an additional 49 potential exons Three exons were identical to known DSCR genes, 6 recognized ESTs (search of 15nov95), and 3 were identical to known human genes not mapped in the DSCR In combination with the data of Lucente et al there is now on average 1 exon per 17 kb of DNA in the DSCR This exon density may be sufficient to identify the majority of genes in this chromosomal region Study of the function of these genes will be important for understanding the pathophysiology of Down syndrome, the most common human aneuploidy

S8.003
A gene from the critical region for X-linked juvenile retinoschisis on Xp22.3 is homologous to the drosophila retinal degeneration C(rdgc) gene.

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Our group is involved in the construction of a transcription map of the human Xp22 region To reach this goal we have constructed a highly annotated physical map using 324 overlapping yeast artificial chromosomes (YACs) which were assembled in a single contig spanning approximately 35 Mb YAC clones have been converted into groups of cosmids which are used for exon trapping and cDNA selection experiments An exon amplification product from YAC clone 939H7 spanning the critical region for X-linked juvenile retinoschisis revealed significant homology to the Drosophila retinal degeneration C(rdgc) gene The rdgc gene encodes a serine/threonine protein phosphatase protein and is required in drosophila to prevent light-induced retinal degeneration We isolated the full-length cDNA for the human gene (HRDGC) which contains a coding region of 1962 bp The overall percentage of similarity between the drosophila and the human protein is 61 7% These data identify HRDGC as a strong candidate for retinoschisis Characterization of the genomic structure and mutation analysis in patients with retinoschisis are in progress

S8.004
Identification and mapping of human ESTs homologous to Drosophila mutant genes: a source of candidate genes for human inherited diseases.

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Expressed Sequence Tags (ESTs) represent a valuable tool for gene identification Currently, over 300,000 ESTs are present in the public databases In order to quickly identify candidate genes for human diseases, we searched dbEST for novel human sequences sharing significant homology with genes already known to cause mutant phenotypes in a well-characterized organism, such as Drosophila We have identified at least 65 different human cDNAs showing homology with Drosophila genes causing aberrant phenotypes in the fly To test the hypothesis that these genes are involved in human inherited disorders, we are mapping these ESTs, using both fluorescence in situ hybridization (FISH) and PCR analysis on a panel of radiation hybrids created at the Stanford Human Genome Mapping Center Ten of these cDNAs were mapped using this combined approach Two of them turned out to be located in chromosomal regions harboring diseases loci whose phenotype is similar to that observed in the corresponding Drosophila mutant Mapping of the remaining ESTs is now in progress Further characterization (full-length sequencing, expression analysis and search for mutations) will be performed on the ESTs mapping in the vicinity of human disease loci

S8.005
*Construction of a transcription map of human chromosome 20p12.

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INSERM U347 affiliée au CNRS, Le Kremlin-Bicêtre, France

Alagille Syndrome (AGS, MIM 118450) is associated with human chromosome band 20p12 AGS is characterised by five major features chronic cholestasis, heart, vertebral and eye defects along with a peculiar facies It is an autosomal dominant trait with quasi-complete penetrance To clone the AGS candidate gene(s), a YAC contig spanning 9 Mb in 20p12 was constructed with sixty-seven YACs A number of markers including new STSs, microsatellites, RFLPs and genes were mapped A NotI and AscI genomic restriction map was generated The AGS candidate region was refined to less than 2 2 Mb and shown to contain 5 putative CpG islands (Pollet et al , Genomics 27 467-474, 1995) The transcription map in 20p12 is currently established using various strategies First, ESTs already assigned to chromosome 20 are localised in the contig Second, several cDNA libraries are screened by cDNA selection with the YACs covering the AGS locus Third, the cloning of putative CpG islands is undertaken Several genes have already been identified in this gene-poor region and their implication in AGS will be tested Supported in part by grants from GREG

S8.006
*Integrated physical and transcriptional map of the Down syndrome Chromosome Region (DCR) between D21S55 and ERG.

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We previously constructed a YAC contig and a high resolution restriction map of the distal DCR between D21S55 and ETS2 This map highlights several CpG islands

and allows to analyze the CG content of this region The study of the patient identifying the distal DCR boundary allowed to reduce the DCR to D21S55-ERG The YAC contig between D21S55-ERG was used as a start point to isolate the coding sequences of this region A cosmid contig was constructed by screening the ICRF chr21 cosmid library with the YACs The cosmids could be organized in 7 contigs with a EcoRI restriction map which cover 85% of the 750kb The 15% remaining are progressively covered by screening P1 and PAC libraries The cosmids were used for exon trapping experiments and cDNA selection cDNAs of three different tissues were used in combination with pooled cosmids The selected cDNAs were subcloned and spotted arrayed on membranes (4 clones/kb cosmid) This library screened with the EcoRI fragments of cosmids and exons or "conserved" sequences will lead to the identification of the transcriptional units, after their verification as unique sequences of chromosome 21 and their sequencing We already identified new expressed sequences of this chromosome with no homology with known genes References Rahmani et al PNAS 86, 5658, 1989, Gosset et al Mamm Genome 6, 127, 1995, Crété et al Eur J Hum Genet 1, 51, 1993, Korn et al Hum Mol Genet 1, 235, 1992

Symposium 9: Molecular pathology

S9.001

*Gene conversions involving the SMN gene in the spinal muscular atrophy (SMA) region at 5q13

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Recently the survival motor neuron (SMN) gene has been described as a determining gene for spinal muscular atrophy (SMA) The SMN gene, at 5q13, has a closely flanking, nearly identical copy (cBCD541) with a more centromeric location Gene and copy gene can be distinguished from each other by sequence differences in exons 7 and 8 DNA analysis of SMA patients reveals homozygous deletions of at least exons 7 and 8 of the SMN gene in the majority of all cases A minority of the patients shows absence of SMN exon 7, but retention of exon 8 We provide evidence that apparent gene conversion events between SMN and the centromeric copy gene may have occurred in all these cases In all 13 such cases we have analyzed we could demonstrate the replacement of the deleted part of the SMN gene by homologous sequences of the copy gene Similar events were also observed among control individuals These results indicate that in the SMA region gene conversions are far from rare and partly explain the frequently observed deletions in SMA patients

S9.002

*A recombination "hot spot" responsible for the inherited peripheral neuropathies CMT1A and HNPP is located near a mariner transposon-like element

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The CMT1A duplication and HNPP deletion are the reciprocal products of an unequal crossing-over event between misaligned flanking CMT1A-REP repeats on chromosome 17p The molecular etiology of this apparently homologous recombination event was examined by refining the cross-over region and determining the nucleotide sequence Through the detection of novel junction fragments from the recombinant CMT1A-REPs in 104 CMT1A duplication and 19 HNPP deletion patients a 1 7-Kb recombination "hot spot" within the ~30-Kb CMT1A-REPs was identified The region encompassing this "hot spot" is >98% identical between CMT1A-REPs implying that sequence identity is not likely the sole factor involved in promoting cross-over events Sequence analysis revealed a mariner transposon-like element (MITE) near the "hot spot" which we hypothesize could mediate strand exchange events via cleavage by a transposase at or near the 3' end of the element during male gametogenesis

S9.003

Loss of heterozygosity in Tuberous Sclerosis hamartomas

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We have previously described in tuberous sclerosis (TSC) hamartomas the phenomenon of loss of heterozygosity (LOH) for DNA markers in the region of both the TSC2 gene on chromosome 16p13 3, and the TSC1 gene on 9q34 We now describe the spectrum of LOH in 45 TSC hamartomas from 31 cases of TSC DNA was extracted from normal tissue and from paraffin-embedded hamartoma tissue, and analysed for 10 markers spanning the TSC1 locus, and 8 markers spanning the TSC2 locus The markers on chromosome 9 were ASS, D9S64, D9S149, D9S150, ABO, DBH, D9S122, D9S298, D9S114, D9S67, and on chromosome 16 were D16S291, D16S665, KG8, a coding EcoRV polymorphism in the TSC2 gene, D16S525, D16S309, D16S85 and HBAP1 Eighteen of 45 hamartomas showed LOH (40%), 13 for markers around TSC2, and 5 for markers in the vicinity of TSC1 No hamartoma showed LOH for markers around both loci Seven of 15 renal angiomyolipomas showed LOH and 5 of 10 giant cell astrocytomas showed LOH LOH also occurred in a cardiac rhabdomyoma, a cortical tuber, two nail fibromas, a renal cell carcinoma, and a shagreen patch All the areas of LOH on chromosome 9 were large, extending beyond the markers D9S149 and D9S114 which flank TSC1 LOH is a common finding in a wide range of TSC hamartomas These data support the hypothesis that both the TSC genes act as tumour suppressors, and that many of the manifestations of TSC are from 'second hit' somatic mutations inactivating the remaining normal copy of the TSC gene

S9.004

The type IV collagen a4 (COL4A4) gene is mutated in familial benign hematuria

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Autosomal dominant familial benign hematuria is characterized by persistent microhematuria and normal renal function Histologically, thinning of the glomerular basement membrane is visible This resembles early stages of the severe renal disorder Alport syndrome The predominant form of Alport syndrome is caused by mutations in the X-linked COL4A5 gene In 1994, we reported the first mutations in the COL4A3 and COL4A4 genes in autosomal recessive Alport syndrome Linkage to the same COL4A3/COL4A4 locus at 2q35-37 has been demonstrated for the autosomal dominant form as well With markers from the COL4A3/COL4A4 genes we were able to demonstrate linkage to familial benign hematuria in a large Dutch pedigree (Zmax=3 58 at Q=0 0) Subsequently, a glycine to glutamic acid substitution was identified in the collagenous region of the COL4A4 gene Homozygozity for a similar COL4A4 mutation was found in a patient with Alport syndrome, which suggests that familial benign hematuria patients can be manifesting carriers of autosomal recessive Alport syndrome Furthermore, similar to other collagen disorders, the clinical expression of type IV collagen mutations varies widely, ranging from very mild familial benign hematuria to severe Alport syndrome with complete renal failure at juvenile age

S9.005
Identification, comparative mapping and developmental studies of the mouse homologue of the SMN gene.

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Spinal muscular atrophy (SMA) is a common recessive autosomal disease, characterized by degeneration of spinal cord motor neurons, resulting in muscular atrophy and paralysis A SMA-determining gene called SMN (Survival Motor Neuron), has been recently isolated by our group, in the telomeric element of a large inverted duplication, and has been found to be lacking in 98,6% of patients (226/229) and patients retaining this gene carried intragenic mutations Cross-species conservation of human SMN gene with rodents has been shown and served to isolate the mouse SMN gene Screening of a mouse fetal cDNA library using human SMN cDNA as probe allowed the isolation of 2 overlapping mouse cDNA clones Sequence analysis of the clones revealed an 864 bp open reading frame (ORF, Figure 1) The ORF encodes a putative protein of 288 amino acids (Figure 1) with an homology of 83% with human SMN aminoacid sequence (Figure 2) The genomic localisation and the expression studies of mouse SMN gene during development and postnatal period will be investigated These approaches will contribute to the understanding of gene function In addition, the mouse SMN cDNA cloning will allow the identification and the characterization of the mouse SMN gene Homologous recombination with disrupted mouse SMN gene and overexpression of the mouse gene by transgenic experiments will be undertaken to obtain a mouse model of the human disease

S9.006
Mutation in the iron responsive element of L ferritin mRNA in a family with dominant cataract and hyperferritinemia.

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The synthesis of ferritin is regulated at the translational level by iron through interaction between the "iron regulatory protein" (IRP) and "iron responsive element" (IRE), a conserved nucleotide motif present in the 5' non coding region of all ferritin mRNAs This region forms a stem-loop structure and when there is a limited supply of iron to the cell, the IRP is bound to IRE and represses ferritin synthesis Here we report the identification of a single point mutation in the IRE of the L ferritin mRNA in members of a three generation family affected by dominantly inherited cataract associated with an elevated level of serum L ferritin This mutation consists in a A to G change in the CAGUGU highly conserved motif which constitutes the loop of the IRE and mediates the high affinity interaction with IRP This mutation abolishes the binding of IRP in vitro and leads to a high constitutive, poorly regulated L ferritin synthesis in culture lymphoblastoid cells established from affected patients This is the first mutation affecting the IRP-IRE interaction and the iron mediated regulation of ferritin synthesis Dysregulation of ferritin synthesis may be a new mechanism involved in the pathogenesis of cataract

S9.007
X-linked inheritance of skewed X chromosome inactivation in a large family with an X-linked recessive deafness syndrome.

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A new X-linked recessive deafness syndrome was recently reported and mapped to Xq22 (Mohr-Tranebjaerg syndrome, MTS) In addition to deafness, the patients had visual impairment, dystonia, fractures, and mental deterioration The female carriers did not have any significant manifestations of the syndrome We therefore examined X chromosome inactivation in 8 obligate and 12 possible carriers using a PCR analysis of the methylation dependent amplication of the polymorphic triplet repeat at the androgen receptor locus Seven of 8 obligate carriers and 1 of 5 carriers by linkage analysis had an extremely skewed pattern in blood DNA not found in 30 normal females The X inactivation pattern in fibroblast DNA from two of the carriers with the extremely skewed pattern was also skewed, but to a lesser degree than in blood DNA One obligate carrier had a random X inactivation pattern both in blood and fibroblast DNA A selection mechanism for the skewed pattern therefore does not seem likely It is concluded that the extremely skewed X inactivation in 8 females of three generations in this family is most probably caused by an X-linked gene which influences skewing of X-chromosome inactivation

Symposium 10: Molecular dysmorphology and developmental genetics

S10.001
Molecular Defects in Malformation Syndromes

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Recent advances in the mapping and isolation of genes responsible for birth defects will be reviewed Groups of disorders where rapid progess has been made include bone dysplasias, craniosynostoses and craniofacial sydnromes

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S10.002
*Cloning of cDNAs for the human SIM1 and SIM2 genes; molecular tools to study the possible implications of human SIM2 in the Down syndrome phenotype.

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Exon trapping was used to capture portions of chromosome 21 genes Among the trapped sequences four exons had strong homology (72%- 96%) with the 5'-half of the drosophila sim (single-minded) gene (Nat Genet 10 9-10, 1995) These exons were used to screen a fetal kidney cDNA library since Northern blot indicated expression in this tissue Sequence analysis of positiv clones (14 of 10^6 pfu) showed that in humans, as in mice (Fan C M and Tessier-Lavigne M unpublished), there are at least two SIM genes, hSIM1 and hSIM2 The amino acid homology between hSIM1 and hSIM2 is almost complete (90%) from aa 1 to 370 of the predicted polypeptide containing bHLH, PAS1 and PAS2 domains Partial sequence at the COOH-terminal end of the predicted hSIM1 and hSIM2 polypeptides (from aa 370) revealed no homology between them or with drosophila sim, however hSIM1 and hSIM2 are very homologous to mouse sim1 and sim2 respectively hSIM2 maps on chromosome 21 between markers CBR and D21S167 in the Down syndrome critical region The Drosophila sim is a regulator for CNS development in drosophila (Cell 67 1157-1167, 1991), this suggests that hSIM2, if present in three copies, may be a candidate gene for some Down syndrome phenotypes We are now, in collaboration with JD Gearhart of Johns Hopkins in Baltimore, constructing a sim2 transgenic mouse to study its overexpression, and a mouse knockout to study the effects of absence of sim2 protein in mouse development

S10.003
*Paternal origin of new mutations in Apert syndrome.

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Apert syndrome is a dominantly inherited disorder characterised by craniosynostosis and syndactyly, with a birth prevalence of ~ 1 in 65,000 Over 98% of cases arise sporadically The mutations responsible are two specific nucleotide substitutions, both C-G transversions, in the fibroblast growth factor receptor 2 gene This implies a germline transversion rate at these sites that is the highest known in the human genome Our aim was to determine the parental origin of the new mutations using a novel application of the amplification refractory mutation system (ARMS) technique Two polymorphic base substitutions were identified by single strand conformation polymorphism analysis of the introns flanking the mutations ARMS primers were designed to amplify selectively one or other variant The phase of the Apert mutations, identified by diagnostic changes in restriction sites, could thus be ascertained with respect to the polymorphic bases In informative families (i e child heterozygous and one or both parents homozygous for at least one polymorphism), this allowed parental assignment of the mutation To date all 46 informative families analysed have shown a paternal origin of mutation We discuss these findings in relation to the paternal age effect previously postulated for Apert syndrome

S10.004
Homeobox gene EMX2 mutations in schizencephaly

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Schizencephaly is a rare cell migration anomaly that causes a full-thickness cleft within the cerebral hemispheres The clefts can be monolateral or bilateral and their size range from extremely narrow (fused lips) to wide separation of the walls (open lips) The walls of the schizencephalic clefts are formed by polymicrogyric cortex and sometime CSF replace the absent cortex The most common clinical signs are neurological and intellectual delay, seizures, motor dysfunction, speech impairment, visual and hypothalamic dysfunction We report that eight out of seven severely affected patients have different de novo heterozygous mutations in the EMX2 homebox gene, the human homologous of the murine Emx2 which is expressed in the neuroblasts of the developing cerebral cortex One of these mutations is a frameshift in the homeodomain, two are 3' splice site mutations that alter the 3' splice site of the first intron upstream from the homeodomain, all probably give a loss of function of the EMX2 gene A fourth mutation involves a deletion of two bases in the second intron within the homeobox The involvement of EMX2 in the formation of the cerebral cortex is supported by the presence of at least three clear detrimental mutations in schizencephalic patients

S10.005
Maternal uniparental disomy of chromosome 7 and Silver-Russell syndrome

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From 1970 the combined term Silver-Russell syndrome (SRS) has been used for individuals of low birthweight and poor postnatal growth, often associated with specific facial dysmorphism, asymmetry, cafe au lait patches and marked fifth finger clinodactyly Most cases are sporadic, although families with apparent dominant, recessive and X-linked forms have been reported A similar phenotype has been described in association with various chromosomal abnormalities Several lines of evidence have implicated maternal uniparental disomy for chromosome 7 as a possible mechanism for this growth disorder, and recently Kotzot described this finding in 3 out of 25 individuals with this diagnosis (1) Using a selection of highly polymorphic PCR primers and VNTRs from chromosome 7 we have analysed 40 SRS families for uniparental disomy All the families have been seen by the same clinician We confirm the presence of maternal uniparental disomy of human chromosome 7 in ~10% of cases This implies that one or more genes on chromosome 7 are imprinted such that only the paternal copy is expressed Lack of the paternal expression leads to the SRS phenotype 1 Kotzot D et al (1995) Human Molecular Genetics 4 4 583-587

Symposium 11: Cancer genetics

S11.001
The molecular epidemiology of breast and ovarian cancer and its clinical application.

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Two genes which predisposed to breast and ovarian cancer have recently been identified BRCA1 and BRCA2 Mutations in these genes confer a lifetime risk of breast cancer of around 80%, and lower risk of epithelial ovarian cancer Epidemiological estimates suggest that about 5% of all breast and ovarian cancer may be attributable to mutations in these genes In the longer term these discoveries may lead to the design of better methods of prevention and treatment, but the immediate application is to the genetic predition of risk for women with a family history When the genetics are clear-cut and there is a well-defined clinical

decision to be made (eg oophorectomy), such genetic testing is almost certainly helpful However, because breast cancer and ovarioan cancer are common, there are a large number of women in the population who have some degree of family history and who may be anxious as a result The role of genetic testing for these women is much less clear, especially since the most part there is at present no clear evidencethat benefits will follow in the form of early diagnosis or prevention A concerted national strategy is needed to deal with this problem

S11.002
Intestinal tumours in the Apc 1638N mouse: aspirin not protective and resistant starch increases small bowel tumours.

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Epidemiological studies suggest that aspirin and resistant starch protect against colon cancer The CAPP study, a randomised control trial involving 37 European registries, is testing these treatments in carriers of familial adenomatous polyposis with 96 recruits to date A parallel study has involved six months treatment of F9 Apc 1638N mice in a C57B1/6 background After genotyping groups of 20 mice were allocated to a high fat, high sucrose "western" diet alone, or supplemented with resistant starch (RS), aspirin, or RS with aspirin A fifth group received a conventional chow diet After six months, almost all animals had developed tumours in the small bowel Aspirin was not protective while the RS group developed a highly significant excess of tumours Like other "mouse models" of FAP, the Apc 1638N develops very few large bowel tumours emphasising the contrast between mouse and man While these surprising results may not apply to humans, they emphasise the need for randomised controlled trials prior to recommendation of new therapeutic interventions

S11.003
A p16 mutation in a family with multiple cancers

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The cyclin-dependent kinase inhibitor p16 (CDKN2) is frequently mutated or deleted in a wide variety of tumours However, germline p16 mutations have been reported in families with melanoma and pancreatic carcinomas only We analysed 80 families with numerous cases of multiple cancers A previously-described Met45Ile mutation was detected in the constitutional DNA of an individual who developed two melanomas at age 41 and a third at 49 The proband's mother was diagnosed with primaries of the breast, colon and mouth at ages 73, 77 and 79, respectively There were also cases of bladder and gall bladder cancer in other relatives We are analysing DNA from normal tissue and tumours from relatives to determine if the Met45Ile mutation is associated with other cancers in the family The Met45Ile mutation segregated with affected individuals in two apparently unrelated Australian melanoma kindreds with distinct haplotypes, suggesting that the mutation has arisen at least twice To further investigate the possibility that the Met45Ile mutation has occurred independently and corresponds to a mutational hotspot, we are haplotyping this family using dinucleotide repeat markers at the p16 locus Our results raise the possibility that inherited p16 mutations may predispose to a wider range of cancers than previously thought

S11.004
*Somatic inactivation of the von Hippel-Lindau (VHL) tumour suppressor gene in familial and sporadic renal cell carcinoma

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Germline mutations in the VHL gene predispose to early onset and multifocal clear cell renal carcinoma In addition we have demonstrated somatic VHL mutations and allele loss in 42% of sporadic clear renal carcinoma (Hum Molec Genet 1994,3 2169-2173) Silencing of VHL gene expression by de novo methylation has also been reported in sporadic renal carcinoma cell lines To further investigate the mechanisms of tumourigenesis in VHL disease we have analysed tumours from VHL patients and sporadic primary renal carcinomas (RCC) for somatic inactivation of the VHL gene Molecular genetic analysis of 45 VHL tumours (28 RCCs, 9 haemangioblastomas, 5 paheochromocytomas, 3 pancreatic tumours) demonstrated loss of the wild type VHL allele in 51% Allele loss was detected in all tumour types 18 tumours without VHL allele loss were analysed for microdeletions/microinsertions and hypermethylation No somatic intragenic mutations were found, but VHL hypermethylation was detected in 4 haemangioblastomas and 2 RCCs Molecular analysis of 38 sporadic RCC without VHL gene mutations demonstrated hypermethylation of the VHL gene in 9 of 27 (33%) clear cell RCC and 0 of 11 nonclear cell RCC All informative tumours with VHL gene hypermethylation also had VHL allele loss These studies suggest that de novo methylation of the VHL tumour suppressor gene is involved in the pathogenesis of familial and sporadic RCCs and also occurs in other VHL tumour types

S11.005
*Sensitive and efficient detection of MSH2 and MLH1 mutations by two-dimensional DNA electrophoresis in sporadic colon carcinomas

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Microsatellite instability is frequently seen in both sporadic and hereditary forms of colorectal cancer The DNA mismatch repair genes MSH2 and MLH1 have been shown to account for a major share of HNPCC In order to assess the role of MSH2 and MLH1 in sporadic, RER+, colorectoral carcinomas we applied two-dimensional (2-D) DNA electrophoresis, including separation by DGGE, in which we separate all multiplex PCR-amplified exons on the basis of both size and base pair sequence under one set of experimental conditions Exons showing a spot position different from normal were sequenced Nine different presumably causative mutations and five polymorphisms have been detected, three in MSH2 and six in MLH1 Seven were found to have somatic mutations Two had germline mutations These mutations together account for 45% (9/20) of unselected RER+ tumours Our results show, first, that a role of MSH2 and MLH1 also in sporadic tumours is much more frequent than previously assessed, second, that mutations in the MLH1 gene are responsible for 2/3 of these cases collected in Finland As we found a much more frequent involvement of MSH2 and MLH1 in sporadic tumours than previously described, we conclude that 2-D DNA electrophoresis is a sensitive and efficient method for mutation screening

Symposium 12: Non-Mendelian disorders

S12.001
The molecular genetics of type 1 diabetes
Bain, SC

Whole genome linkage analysis of type 1 diabetes using affected sibling pairs and semi-automated genotyping and data capture has facilitated the genetic dissection of this common autoimmune disease
A major proportion of the familial clustering of type 1 diabetes can be accounted for by sharing of alleles at susceptibility loci in the major histocompatibility complex (termed HLA in humans) on chromosome 6 (*IDDM1*) and at least 11 other loci on nine chromosomes Primary aetiological components of *IDDM1* and *IDDM2* have been identified, these are the HLA-DQB1 and DRB1 class II immune response genes (*IDDM1*) and the insulin gene region variable number tandem repeat sequence (*IDDM2*) Identification of the other loci will involve linkage disequilibrium mapping and the sequencing of candidate genes in regions of linkage

S12.002
The genetics of schizophrenia
McGuffin, Peter

S12.003
Endothelin-signaling pathway mutations in Hirschsprung's disease.
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Hirschsprung's disease (HSCR) is a frequent congenital malformation related to abnormal migration of neural crest cells towards the hindgut Significant proportions of sporadic (15-20%) and familial (50%) HSCR cases have been ascribed to mutations of the RET proto-oncogene However, several lines of evidence supported the involvement of other suceptibility genes, including i) segregation analyses, ii) chromosomal anomalies (trisomy 21, 13q deletions), iii) RET exclusion in some HSCR families, and iv) the existence of several mendelien models for megacolon in mice Recently, mutations of the endothelin B receptor (EDNRB) and the endothelin 3 (EDN3) genes were shown to account for megacolon and pigmentary anomalies in the piebald-lethal and lethal spotting mouse mutants respectively Here we report on mutations of the EDNRB and EDN3 genes in a series of 160 HSCR probands Interestingly, homozygous mutations of either EDNRB or EDN3 seem to predispose to features of the Waardenburg syndrome in addition to HSCR Conversely, heterozygous EDNRB mutations were found in isolated HSCR patients These data suggest that EDNRB mutations are dosage sensitive and give support to the role of the endothelin-signaling pathway in the development of neural crest-derived enteric neurons

S12.004
An empirical approach to distinguish true and false positive lodscore peaks
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Lodscore methods have been used to map complex traits by using an appropriate one locus model However, the interpretation of weakly positive lodscores become difficult because statistical methods capable of distinguishing true from false positives are not available We propose to use the length of positive region in addition to the original lodscore statistic to investigate whether there is a difference between true and false lodscore peaks We simulated an oligogenic trait and performed a genome search with markers which were 1cM apart on all chromosomes We performed two point analyses using one locus model for all markers, recorded all lodscores above 1 We defined the number of markers included in a ositive regions as length Our results show that the difference in length of a true or false positive region is highly significant A new test statistic based on maximum lodscore and length has been developed based on logistic analysis This statistic can be used to distinguish true positives from false positives in a real dataset

S12.005
Transient neonatal diabetes mellitus- a cytogenetic and molecular study.
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Transient neonatal diabetes mellitus (TNDM) is a rare (1/400,000 UK births) but well characterised form of diabetes which presents within 6 weeks of life with intrauterine growth retardation, dehydration and mild ketosis Recovery is usually complete by 1 year of age In a preliminary study[1] we showed 2 of 3 patients with TNDM to have paternal uniparental isodisomy of chromosome 6 Three other children with neonatal diabetes have now been shown to have paternal UPD Ten cases have so far been ascertained in the UK The median duration of exogenous insulin requirement was 3 months (range 1-8 months) Islet cell antibodies were negative and DQ alleles were not consistent with those usually seen in type 1 diabetes Three patients have developed early onset type 2 diabetes in adult life at 13, 17 and 20 years requiring intermittent insulin or oral therapy Using multiple polymorphic microsatellite repeat markers for chromosome 6 wo cases were shown to have paternal uniparental isodisomy and eight cases have biparental inheritance of chromosome 6 Routine cytogenetic analysis has failed to reveal any structural rearrangements on chromosome 6 in this group A search for molecular rearrangements is underway 1Temple IK et al Nat Genet 9,110-112,1995

Symposium 13: Neurogenetics

S13.001
Genes with trinucleotide expansion and their proteins
Mandel, Jean Louis

S13.002
Molecular genetic dissection of Alzheimer dementia
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Molecular genetic studies in Alzheimer's disease (AD) families with polymorphic DNA markers have identified 4 genetic loci predisposing to familial AD on chromosomes 1, 14, 19 and 21 The chromosomes 1 (AD4), 14 (AD3), and 21 (AD1) are responsible for familial early-onset (<65 years) AD (estimated 10% of all AD cases), while the chromosome 19 (AD2) locus was identified in late-onset (>65 years) AD families The AD1 locus was the first to be identified and coincided with the location of a candidate gene i e the gene coding for the amyloid precursor protein (APP) located at 21q21 2 Mutations were found in APP coding in part for βA4 amyloid, the major constituent of senile plaques in AD brains However, APP mutations are rare genetic causes of AD since they are detected only in familial early-onset AD cases at a frequency of at most 5% The 2 remaining genes for early-onset AD, presenilin-1 (PS-1) on chromosome 14 (AD3) and presenilin-2 on

chromosome 1 (AD4), were identified only recently Preliminary data indicates that PS-1 gene harbours an estimated 70% of the disease-causing mutations making it the major gene for familial early-onset AD The PS proteins share a very high homology and are predicted to be integral membrane proteins with at least 7 transmembrane domains The PS mutations are scattered over the protein indicating that almost all regions of the protein are functionally important However, 2 clusters of mutations have been identified in respectively transmembrane domain 2 and hydrophilic loop 6 The high sequence homology between the 2 PS proteins suggests that they have a similar biological function However, a mutation in one PS gene inevitably leads to AD pathology The E4 allele of the apolipoprotein E gene (APOE4), located in the AD2 candidate region, was identified as a major risk factor for AD The exact role of the PS proteins and APOE in AD pathology is not yet understood, however, preliminary data indicates that they may have a role in the βA4 amyloid deposition in AD brains

S13.003

Gene dosage effect at the Dentatorubral-pallidoluysian atrophy gene locus.

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DRPLA is an autosomal dominant neurodegenerative disorder the genetic defect of which has been identified as an unstable expansion of a (CAG)n repeat within exon 5 of the gene (CTG-B37) Recently homozygosity for the CAG expansion in Machado-Joseph disease has been reported to result in an additive effect on the age of onset This contrasts with previous reports suggesting that homozygotes for Huntington's disease are no more severely affected than heterozygotes We now report evidence of a gene dosage effect at the DRPLA locus A female child who had psychomotor retardation with neurological deterioration including recurrent vomiting (but no myoclonus) from age 2 years until death at 4 years was found posthumously to be homozygous for DRPLA with 64 and 72 repeats in all tissues tested Post-mortem showed degeneration of gracile and cuneate nuclei and dentate hila Her mother and father at age 30 years and apparently asymptomatic at the time of diagnosis in their child were found to carry repeats of 63 and 65 respectively A paternal relative who presented with symptoms at age 42 years has subsequently been found to carry 63 repeats The extended paternal rural family were well known to clinicians in the region because of their neurological problems The origin of the homozygosity in the family is believed to be previously unrecognised consanguinity Given the observed general inverse correlation of repeat size with severity and the proposed toxic consequence of the mutation perhaps it is not surprising to observe such a gene dosage effect

S13.004

Cloning of the XNP gene involved in the ATRX and related MR syndromes.

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During the construction of an extensive transcriptional map of the Xcen-q21 region we isolated a new gene, called XNP, which encodes a putative nuclear protein belonging to the SNF2/SWI2 helicase family This gene, which extends over 350kb, contains more than 30 exons and is widely expressed Preliminary results suggested that its function might be correlated to cell division and differentiation The combination of its strong expression in the developing brain and its localisation in Xq13 3 strengthened our idea that XNP could be involved in syndromic MRs assigned to this part of the X chromosome A collaboration with the group of D Higgs (Oxford, UK) has recently demonstrated that XNP mutations are responsible for the X-linked a-thalassemia/mental retardation syndrome (ATR-X,), a severe mental retardation condition with dysmorphic features Moreover, exploration of additional families with MR linked to this region revealed that the XNP gene is also associated with MR without any signs of a-thalassemia In addition, we report on a point mutation in the XNP gene co-segregating with Juberg-Marsidi syndrome in a large family These findings suggest that the XNP gene is probably one of the major MR genes in the proximal long arm of the human X chromosome

S13.005

Isolation and analysis of a novel family of genes (presenilins) bearing mutations in Familial Alzheimer's disease.

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Alzheimer's disease is a common degenerative disorder of the central nervous system We identified a novel genes for Familial Alzheimer's disease (FAD) First, after initial mapping of the FAD3 locus (14q24 3), we investigated additional genetic markers and constructed a YAC and cosmid physical contig overlapping this locus To isolate expressed sequences we used direct cDNA selection, exon trapping and 5'- gene region search strategies More than 900 cDNA sequences were recovered from this region Transcripts of these genes were isolated from tissues or cell lines of affected members of linked pedigrees and than were screened for mutations These studies led to the discovery of different missense mutations in a novel gene S182 (PS-1) (Nature, 375, 754-760, 1995) By additional screening of cDNA libraries we found a homologous gene E5-1 (PS-2) which we mapped on chromosome 1 Analysis of PS-2 gene led to the discovery of 2 mutations in affected members of FAD pedigrees (Nature, 376, 775-778, 1995) Both of these genes PS-1 and PS-2 are predicted to be integral membrane proteins with at least seven similar membrane-spanning domains and differences in a large exposed hydrophilic loop We deduced the complete structure and expression of PS-1 gene (submitted) The PS-1 promoter resembles the promoters of "housekeeping" genes The study revealed 13 exons in PS-1 gene and alternative spliced exons in both untranslated and translated regions Analysis of PS-2 suggested a similar organization, but differential expression in some tissues The transcripts of both genes PS-1 and PS-2 are alternatively spliced in the same region which affects the size and structure of a large hydrophilic loop domain Sequences surrounding this loop domain are also the site of a preponderance of FAD related mutations These observations and obvious similarity to C elegans sel-12 gene imply the functional role of presenilins

Symposium 14: Gene identification

S14.001

Cloning of PWP2, a candidate gene for progressive myoclonus epilepsy (EPM1), that encodes a member of the WD-repeats protein family.

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Progressive myoclonus epilepsy (EPM1) is an autosomal recessive disorder, characterised by severe, stimulus-sensitive myoclonus and tonic-clonic seizures Linkage analyses mainly in Finnish families have mapped the EPM1 locus to within 0 3cM from PFKL in chromosome 21q22 3 In order to identify transcriptional units from the region MX1-D21S112 near the 21q telomere, we have used exon trapping from appropriate cosmids, P1s, and PACs From the 53 different potential exons identified, with either no homology to known genes or homology to ESTs, 27 are candidates for the EPM1 gene based on their position on the physical map The predicted polypeptides of 4 exons showed homology with PWP2, a recently

identified yeast protein with unknown function We have isolated the cDNA of the human PWP2 gene, which encodes a protein belonging to the WD-repeats family This gene maps approximately 200 kb proximal to PFKL Northern blot analysis showed a 3 7 kb mRNA species in all fetal and adult tissues tested The human PWP2 is most homologous to the b subunit of the trimeric G proteins Because of its potential function in the signal transduction pathway, the human PWP2 gene is a prime candidate for EPM1 Mutation search is being carried out, using RT-PCR and single-stranded conformation analysis (SSCA) in RNA from patients with different ethnic backgrounds to evaluate the involvement of PWP2 gene in EPM1

S14.002

*Cloning the human Na-Cl thiazide-sensitive cotransporter gene: mutations in Gitelman syndrome

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Na-(K)-Cl cotransporters promote the electrically silent movement of chloride across the membrane in absorptive and secretory epithelia We have cloned the human cDNA coding for the renal Na-Cl cotransporter (hTSC), selectively inhibited by the thiazide class of diuretic agents We consider hTSC as a candidate gene for Gitelman syndrome (GS), a defect of ion reabsorption in the nephron distal tract The predicted protein sequence of hTSC spans 1021 amino acids (112 kDa) and shows a structure common to the other members of the Na-(K)-Cl cotransporter family a central region harboring twelve transmembrane domains and two intracellular hydrophilic amino- and carboxyl-termini The expression pattern of hTSC is strictly kidney specific The gene was mapped by FISH to human chromosome 16q13 The genomic structure is being characterised and more than twenty exons have been identified so far SSCP analysis of the coding regions allowed us to identify a two base-pair deletion within the first exon in two GS monozygotic twins At present, the molecular analysis of 18 additional GS families is in progress

S14.003

*Molecular genetics of Sanfilippo A syndrome (mucopolysaccharidosis type IIIA)

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Sanfilippo A syndrome is one of the four Sanfilippo syndromes (A, B, C and D or mucopolysaccharidosis (MPS) type III A, B, C and D) that result from deficiencies of different enzymes involved in the lysosomal degradation of heparan sulphate, leading to its storage and patients with mainly neurological symptoms All MPS-III sub-types are inherited as autosomal recessive disorders with considerable variation in clinical severity The incidence of the MPS-III is approximately 1 24000 in the Netherlands with MPS-IIIA the most common Due to the mild somatic disease compared with other MPS disorders, difficulty in diagnosing mild cases of MPS-III may lead to its underdiagnosis in patients with mild mental retardation We had previously isolated the sulphatase deficient in MPS-IIIA, sulphamidase, to homogeneity Isolation of the gene for sulphamidase is prerequisite for genotype to phenotype correlation, and possible therapies such as enzyme replacement therapy and gene replacement therapy Using degenerate oligonucleotides designed to N-terminal amino acid data and RT-PCR, we have isolated, sequenced and expressed the cDNA for sulphamidase In addition, we have mapped the gene to chromosome 17q25 3 by FISH Preliminary mutation analysis in MPS-IIIA patients has detected several possible mutations including an 11-bp deletion Further mutation analysis and characterisation of the genomic structure of the sulphamidase gene are ongoing

S14.004

The X-linked myotubular myopathy gene encodes a putative signal transduction protein conserved in yeast

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Myotubular myopathy (MTM1) is a very severe X-linked recessive disease characterized by impaired maturation of muscle fibers, as indicated by the presence, in affected newborn males, of myotubes with centrally located nuclei The biochemical defect is completely unknown We have decided to go for positional cloning of the MTM1 gene We characterized three deletions in unrelated MTM1 patients included one girl with a preferential inactivation of thenormal X chromosome Together with linkage analysis of several recombinant families, the candidate region was reduced from 10Mb to 280Kb, almost entirely covered by cosmids We performed various strategies to isolate putative coding sequences (exon-trapping, cDNA selection, random sequencing and exon prediction) Two genes were characterized and one of them was mutated in MTM1 patients The MTM1 gene, with an ubiquitous expression, shows a muscle specific alternative transcript and codes for a protein most probably implicated in signal transduction The MTM1 gene product (or myotubularin) is highly conserved in yeast, which is very syrprising for a muscle specific disease At least three new homologous human genes were found and might account for the autosomal forms of myotubular myopathy or might be involved in cell maturation in other tissues The cloning of the MTM1 gene will greatly improve diagnosis Elucidation of the enzymatic activities, the signaling molecule(s) and the substrate(s) on which it operates are now required to understand the role of myotubularin in muscle maturation

S14.005

Characterisation of a novel human dystrophin homologue expressed in brain

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Dystrophin and utrophin/dystrophin-related protein (DRP) are large membrane-associated cytoskeletal proteins whose functions are unclear Loss of dystrophin as a consequence of genetic mutation results in the complex human disorder Duchenne muscular dystrophy, whose symptoms include skeletal myopathy, mental retardation and electroretinopathy During an evolutionary study of dystrophin-like sequences in a range of vertebrate and invertebrate animals (using degenerate RT-PCR), we identified transcripts encoding a novel class of dystrophin-related proteins, conserved throughout the vertebrate clade Further study of the human sequence, named DRP2, showed it to resemble only the most C-terminal region of dystrophin (including two spectrin-like repeats) DRP2 is encoded by a small X-linked gene (23 exons, c 30 kb), and is much more similar to dystrophin and utrophin/DRP than is the 87K tyrosine kinase substrate Studies in mouse and rat by RT-PCR and in situ hybridisation revealed expression in specific regions of the brain (including the cortex, hippocampus and olfactory bulb), in testis, ovary, and adrenal and salivary glands We believe that study of DRP2 will not only serve to shed further light on the function of dystrophin, but may also prove it to be biologically important in its own right

S14.006
Homogentisate 1,2-dioxygenase: Cloning of the key enzyme of Alkaptonuria in mouse and man

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The genetic analysis of alkaptonuria (AKU, OMIM No 230500) marks three historical firsts in the history of human genetics: (i) Bateson noted its recessive mode of inheritance and concluded that Mendel's laws apply to humans, too, (ii) Garrod made it one of his paradigms for an inborn error of metabolism, (iii) La Du demonstrated a specific enzyme defect, the lack of homogentisate 1,2 dioxygenase (HGO) activity, as the cause of the disease We have previously purified HGO from mouse liver to homogeneity and now report on the cloning and sequencing of the corresponding genes in mouse and man Partial amino acid sequence information of the monomeric 49 kD protein was used to design degenerate PCR primers which were able to amplify parts of the cDNA from liver mRNA With this probe a full-length cDNA clone of 2 kb was isolated from a mouse liver cDNA library and sequenced The sequence predicts a protein of 50 kD and shows partial homology to other dioxygenases and dehydrogenases from lower eukaryotes Several ESTs were identified in the genome databases which were helpful in assembling the human cDNA sequence FISH and chromosome 3-specific cosmids map the cDNA to chromosome 3q13, the locus of the human AKU gene An antiserum, raised against the purified protein, demonstrates the absence of cross-reacting protein in livers of alkaptonuric mice We conclude that we have cloned the gene for homogentisate 1,2 dioxygenase, the last gene which was missing in the degradation pathway of aromatic amino acids

S14.007
Isolation of genes from the DiGeorge syndrome critical region.

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CATCH 22 is a developmental defect that covers a wide range of clinical abnormalities including DiGeorge syndrome (DGS), Velo-Cardio-Facial syndrome, isolated conotruncal heart defect (either sporadic or familial) and the Conotruncal Anomaly Face syndrome This syndrome has been associated with haploinsufficiency for a 2 to 3 megabases long chromosomal region in 22q11 2 for most cases studied, but a critical region of 500 kb in the centromeric side of the deletion has been defined We have previously reported the cloning of a balanced translocation breakpoint carried by a DGS patient by chromosome walking, and establishment of cosmid contigs Search for genes interrupted or in the vicinity of the balanced translocation breakpoint are in progress using the xon trapping technology and search for phylogenetically conserved sequences with the aim of cloning the major gene involved in CATCH 22 One gene, isolated from the DGS critical region (DGCR), and showing homology to laminin is presently being characterized The possible role of this gene and others mapping within the DGCR will be discussed

Poster Presentations

Section 1: Developmental genetics

1.001
Isolation of a human homologuos to the Drosophila dishevelled gene from the DiGeorge critical region.

Mari, Aldo,Pizzuti, A, Novelli, G, Ratti, A, Colosimo, A, Amati, F, Sangiuolo, F, Calabrese, G, Palka, G, Gennarelli, M; Mingarelli, R, Scarlato, G, Scambler, P, Dallapiccola, B
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DiGeorge syndrome (DGS) is a developmental defect of some of the neural crest derivatives, presenting clinical overlap with Velo-Cardio-Facial syndrome (VCFS) Microdeletions of 22q11 have been detected in the majority of these patients, indicating that monosomy at this locus is a major factor in the etiology of these diseases About 30 genes or transcripts have been identified in the DGS/VCFS critical region, suggesting that the phenotype probably results from contiguous-gene deletions We have isolated from the DGS/VCFS critical region a human cDNA (DVL-22) homologous to the Drosophila dishevelled (dsh) segment polarity gene FISH experiments and Southern blotting analysis have shown that this cDNA is deleted in DGS/VCFS patients DVL-22 is expressed in two forms of 3 2 and 5 0 Kb, in several fetal and adult tissues, including the thymus and, at high levels, the heart DVL-22 exhibits high amino acid homology (>85%) with other dishevelled transcripts isolated from mouse and Xenopus Since DGS is due to perturbation of differentiation mechanisms at decisive embryological stages, the DVL-22 gene, similarly to Dsh genes, might be a candidate for the pathogenesis of this disorder Work supported by EEC, Biomed-1 and ASM, Milan

1.005
Scatter factor/hepatocyte growth factor as an in vivo regulator of morphogenesis and cellular migration.

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Scatter factor/hepatocyte growth factor (SF/HGF) stimulates mitogenic, motogenic and morphogenetic activities in a variety of cultured epithelial cells, yet its role in vivo is still poorly understood To elucidate physiologically relevant functions of SF/HGF, transgenic mice were generated expressing highly elevated levels of SF/HGF transcripts in fetal and adult tissues using the mouse metallothionein promoter and locus control regions SF/HGF protein levels were greatly enhanced in the transgenic serum and urine Transgenic mice overexpressing SF/HGF exhibited developmental abnormalities of the mammary gland and olfactory mucosa, and died prematurely due to sporadic gastrointestinal obstruction and renal failure Significantly, inappropriate expression of SF/HGF in transgenic embryos influenced the development of two distinct migratory cell lineages, resulting in ectopic skeletal muscle formation and melanosis in the central nervous system, and patterned hyperpigmentation of the skin In situ hybridization analysis of midgestation embryos demonstrated aberrant localization of committed melanoblasts in and around the transgenic neural tube, which overexpressed SF/HGF Our data strongly suggest that SF/HGF possesses in vivo "scatter" activity, and functions as a genuine morphogenetic factor by regulating migration of select populations of premyogenic and neural crest cells during normal mammalian embryogenesis

1.007
Thymic involution in hSOD-1 transgenic mice: A model for the immunopathophysiology in Down's syndrome.

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As an approach to the development of an animal model for Down's syndrome, we have generated heterozygous B6/D2 and FVB/N transgenic mice for the entire human SOD-1 gene, a key enzyme in the metabolism of free radicals and located in 21q22-1 Thymus of 15 days old to one year old animals from both transgenic lines over-expressing the hSOD-1 by a factor 1 5 to 7 were studied by electron microscopy As early as in 1 month old thymus, we observed typical microenvironments characteristics of a premature involution, usually present in one year old normal mice This thymic premature involution is similar to that previously observed in light microcopy for thymus of Down's syndrome patients Moreover the total number of thymocytes is reduced in transgenic mice in comparison to non transgenic littermates and this reduction is even more drastic in homozygous FVB/N transgenic mice Preliminary results on thymocytes subpopulations suggest a higher proportion of immmature thymocytes and a delaly in thymocytes maturation In other hSOD-1 transgenic mice impaired neuromuscular junctions and decrease in blood platelets of the serotonin neurotransmitter have been demonstrated All these results are consistent with the role of over-expression of hSOD-1 in some impaired biological functions and premature ageing observed in Down's syndrome patients Thus hSOD-1 transgenic mice provide a good animal model for studying premature ageing in different tissues

1.008
Cloacal dysplasia and pseudo-hermaphroditism.

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External genitalia embryology is based on an endocrinological determination Several pathological situations are not well explained by the endocrinological models and support the inductive role of clocal membrane Case 1 · Anal imperforation, ambiguous external genitalia, 46 XX Cloacal formation, normal ovaries Case 2 Female pseudohermaphroditism, 46 XX Pregnancy terminated at 24 weeks Anal imperforation, fusion of labia majora Cloacal formation Cases 3 and 4 · Antley Bixler syndrome Case 3 oligohydramnios, renal agenesis, 46 XY. Imperforate anus, scrotal hypoplasia Case 4 2nd pregnancy 46 XX Elected termination at 22 weeks Perineal imperforation with dysplastic epithelial structures These patients have anal imperforation with abnormal external genitalia This association cannot be related to androgen abnormalities Animal data support an active role of the cloacal plate in anal and uro-genital compartments The cloacal plate may be under the dependency of genes regulated directly by the SRY or other genes Female normal pattern of gene expression leads to a full disparition of the urogenital plate, in male the urogenital plate may be partly saved allowing for the secondary endocrinologically-regulated "fusion" of the lateral anlagen In these cases, the abnormal pattern of genetic regulation of urogenital plate regression may allow for an endocrinologically-independent "fusion" of lateral anlagen leading to female pseudo-hermaphroditism

1.009

Expression of a New Growth Factor gene in the Developing Mouse

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We have identified a novel mouse gene that is closely related to the PDGF gene family The human gene was initially isolated using an 11q13 specific cosmid probe (D11S750) Subsequently the human gene was used to isolate the corresponding mouse gene Here, we report the spatiotemporal expression pattern during prenatal mouse development Gene activity starts early in fetal development At gestational day 14 it is expressed in most cells of the embryo although the heart, spinal cord and the brain cortex shows significantly higher levels of expression than other tissues At gestational day 17 expression is almost exclusively seen in heart, fat and spinal cord, with the expression in other tissues markedly reduced The time pattern of expression and its confinement to heart, fat and the nervous system suggest that it may play a role as a growth factor in these tissues

1.010

Altered fertility patterns and maternal transmission in Irish families with neural tube defects: A pilot study

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Earlier work by this author and collaborators in Italy showed a pattern of maternaltransmission of neural tube defects in the families of children who had a high spinal NTD lesion Expression of the trait was not in the form of NTDs but of altered fertility Specifically,maternal grandparents had smaller families than paternal grandparents (p< 001) Evaluationof the earlier literature revealed that preferential maternal transmission was present in most, ifnot all, studies that included extended families in the form of increased NTD risk amongmothers' sisters' children As a pilot for a larger study, we evaluated familial patterns in 48Irish families with NTDs and found, as in Italy, that maternal grandparents had smaller families But, in addition, childlessness was significantly more common among mothers' brothers thanamong mothers' sisters (1-tailed p= 008) These results and others strongly suggest thatmultigeneration transmission of a trait or traits for NTDs can be detected on the mother's side Expression is not limited to females, but can be detected in males also Studies of genesassociated with NTDs should incorporate studies of preferential maternal transmission

1.011

Association of omphalocele and neural tube defects in multiply malformed infants: a survey of 3 million births in Europe (1980-1990)

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Among 732 cases of omphalocele (O) identified in Europe (prevalence rate 2 52 per 10,000), 335 isolated cases and 397 cases associated with other defects (196 recognized conditions, and 201 multiply malformed) were registered There was a significant difference between multiply malformed in the registries of the United Kingdom and Ireland (UKI) (116/906,805, 1 3 per 10,000) and registries in Continental Europe and Malta (CEM) (85/1,999,059, 0 43 per 10,000)(chi-square= 65 78 df 1, P<0 01) due to a specific association of NTD and O in the UKI (64 cases among 116 in UKI as compared to 14 of the 85 registered in CEM, chi-square= 29 33 df 1, P<0 01) The classification of multiply malformed cases according to sex, type of NTD, and geographical area is shown in the table

	UKI		CEM	
	N°	M/F	N°	M/F
Anencephaly	35	0 36	3	2/0
isolated	11	0 38		
with spina bifida	21	0 40	3	
with iniencephaly	3	0 0		
Spina Bifida	19	1 14	10	1
Encephalocele	3	1		
Iniencephaly	7	0 75	1	0 0
Total	64	0 57	14	1 25

The table shows both a specific association between O and anencephaly and an excess of females among the cases in the UKI, which suggest common factors involved in the etiology of NTD and omphalocele Work supported by MURST and Emilia Romagna Region

1.012

Ventricular septal defects in the Emilia Romagna congenital malformation registry (IMER), Italy: epidemiological study in 330,017 consecutive births.

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Among 1,546 cases of congenital heart defects (CHDs)(total prevalence rate 44 7 per 10,000) identified by IMER registry (1980-1994), 1,148 (74,25%) isolated cases (without extracardiac malformations) and 398 (25,75%) cases associated with extracardiac malformations were recorded Total birth prevalence of CHDs varied from 31 3 per 10,000 in 1980 to 63 3 in 1994 (chi square= 16 45, p<0 01 df 1) These data are in keeping with those reported by BWIS and EUROCAT-CHD registries. In the study period, 705 cases of ventricular septal defect (VSD) (45 6% of total CHDs), excluding those occurring as components of complex CHDs, were detected (birth prevalence 21 4 per 10,000) VSD was the only heart defects in 607 cases (39 3%), in 93/607 (15,5%) there were associated extracardiac malformations (59/93 multiply malformed infants, 25/93 chromosomal anomalies, and 9/93 recognized conditions) Birth prevalence of VSD (without other CHDs) changed from 10 4 per 10,000 in 1980 to 23 2 per 10,000 in 1994 (chi square=6 55, p<0 05 df 1). A similar temporal trend was not osbserved for VSDs with extracardiac malformations This increase of prevalence is explained by the progress obtained in the diagnosis of CHDs due to the widespread of ultrasonography, expecially in late eighties Work supported by MURST and Emilia Romagna Region

1.013

The choroideremia mutation is embryonic lethal in hemizygous mice and results in photoreceptor cell degeneration in chimaeric mice.

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Choroideremia (CHM) is a progressive X-linked eye disease The gene defective in CHM has been cloned and its protein product (REP-1) has been shown to be involved in the geranylgeranylation of Rab proteins Still, the pathogenesis of CHM remains unclear We therefore tried to generate an animal model for this disorder by targeted disruption of the Rep-1 gene in mouse embryonic stem (ES) cells and subsequent injection of targeted ES cells into host blastocysts We obtained transmitting male chimaeras and heterozygous females but, surprisingly, neither affected male nor carrier female offspring from these heterozygotes Via nested PCR we could demonstrate the targeted Rep-1 allele in male as well as female

blastocysts isolated from a carrier mother Thus, it appears that disruption of the Rep-1 gene causes embryonic lethality in males, in females it is lethal only if inactivated Rep-1 gene is present on the maternal X A plausible explanation for this observation would be that in mice the Rep-1 gene is required in extraembryonic membranes, since it is well established that in these tissues the paternal X is preferentially inactivated Compared to control mice, several chimaeric animals showed significant deficiencies in the b-wave of their electroretinograms Histologic examination of the retina revealed a patchy degeneration with absence of the photoreceptor cell layers

1.014
Collection of fetal cells from the cervical canal during the first trimester of pregnancy: Sampling techniques, molecular analysis and isolation of purely fetal material

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Fetal cells can be detected in samples taken from the cervical canal from pregnant women Different techniques for retrieving fetal cells transcervically were investigated uterine lavage and cervical mucus aspiration Whole samples and cell clumps isolated by micromanipulation were analyzed using molecular cytogenetics and PCR An assessment of the safety of the mucus aspiration before transcervical chorion villus sampling in continuing pregnancies (130 study cases and 140 control cases) was carried out Normal and chromosomally abnormal fetal cells can be detected by fluorescent in situ hybridization in samples taken from various levels along the cervical canal Clumps of fetal cells from transcervical samples were isolated by micromanipulation and tested by FISH and PCR Out of 129 cell clumps isolated from mucus aspirates and transcervical lavages from 29 patients, 29 clumps from 11 patients were found to be of exclusively fetal origin as judged by the detection of chromosome 21-specific polymorphic DNA markers and Y-derived DNA sequences by PCR and multicolour FISH One case of a male triploid fetus, diagnosed by the analysis of a transcervical sample obtained by mucus aspiration and lavage, was confirmed by testing clumps of cells isolated by micromanipulation These results demonstrate the potential of the sampling technique with a view to the diagnosis of chromosomal anomalies and single gene disorders

Section 2: Chromosome structure and function

2.001
Analysis of low and high GC Xq DNA regions by random and ordered shotgun sequencing and computer-assisted gene finding.

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Regions of at least 140 kb in Xq25, Xq26, Xq27 and distal Xq28 were analyzed either from cosmids by standard random shotgun, or from BACs or YACs by ordered shotgun sequencing [Chen, Schlessinger and Kere, Genomics 17, 651-656 (1993)] Ordered shotgun methods subcloned larger clones to lambda phage with 7-10 kb inserts, amplifying the inserts by PCR, carrying out end-sequencing on the subclones to assemble a minimal tiling path, and either filling in gaps with more end-sequences or subcloning a minimal number of lambda phage into plasmids and sequencing those Individual technical staff can currently sequence 10 kb/week, and a group of three can carry out the subcloning, recursive map building, and assembly required to sequence 1 to 2 Mb per year Concerning sequence content, the large clones were chosen from isochores that varied in GC level from 40 to 58% The content of repetitive sequences and genes varied correspondingly In particular, gene content, assessed by consensus elements, EST/gene hits, and computer-assisted predictions, ranged from 1/100 kb to 1/12 kb (the latter in the gene-richest portion of Xq28 between the color vision and G6PD loci)

2.002
Tissue-specific differences of C-banded heterochromatin in human embryos: the possible role of DNA methylation.

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Sizes of C-band heterochromatin regions were estimated in embryonal and extraembryonal tissue lineages of 82 human spontaneous and medical abortuses These regions in chromosomes 1 and 16, containing mainly satellite II DNA, were significantly longer in extraembryonic tissues than in embryonic ones The differences in length between chromosome 9 C-band heterochromatin, which contains satellite III DNA, and Y chromosome C-band heterochromatin, which contains all four classical satellite types, were not significant between the two tissues Our data, together with other findings on the correlation between constitutive heterochromatin compactization and DNA methylation, as well as data on mutation of DNA methylation in ICF (immunodeficiency, centromeric instability, and facial abnormalities) syndrome patients, indicated that the observed variations in C-band length reflected a tissue-specific pattern of heterochromatin methylation Satellite II DNA probably is the most sensitive target for the decondensation effect of DNA hypomethylation compared with other satellite types

2.003
Cosmid contig construction in the chromosome 21 ETS2-HMG14 region

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The present work was initiated with the goal of generating a continuous, ready to sequence, cosmid contig covering the ETS2-HMG14 region of chromosome 21 111 cosmid clones corresponding to cosmid pockets 121, 122, 123 and 124 (Nizetic et al , 1994, Hum Mol Genet 3, 759-770) were grown, the plasmid DNA was digested with EcoRI, and the digestion products separated on a 0 7% agarose tris-acetate, 30 cm long, gels The ethidium bromide stained gels were digitized and analyzed with the Intelligent Quantifier program from Millipore to size the different bands Two internal standards corresponding the common vector bands were considered, in addition, in each lane to correct for slight relative differences in migration between different gels Coincidence of two or more sized bands between cosmids was taken as evidence of overlap 41 cosmid clones have been organized into 6 different contigs containing an average of 7 cosmids each A EcoRI map is also available for some of the contigs In order to anchor the contigs into the existing physical and genetic maps, we are testing the following chromosome 21 markers on each of the 111 cosmid clones arranged into 11 different pools containing 10 clones each S168, S3, S259, S270, S267, S1252, S1255, S198, HMG14, S268, S231, S1260, S1412, PFKL, S266, S1259, S212, S1411, IFNAR, S171, ETS-2 The end fragments of the clones from the contig ends are now being used as probes to extent the contigs In addition selected cosmid fragments are being used as hybridization probes against Southern blotted EcoRI gels and against a dot blot matrix containing all cosmid clones in order to confirm the contigs Supported by a grants from the European Union BIOMED1-GENE-CT93-0015 and Fundació Catalana Síndrome de Down

2.004
Delineation of the genetic basis of azoospermia

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About 2% of human males suffer from infertility Aetiological factors include nongenetic and genetic causes, namely bacterial or viral infection, ejaculatory system obstruction, varicocele, drug toxicity, toxins, immunological and endocrine disorders, sex chromosome triploidy, deletion of the pseudoautosomal regions or of Yq, disrupting structural genes required for spermatogenesis We studied 20 azoospermic adult males and a growth retarded boy (GRD) by standard cytogenetic methods (G, C and Q banding), hormone assays (FSH, LH, and T) and DNA (Southern-blotting and PCR) analysis Different markers or genes located throughout the Y chromosome, (including, PABX/Y, SRY, ZFY, AMGY, Ycen, STSP, YRRM, DAZ and DYZ1) were analysed We have found numerical and structural abnormalities of the X and Y chromosomes or abnormal hormonal levels in 16 out of 21 patients 6 with 47,XXY karyotype, 3 with a mosaic chromosome constitution (45,X0/46,XYq-, 45,X0/46,XYq-, 46,XY/47,XXY), 1 with 46,XYq- (the GRD boy), 1 with a microdeletion of the 6D Y subinterval including the DAZ gene, 1 with an interstitial deletion (0 8 Mb) in the PABX region, 3 with isolated FSH elevation and normal LH and T levels, suggesting a severe damage of the Sertoli cell germinal compartment, and 1 with hypogonadotropic hypogonadism with very low FSH, LH and T levels The 5 remaining patients (46,XY) were diagnosed as idiopathic azoospermic This could be due to i) point mutations in AZF candidate genes (YRRM family or DAZ), ii) microdeletions or point mutations in other(s) AZF gene(s), and iii) anatomical obstruction in the ejaculatory system of difficult clinical diagnosis. We emphasize the importance of clinical, endocrine, cytogenetic and molecular studies to elucidate the aetiology of male infertility

2.005
New genes located to the distal part of human and mouse MHC : structural functional and evolutionary analysis.

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We have precise the structure of distal half of human major histocompatibility complex (MHC) The MHC map is now continuous from HLA-E to 1000 Kb telomeric of HLA-F, and includes several new genes, in particular olfactory receptor genes Somes of new genes have furthermore been analysed at structural, functional and evolutionary level In addition the corresponding sequences that were

not previously mapped in the mouse genome have been located The human and the mouse organizations have therefore been compared This comparison allows us to demonstrate that the structure of distal part of the MHC is similar in two species In addition this comparison shows the presence of breakpoint of synteny telomeric of distal part of H-2 region Indeed the region telomeric of HLA in human is found on chromosome different from the one carrying H-2 in mouse This evolutionary breakpoint was precisely mapped in human and the surrounding region was cloned into yeast artificial chromosomes We show also that the region found around the breakpoint was involved several times in chromosome recombinations in the mouse lineage as it seems to correspond also to the t-complex distal inversion point

2.007
Polymorphism of Ag-NOR patterns in human foetuses

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Ribosomal gene transcription of each NOR bearing chromosome is genetically determined and inherited in a Mendelian fashion, and Ag-staining intensity is considered to be an indicator of the NORs activity However, Ag-NOR pattern variability is known dependent on different reasons including the stage of development NORs activity in human embryogenesis has not been examined in detail so far The functional polymorphism of Ag-NORs was studied in diagnostic culture sof cord blood lymphocytes obtained by cordocentesis from 43 foetuses at 22-26th week of pregnancy Mothers admitted fetal blood sampling because of high risk for chromosome disorders - 22 cases (1), foetal malformations (anencephaly, neural tube defects, etc) - 8 cases (2), high risk for monogenic and X linked disorders (control cohort) - 13 cases (3) The mean number of chromosomes with positive Ag-NORs per cell has been significantly ($p<0,01$) increased in the foetuses of group 1 as well as in the foetuses of the group 2 compared to control group 3 (8 74, 8 37 and 7 25 respectively) The difference between groups 1 and 2 was not significant Variable distribution patterns of the total cellular NOR activity in the all groups has been registered More than one mechanism underlying the Ag-NOR pattern polymorphism in human foetuses was suggested

2.008
Isolation and FISH analysis of alpha-satellites from chromosomes 13, 21 and 22 specific cosmid libraries

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Molecular and cytogenetic analysis of heterochromatic regions in human chromosomes is complicated due to repetitive nature and heterogeneity of DNA sequences, forming this parts of genome Isolation of large insert DNA clones from chromosome-specific libraries can assist in further analysis of heterochromatin structure and function Here, we identified and mapped by FISH 116 alphoid clones presented in 36,000 screened cosmids of chromosomes 13, 21 and 22 specific libraries Centromeric clones, containing different elements of alphoid DNA arrays, associated with interspersed, classical satellite, ribosomal and telomeric-like DNA sequences were detected Most of all alphoid cosmids contain interspersed repetitive elements, indicating on often interruption of long tracts of tandemly repeated alpha-satellite by non-alphoid insertions at least in each 10-20 kb Alphoid cosmid clone, containing telomeric repetitive elements, hybridized to pericentromeric regions of many chromosomes as well to regions of "ancient" centromeric sequences at 2q21 and 9q13 These results confirm and extend the data that heterochromatic regions on acrocentrics chromosomes are extremely heterogeneous in composition and organization, and include repetitive sequences with "relative chromosome specificity" from different subfamilies of alphoid DNA The pools of centromeric alphoid cosmids are useful resource for further studies of the structural and functional organization of heterochromatic DNA, and may be applied for contig assembling throughout heterochromatin for high resolution mapping and massive sequencing experiments

2.009
Differential mechanisms of X-inactivation in the soma and germ-line

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It has been suggested that the X-inactivation of the single X in the male germ-line could have been a starting point for evolution of somatic X-inactivation seen in females To-date there is no clear indication of a differential germ-lin and somatic role of the X-Inactive Specific Transcript (XIST/Xist), presumed to initiate X-inactivation On the other hand we have found evidence that the mechanisms for maintenance of X-inactivation may differ with respect to patterns of histone acetylation It has previously been shown by immunocytochemistry that the somatic inactive X in human and mouse females is marked by under-acetylation of histone H4 isoforms, and it has been suggested that this may be important for regulation of transcription (Jeppesen, & Turner 1993, Cell 74, 281-288) We have now investigated X-inactivation in the male germ-line of the mouse with antibodies to H4, acetylated at lysine positions 5, 8, 12 and 16 and demonstrate that there is no accompanying histone H4 under-acetylation We conclude that there is a differential germ-line strategy for maintenance of X-inactivation, where the acetylation may be associated specifically with the permanent marking of inactive X chromosomes in the female soma rather than the direct silencing of X-linked genes

2.010
Immunocytology and In-situ hybridization of surface spread human spermatocytes.

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The meiotic process involves homologous chromosome pairing, synapsis and recombination as a prerequisite to reductional segregation It is generally accepted that the proteinaceous tripartite structure, the synaptonemal complex (SC) serves to bring homologous DNA sequences together and support recombination The SC associates with only a fraction of the genomic DNA at the bases of chromatin loops The amount of DNA available for recombination is therefore believed to be very small The nature of this recombinational DNA and the mechanisms involved are largely unknown Previous investigations of human surface spread SCs have been performed by LM and EM, using PTA or silver stained testicular and ovarian material We present the results of and LM approach involving the combination of surface spreading, immunocytology with a fluorescently labelled antibody against the SC, and fluoresence in-situ hybridisation (FISH) using both repetitive and single copy DNA sequences In this initial study we demonstrate that telomeric sequences are closely associated with the SCs, while the signals corresponding to the heterochromatic blocks 1qh, 9qh, and 16qh as well as the single copies 21 contig (Cambio Ltd) and the bcr/abl (Oncor Ltd) are associated with chromatin loops We have thus shown the feasibility of using this approach for human material Our long term aim is to further the understanding of the spatial relationship between the SC and DNA sequences relevant to spermatogenesis and recombination

2.011
Models of meiotic recombination and segregation

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Most constitutional reciprocal translocations in humans are unique with respect to the chromosomes and breakpoints involved. The only outstanding exception to this general rule concerns the translocation t(11,22)(q23,q11) of which to-date several hundred unrelated families have been identified Ascertainment has usually been through a dysmorphic mentally handicapped child with the unbalanced 22 derivative originated by parental first meiotic 3 1 segregation A number of models on meiotic recombination and segregation have been proposed to explain this situation (see e g Koduru and Chaganti, 1989, Genome 32, 24-29) We have used dual colour FISH with chromosomes 11 and 22 paints (Cambio Ltd) on meiotic chromosome preparations made from testicular biopsy material from a carrier with the karyotype 46,XY,t(11,22)(q23,q11) This family was ascertained in the usual way with the brother of our Case having the karyotype 47,XY,+der(22),t(11,22)(q23,q11)mat The meiotic analysis of first and second metaphases showed that the recombination patterns and segregation of this translocation conforms to those previously investigated in our Laboratory (Golman et al, 1993, Cytogenet Cell Genet 63, 16-23, Goldman et al, 1993, Chromosoma 102, 102-11, and refs therein) We conclude that the +der(22) constitution that is generally seen in the dysmorphic, mentally handicapped children in these families is not due to an unusual meiotic behaviour of the translocation but likely to be the result of postzygotic selection against other unbalanced karyotypes

2.012
Analysis of chromosome pairing in early female meiosis of the mouse using FISH

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Prior to the development of fluorescence in situ hybridisation (FISH), the initiation of homologous chromosome pairing in mammalian foetal oocytes proved difficulty to study Using FISH, the role of particular chromosomal segments in initiating pairing can now be investigated The early events of pairing in female germ cells are being studied using the laboratory mouse, as oocytes from pre-meiotic interphase to diplotene can easily be obtained from foetuses at particular days of gestation The centric ends of the mouse acrocentric chromosomes were found to associated in varying numbers of clusters from pre-meiotic stages onwards However, using a probe specific for the proximal end of the X, these clusters appeared to be non-specific and therefore unlikely to play a significant role in the initiation of pairing The distal telomeres of the mouse acrocentric chromosomes also show clustering in zygotene cells Whether this is a means of bringing together the distal ends of homologous chromosomes for pairing initiation is currently being investigated Preliminary observations using whole chromosome paints show that homologues usually lie in different domains in leptotene and early zygotene cells with no apparent indication of pre-meiotic alignment

Section 3: Cytogenetics

3.001
Two cases of satellite chromosomal region aberations.

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We report a girl with de novo derived unbalanced translocation 15,22(q13,p13) She showed a mental retardation and various dysmorphic features Ag-staining combined with G-banding technique revealed a breakpoint at a satellite region of chromosome 22 The second reported case presented an inversion of chromosome 21 transmited from a father to his son Paternal karyotype showed a balanced inversion 21 (p13,q22) The boy was with Down syndrome and demonstrated an unbalanced inversion 21(p13,q22) having a partial duplication 21(qcen-q22) as a result of unequal mejotic recombination event in his father Again Ag/G-banding exhibited a breakpoint at a satellite region, a very rarely affected area

3.002
A complex chromosome rearrangement leading to a balanced reciprocal translocation: elucidation by means of FISH

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A familial complex chromosome rearrangement (CCR) involving four breakpoints in three chromosomes is reported The index patient was a pregnant woman referred because of repeated spontaneous abortions Cytogenetic investigation of the family showed that the index patient, her mother and grandmother were carriers of a CCR between chromosomes 2, 3 and 8 Use of the FISH technique revealed that four breakpoints were involved The karyotype of the women in three generations is as follows
46,XX,t(2,3,3,8)(2pter->2q23 3q13 2->3qter,3pter->3q13 2 2q23->2q33 8q13->8qter, 8pter->8q13 2q33->2qter)
Prenatal chromosome analysis on amniocytes from the index patient showed an apparently balanced reciprocal translocation between chromosomes 2 and 3 There was a der(2) and a rec(3) The der(2) was the same as that in the mother but the rec(3) must have arisen as a result of a crossover during meiosis in the mother The pregnancy, that is being monitored by regular ultrasound examination, appears to be proceeding without problems The occurence of a CCR is extremely rare Origin of a balanced translocation resulting from a crossover in a CCR must be even rarer and as far as we know has never been reported before

3.003
Identification of four cases of chromosomal rearrangements by Fluorescence in situ hybridization.

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Two cases with chromosomal translocations and two cases with small supernumerary marker chromosomes are presented Routine cytogenetics was complemented by Fluorescence in situ hybridization (FISH) to give accurate information in these cases Case 1 The 15 years old patient with dysmorphic features, growth and mental retardation had the karyotype 46,XY,18p+ (de novo) revealed by cytogenetic studies Using the Y-heterochromatic probe pYZ3 and FISH, the variant 18 was identified as a t(Y,18)(q11 23,p11 3) Case 2 The family had the complex autosomal translocation involving chromosomes 6, 18 and 21 confirmed by FISH with chromosome 6 painting probe The mother with the karyotype 46,XX,t(6,18)(p12,q23),t(6,21)(q23,q22) had no clinical symptoms Her daughter with developmental abnormalities showed an unbalanced chromosomal arrangement with der (21),t(6,21)(q23,q22)mat The prenatal diagnosis of second pregnancy was performed and the fetus had the same balanced translocation as his mother This pregnancy continued uneventfully and a phenotypically healthy normal male baby was born Case 3 The girl with some dysmorphic features and growth retardation had the karyotype 45,X/46,X,+r(?) This ring marker was derivative of chromosome X defined by FISH with X-centromeric probe Case 4 The 18 years old patient with growth and mental retardation had the karyotype 46,XY/47,XY+mar FISH analysis with chromosome 15 painting probe confirmed the origin of marker from chromosome 15. Our cases illustrate the effective use of FISH as a rapid accurate technique in cytogenetics for diagnosis of chromosomal rearrangements

3.004
A case of Prader-Willi syndrome arising as a result of familial unbalanced translocation t(11;15)(q25;q13)

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The majority of patients with Prader-Willi syndrome (PWS) have been shown to have a cytogenetic or molecular deletion on the paternally derived chromosome 15 We report on a case of PWS in which this deletion occurs as a familial unbalanced translocation, 45,XX,-11,-15,+der(11),t(11;15)(q25,q13)pat The proband was diagnosed clinically as having unusually severe PWS She has been susceptible to infections since infancy because of severe hypogammaglobulinemia She is severely mentally retarded At the age of 3 years she is still hypotonic and can neither walk alone nor talk Molecular studies revealed loss of the paternal methylation pattern at locus D15S63 and a deletion encompassing the loci from at least D15S10 to D15S97 The proband's father, the father's sister and their mother are all carriers of the same balanced translocation t(11,15)(q25,q13) The father's sister had two spontaneous abortions, two twin babies from the third pregnancy died soon after birth By genomic imprinting we would expect that if she would give birth to a child with the same unbalanced translocation as the proband - it would be affected by Angelman syndrome So far, a similar familial unbalanced translocation due to loss of the small chromosome 15 derivative has never been described

3.005
The chromatid type exchange aberrations in liquidators

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At cytogenetical investigation of 257 liquidators of Chernobyl accident long time (6 and more years) after exposure the different types of chromosomal aberrations were found It was shown that not only high level of radiation markers but another type of chromosomal aberrations - chromatid exchanges- were revealed in liquidators Among all liquidators two groups - 31 individuals without any additional mutagenic influences and 51 individuals contacted with infavorite environmental factors after accident - was picked out The control group (59 individuals) was generated by the same principles The frequency of chromatid exchanges in liquidators contacted with additional infavorite factors was significantly higher than in control group connected with similar environmental factors (0 21±0 09% and 0 00±0 04%, $p<0.05$) It is possible the increased level of chromatid type exchange aberrations in liquidators is a result of alteration chromosome sensitivity to action of environmental mutagenes

3.006
Chromosomal abnormalities at the liquidators in a remote period after Chernobyl accident.

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Chromosomal aberrations in blood lymphocytes of the liquidators 6 and more years after Chernobyl accident were investigated The results of cytogenetical analysis and the dates of detailed life style questionnaires were entered to a computer

database. 257 liquidators were examined The documentary registered physical doze of radiation exposure did not exceed 25 cGy It was shown, that in a remote period after accident the total frequency of chromosomal aberrations (2 73±0 21%) did not significantly exceed a control level (2 22±0 13%) The reasons of high frequency of the chromosomal aberrations in control group of St -Petersburg inhabitants are discussed. The statistically significant differences between the liquidators and the control group were revealed in a spectrum of chromosomal aberrations the frequency of dicentric, ring and translocated chromosomes increased in exposed group. The results of the statistical analysis of the database in order to establish the reasons and mechanisms of long-term existence of cytogenetical radiation markers at liquidators are represented. The connection between genome instability and the health of the carriers of cytogenetical radiation markers is discussed

3.007

Micronucleus test and chromosomal analysis in liquidators of theChernobyl accident.

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The parameters of chromosomal instability of lymphocytes from persons suffered under irradiation 6-8 years ago and the control group were detected with cytokinesis-block micronucleus assay and conventional staining The frequency of chromosomal aberrations in the liquidators (2,73±0,21%) did not significantly exceed the control dates (2,22±0,13%) In spate of low doses of radiation and long period after exposure the radiation markers - dicentric and ring chromosomes were found during cytogenetical analysis 0,20±0,04% The frequency of chromosomal damages in control group was 0,04±0,02% (p<0,001). The micronucleus assay was performed in group of 22 liquidators and 14 control persons. The frequency of micronucleus was 12,3±1,24 and 10,6±1,72 in 1000 binucleated cells accordingly. It was not shown any differences between two groups. The high inter-individual variations of this parameter was found in each groups. In irradiated persons - 0 till 28 and control - 2 till 22 micronucleus per 1000 cells. Thus micronucleus assay in remote period did not allow to detect differences in genome stability of liquidators and control group which have been shown by conventional staining.

3.008

Sacral agenesis in a fetus monosomic for 7q36.1→qter

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We describe a fetus ascertained during amniocentesis on a 29 year old woman referred because of a raised maternal serum AFP The fetus had inherited an unbalanced form of a balanced paternal 7;19 translocation resulting in functional monosomy for 7q36 1→qter FISH with a 7q36 specific cosmid (D7S427) confirmed the deletion in the fetus Ultrasound studies had suggested sacral agenesis and, following termination, the autopsy confirmed severe type II sacral agenesis together with intrauterine growth retardation The critical region for autosomal dominant sacral agenesis has recently been mapped to 7q36, and this case provides further evidence for a sacral agenesis locus in this region and may help to further refine the critical region

3.009

A 45,X male with a cryptic Y;21 translocation and a ring chromosome 22.

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We present a unique case of a one year old male referred with full cheeks, a long philtrum, developmental delay and a possible diagnosis of Williams syndrome Fluorescence in situ hybridisation (FISH) with a Williams syndrome probe (WSCR) showed no evidence of a deletion Chromosome analysis demonstrated a chromosome complement of 45 including a single X chromosome and a ring chromosome 22 In addition, FISH studies demonstrated a subtle whole arm translocation, in which the short arm of one chromosome 21 was replaced with the short arm of a Y chromosome, including the SRY locus Signals for both centromeres (DYZ3 & D13Z1) were juxtaposed suggesting the translocation had arisen as a result of centromere fusion Despite his young age, he already has phenotypic features consistent with previously reported cases of both ring chromosome 22 and Y deletion This is, to our knowledge, the first reported case of a cryptic Y, autosome translocation involving chromosome 21 The parental chromosomes were normal The parental origin of both these de novo abnormalities is currently under investigation

3.010

«Pseudo-recessive» private mca syndromes may result from unbalanced cryptic translocations: the Lambotte syndrome as an illustrative case

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In 1990, one of us reported as probable autosomal recessive syndrome a MCA/MR syndrome observed in 4 out of 12 sibs from a probably consanguineous mating (Verloes et al., Am J Med Genet 1990,37,119-123) Major manifestations included IUGR, microcephaly, large soft pinnae, hypertelorism, beaked nose, and extremely severe neurologic impairment, with holoprosencephaly in one instance After the observation of a further affected child born of one unaffected sister, in situ analyses revealed a cryptic t(2,4)(q37,p16) in the mother, letting us suspect a combination of 2q / 4p trisomy/monosomy in all the affected children of the sibship. Many private recurrent MCA/MR in the literature could result from similar symmetric translocations, undetectable by conventional banding techniques

3.011

Replication timing of untranscribed allelic DNA sequences associated with human centromers

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Replication timing and genetic transcriptional activity are correlated at the allelic level ; genes that display allele-specific expression (imprinted genes) replicate asynchronously, whereas most of the other genes (house keeping as well as tissue specific) replicate synchronously The present study was conducted to reveal the pattern of replication timing of allelic DNA-sequences not involved in transcriptional activity Hence, the pattern of replication timing of centromer-associated α-satellite DNA-sequences of chromosomes 10,11,17 and X was studied The analysis was performed on human female cells (PHA-stimulated lymphocytes) using the FISH technique The two allelic sequences of each autosome replicated highly synchronously, whereas the two allelic sequences of the X-chromosome revealed a pattern of early and late replication Evidently, autosomal allelic sequences not involved in transcriptional activity kept synchrony in replication timing while such

sequences on the X-chromosome were subjected to X-chromosome inactivation It appears therefore that replication timing in addition to being related to gene activity, reflects certain genomic organization associated with whole chromosome behaviour

3.012

Clinical features and molecular cytogenetic characterization of a de novo inverted duplication with a concomitant deletion of chromosome 3p26

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In a child with severe hypotonia, psychomotor retardation and congenital heart disease an inverted duplication of chromosome 3 with breakpoints in p21 3 and p26 was cytogenetically diagnosed The striking craniofacial features included a square-shaped face, frontal bossing, temporal indentation, down-turned corners of the mouth as described in other patients with trisomy 3p syndrome The cytogenetically observed inverted duplication was confirmed by CISS-hybridisation and multicolor fluorescence in situ hybridization (FISH) with yeast artificial chromosome (YAC) clones from the Centre d'Étude du Polymorphisme Humain (CEPH) library A subtelomeric deletion was diagnosed in the aberrant chromosome 3 with a newly constructed YAC clone from the 3p26->pter region (Helen Donis-Keller, Washington University School of Medicine, St Louis, MO 63110, USA) The telomeres were present on the recombinant chromosome 3 We propose an overall mechanism to explain inverted duplications with a simultaneous deletion in the distal breakpoint region

3.013

Analysis of small ring X chromosomes in patients with ring X Turner syndrome.

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Five patients with 45,X/46,X,r(X) karyotype were studied The ring in each case was smaller than a G group chromosome The rings were confirmed to be X in origin by FISH with an X centromere probe and X paint They were investigated for the presence of the XIST (Xi Specific Transcript) locus using FISH The replication status and transcriptional activity of the rings were investigated using a late pulse of BrdU and immunofluorescence with antibodies to acetylated histone H4 isoforms respectively The inactive X chromosome in somatic cells of females is marked by lack of H4 acetylation All rings were shown to contain XIST Four rings were shown to be late replicating and one ring was early replicating by BrdU H4 acetylation studies demonstrated bright fluorescence in the early replicating ring and lack of fluorescence in late replicating rings Magnetic resonance imaging was performed on two patients and structural brain abnormalities were detected The patients were assessed for learning disability There was a clear correlation between moderate mental handicap and an early replicating ring chromosome It has been previously suggested that ring X chromosomes deleted for XIST may be unable to inactivate and overexpression of X chromosome genes may lead to learning disability However in our case the early replicating ring in the patient with moderate mental handicap contained the XIST locus Although we have not yet assessed XIST expression our results suggest that there may be other sequences besides XIST involved in X chromosome inactivation

3.015

Trisomy 18 mosaicism revealed by hypofertility in a normal adult woman.

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A twenty-two-years-old woman searching medical assistance for hypofertility after two miscarriages had very slight facial anomalies ie mild macrogenia and prognathism, mild temporal depilation and no visceral defects (cardiac, renal, skeletal, cutaneous) Karyotype on peripheral lymphocytes revealed a 46, XX / 47, XX, +18 chromosomal constitution with four trisomic cells out of fifty Fibroblastic karyotype disclosed the following mosaicism 46, XX / 47, XX, +18 / 45, XX, -18 with only one monosomic and one trisomic cell out of a hundred. This is the tenth case (two men and eight women) of low level mosaicism for trisomy 18 and normal intelligence Three of these cases had led to trisomic 18 fetuses As five of them have been reported in the last two years, this particular chromosomal anomaly may have been overlooked and may be not so exceptional and should be searched in case of hypofertility with slight dysmorphism

3.016

Detection of Y chromosome sequences in patients with Turner syndrome

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It has been speculated that liveborn individuals with Turner syndrome arise from mitotic error in an early zygote and are actually mosaic This mosaicism may be of a low level and is thought to give a fetoprotective effect for survival during pregnancy, due to the presence of a locus or loci on a second sex chromosome (X or Y). Detection of Y sequences in the second cell line are of crucial diagnostic importance because phenotypic females with a Y chromosome and gonadal dysgenesis, have about a 20% risk of developing gonadoblastoma Here we present a PCR method to detect low level mosaicism of Y chromosome material in 45,X patients, who had no cytogenetic evidence of a second cell line To date, eight patients have been studied and one has been shown to be positive for Y sequences

3.017

A 46,XX 18p-/46,XX 18q- mosaic with a 46,XY,18p- child.

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A 15 year old boy presented with longstanding developmental delay, a severe communication disorder, seizures and hyperactivity He was in residential care, with very little speech, and had major aggressive outbursts He had moderate short stature, with large posteriorally rotated ears, coarse facies, a thin philtrum and upper lip, and short hands He had no major malformations A karyotype performed at age 12 was 46,XY,del(18)(qter→p11 2), and his clinical features were consistent with 18p- His father and normal brother both had normal karyotypes His mother had two previous miscarriages, and had mild short stature of 150 cms. She led an independent life, and had no major schooling or behaviour problems She had a normal head circumference, a left single palmar crease, a slightly protruding lower lip, and a smooth upper lip Her lymphocyte karyotype showed 198 cells with a 46,XX,del(18)(qter→p11 2), and 2 cells 46,XX,del(18)(pter→q21 3) Skin fibroblast culture of 200 cells showed 68% cells with 46,XX,del(18)(qter→p11 2), and 31% 46,XX,del(18)(pter→q21 3), with 1% 47,XX,del(18)(qter→p11 2) Her

parents and sibs were not available for karyotype analysis Her clinical phenotype was not consistent with either 18q-, 18p- or r(18) syndrome This is the first report of a healthy person with mosaic 18p-/18q-, who has a a child with characteristic 18p- syndrome. The mother's mosaic karyotype may have arisen from fission of a r(18) However, in either case there is a striking contrast between her relatively normal phenotype and a karyotype which would predict severe consequences

3.018

Follow-up investigation of confined placental mosaicism for autosomal trisomies detected on chorionic villus sampling .

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The principal disadvantage of chorionic villus sampling (CVS) as a method of prenatal diagnosis is the observation, in 1-2% of samples, of apparent feto-placental cytogenetic discrepancies. These discrepancies, which often involve mosaicism for autosomal trisomies, are most often due to confined placental mosaicism (CPM). CPM, at least for some chromosomes, is associated with an increased risk of gestational pathologies such as intrauterine growth retardation and fetal demise Additional factors important in determining potential negative effects of CPM include the proportion of abnormal cells, the potential presence of uniparental disomy (UPD) and, perhaps, the parental origin of the chromosomal copies. We have collected samples from 15 placentas from gestations with feto-placental discrepancies involving autosomal trisomies, detected on CVS The chromosomes involved were 2,3,7,13, 15 and 16 Cytogenetic analysis of multiple placental biopsies includes classical cytogenetics and/or interphase fluorescent in situ hybridization (FISH) using alpha-centromeric probes. The fetal karyotype is also confirmed postnatally or after abortion CPM has thusfar been confirmed in term placenta for cases involving chromosomes 3,15 and 16 Fetal UPD was present in two cases with CPM for trisomy 16. Clinical and histological characteristics are currently being analyzed in the light of cytogenetic characteristics (This work was supported by Swiss FNRS grant no 32-40 86294)

3.019

Uniparental disomy research after ambiguous results on CVS.

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Feto-placental cytogenetic discrepancies, observed in 1-2% of chorionic villus samples, reflect either meiotic or mitotic errors. Aneuploidy of meiotic origin carries an increased risk of fetal uniparental disomy (UPD), through "trisomy rescue" One of the ancillary studies of the European collaboration on prenatal diagnosis (EUCROMIC) is the search for UPD in children/fetuses whose gestations were ascertained through mosaic or nonmosaic feto-placental discrepancies Ninety UPD tests have been performed, using polymorphic PCR-based markers for the chromosome originally implicated in the CVS aberration The fourteen cases of maternal UPD revealed involved chromosomes 9,14,15,16 and 22 The highest proportion of positive results was found in cases where the ambiguous result involved chromosomes 15 or 16 No fetal UPD was revealed for some karyotype members, such as 7 and 13, suggesting that feto-placental discrepancies for these chromosomes may frequently have a mitotic origin The results of UPD testing have been correlated with clinical and cytogenetic criteria indication for CVS, type of CVS preparation, proportion of abnormal cells and pregnancy outcome According to these data, those gestations in which fetal UPD is most likely are those having a high proportion (>70%) of abnormal cells on CVS and those in which CVS was performed because of an abnormal gestational phenotype (supported by the European Union, BMH1-CT93-1673 and the Swiss OFES, 93 0337 2057)

3.020

Unusual karyotype in the offspring of an 11;22 translocation carrier.

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Only one type of unbalanced karyotype is seen in viable offspring of 11,22 translocation carriers This karyotype 47, XX or XY,+der(22) is associated with a fairly distinct phenotype We report a child with a similar phenotype born to a mother carrying an 11,22 translocation, whose karyotype was 46,XY/47,XY,+mar The extra chromosome which was smaller than the standard der(22) described was shown to contain material from both chromosome 11 and 22 by FISH The proband, S A , was referred at 2 5 years of age with severe developmental delay and facial dysmorphism He was the first of twins and his twin brother was unaffected. A cleft palate was repaired aged 2 years Weight was between the 3rd and 10th centiles and length and head circumference <3rd centile Scalp hair was sparse with two posterior hair whorls The forehead was broad and hirsute with an upswept anterior hairline. There was hypertelorism, flat nasal bridge, downslanting palpebral fissures, right-sided preauricular pit small mouth, small penis, alternating divergent squint and marked hypotonia. Cytogenetic analysis showed mosaicism for a marker chromosome. Of 106 metaphases examined 78 showed 47,XY,+mar and 28 showed 46,XY . Paternal karyotype was normal and maternal karyotype was 46,XX,t(11;22)(q23,q11 23) FISH studies showed that the marker chromosome contained the D22S75 sequence of chromosom region 22q11 as well as chromosome 11 material (whole chromosome 11 paint) The additional chromosome was felt to be too small to be the standard derived chromosome 22 found in offspring with unbalanced karyotypes inherited from 11,22 translocation carriers

3.021

Familial trisomy 12p due to a direct duplication.

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Trisomy of 12p is rare and only a few cases have been reported We report on a mother and daughter with trisomy of terminal 12p due to a direct duplication of this region The proband, N M , was referred at 19 months of age with global developmental delay and mild facial dysmorphism Birth weight was 2 63 kg Bilateral congenital dislocation of hips and positional talipes of feet were managed conservatively A right sided inguinal hernia was repaired aged 18 months Weight and length were between the 10th and 25th centiles and head circumference was less than the 3rd centile The nasal bridge was depressed and the ears were low set with overfolded helices The anterior hairline was upswept and the palpebral fissures were large with long eyelashes There was mild hypotonia Both parents were also examined. Her mother, M M , aged 19 years had a head circumference of 52 cm which was less than the 3rd centile She appeared to be slightly slow and had low set ears with overfolded helices which were very similar to her daughter's Cytogenetic analysis showed that the proband's and her mother had identical karyotypes [46,XX,dir dup (12)(p13 32->13 33)] Father's karyotype was normal Further family studies are in progress to see whether anybody else also has the terminal 12p duplication This family illustrates both the non-specific and mild effects of terminal 12p trisomy

3.022

Anomalies of thyroid function in Down syndrome children.

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Abnormal function of thyroid, especially hypothyroidy, was shown This study was undertaken in order to precise these anomalies Thyroid function of 105 children with Down syndrome (DS) aged 3 months to 20 years was studied by evaluation of serum concentration of zinc, thyrotropin, free T4 (FT4), free T3 (FT3), reverse T3 (rT3) insuline-like growth factor (IGF1) and antithyroid antibodies Twenty six of these children had a pituitary stimulation test Each DS child was matched to a control of the same age The mean concentration of thyrotropin of children with DS was increased while the mean concentration of rT3 of the DS children was significantly decreased compared to the controls, as was the ratio rT3/TSH When DS children are split into two groups, those with and those without increased thyrotropinemia, a significant decreased of the ratio rT3/TSH appeared in DS children with increased thyrotropinemia whereas there is no difference between these two groups regarding to the level of FT4, FT3, rT3 and zincemia However in all DS children serum zinc levels were lower than in controls Thyrotropinemia became normal soon after thyroxin treatment This study demonstrated that one half of the DS children had increased thyrotropinemia wich must be treated. This increase of thyrotropinemia is the result of an abnormal regulation of the hypothalamo-pituitary, axes as the pituitary answer after stimulation with TRH was normal

3.023

Mapping of the breakpoints of Robertsonian translocation chromosomes

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The short arms, the pericentromeric and centromeric regions of human acrocentric chromosomes are often involved in Robertsonian translocations, the most common balanced structural chromosome abnormality in all human populations Due to the high frequencies observed (0,1 % in lifeborn), and the fact that they often result in partial trisomies they play an important role in the etiology of Down syndrome The exact location of the breakpoints and the sequences involved in the crossover process are still unknown, because the short arms of the acrocentrics are almost completely composed of various classes of satellite sequences which cannot be used as markers Now, we were able to localize copies of the long range repetitive DNA-family chAB4 in direct neighbourhood to the centromere of chromosome 22 and most probably of all acrocentric chromosomes chAB4 is organized as a huge palindromic structure of approximately 250 Kb About 50 copies of the sequence are distributed to at least 15 different chromosomal loci Using chAB4 sequences as probes for FISH-analysis of 5 different Robertsonian translocations we were able to localize the breakpoints directly to centromeric sequences in all cases, as chAB4 was absent from all translocation chromosomes analysed Results obtained with a larger number of probands will be shown

3.024

Comparison of chromosome 21 specific probes developed for rapid prenatal detection of Down syndrome.

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We report on a study which compares rapid prenatal diagnosis of Down syndrome using fluorescence in situ hybridisation (FISH) with a chromosome 21 specific cosmid contig (cCMP21 a) and 21 specific Alu - PCR products from yeast artificial chromosome (YAC) clones (831 B9) Amniocytes were collected from 2 mls of unspun amniotic fluid Cells were hypotonically treated then fixed on aminoalkylsilane - treated slides Biotinylated probe DNA was denatured and preannealed with competitor DNA Cell DNA was denatured and hybridised overnight with the probe Signals were detected using alternating layers of fluoresceinated avidin and biotinylated anti - avidin. Cells were counterstained with DAPI and propidium iodide and 50 nuclei were scored from each sample. Hybridisation of 500 amniotic fluids with cCMP21 a produced 2 distinct signals on an average of 74% of cells from normal cases. An average of 50% of cells from trisomy 21 cases displayed 3 distinct signals Hybridisation of 250 amniotic fluids with YAC 831 B9 produced 2 distinct signals on an average of 89% of cells from normal cases 72% of cells from trisomic cases displayed 3 distinct signals. We conclude that YAC 831 B9 derived products along with FISH can be used satisfactorily for prenatal diagnosis of Down syndrome

3.025

Inverted duplicated chromosome 15(q12-pter) in proband and mother

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The proband presented at 3 years with developmental delay and non specific dysmorphism , no Prader-Willi or Angelman syndrome features were present Chromosome analysis of lymphocyte cultures revealed a 47,XX,inv dup(15)(q12-pter) karyotype in both the proband and the mother Subsequent analysis of other family members revealed normal karyotypes in 2 normal sibs, father and maternal grandparents Fluorescence in-situ hybridisation was performed using PWS/AS cosmids for regions GABRB3 and SNRPN Results indicated that the proband and mother showed two signals on the inverted duplicated chromosome PCR analysis using dinucleotide repeat markers from the PWS/AS region (D15S113,GABRB3) and 15p(D15S218) confirmed the presence of at least one extra copy in both mother and daughter Southern analysis with the probe PW 71 indicated that the probe hybridisation site(s) on the marker were methylated in both mother and daughter A differing banding pattern was however observed with the second methylation sensitive probe, Y48 5 which detects 3 bands - a constant band at 2 9kb, a paternally-derived band at 2 5kb and a maternally-derived band at 1 0kb The mother showed 2 9=2 5>1 0 while the daughter showed 2 9>>2 5<1.0 (controls 2.9>2 5=1 0) Future work using PWS/AS cosmids for different loci in 15q11-q13 region may possibly help to determine if the marker is identical in both patients

3.026

An 8;22 balanced translocation associated with profound B-cell immunodeficiency.

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We have investigated a patient (GB) with developmental delay, mild hemihypertrophy, haemangiomata and a profound B-cell immunodeficiency A remarkable feature of this case is that there were no signs of immunodeficiency until age 12, when the patient presented with recurrent infection Virtually no B-cells or B-cell precursors were detectable in blood, and analysis of a bone marrow aspirate suggested a block in B-cell differentiation prior to that seen in XLA. A karyotype revealed an apparently balanced translocation (46XX, t(8,22)(q24 1,q11 2) in both T-cells and fibroblasts As this is indistinguishable from the 8,22 translocation seen in Burkitt's lymphoma (BL) molecular cytogenetic investigations were undertaken FISH indicates that the chromosome 22 breakpoint occurs in the region commonly deleted in DiGeorge syndrome, but distal to the critical region, and proximal to the region involved in BL This rules out haploinsufficiency of a gene at 22q11 as a cause of the features described above The chromosome 8 breakpoint maps between D8S284 distally and D8S198 proximally A YAC from this interval has been shown to span the translocation breakpoint, this YAC contains the cMYC gene While mutation and/or dysregulation of cMYC is normally associated with B-cell over-proliferation (lymphomas), we suggest that in GB the translocation causes B-cell death Continuing work might elucidate further the role of cMYC in B-cell differentiation and apoptosis

3.027

Cytogenetic and molecular study on Angelman syndrome and Prader-Willi syndrome patients.

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We report on the cytogenetic and molecular study on 22 AS and 64 PWS typical patients. All the patients were clinically ascertained by the same physicians according to the Consensus Diagnostic Criteria. High resolution banding was performed on 18 AS and 52 PWS patients and the 15q11-13 deletion was found in 9 (50%) and 26 (50%) respectively. FISH analysis with specific cosmids was carried out on 20 AS and 40 PWS patients, allowing to demonstrate the deletion in 12 (60%) AS and 23 (57 5%) PWS In the PWS group two patients, one deleted and one with maternal uniparental disomy (UPD), showed a mosaic karyotype for the presence of an extra small, non satellited, marker chromosome By means of CISS hybridization we demonstrated that the small marker in the deleted and UPD patients originated from chromosome 15 and X respectively Another PWS patient was found to be carrier of a balanced 15q15q translocation Evidence provided by molecular studies indicated that the 15q15q translocation is an isochromosome of maternal origin Apart from this case, the UPD investigation in 7 PWS showed 5 patients with maternal heterodisomy and no UPD in 5 AS so far tested The nondeleted nondisomy individuals could represent a selected sample to be further investigated to identify rare mutational mechanisms underlying AS and PWS

3.028

Case of Xp+ characterized by CISS analysis.

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High-resolution chromosome banding and chromosomal in situ suppression hybridization (CISS) were used to identify a familial Xp+ chromosome in a 20 month-old female patient, with retarded growth and slight dysmorphism Replication study of X chromosome showed the abnormal X to be late replicating in 88% of cells, except for the distal part of the short arm, corresponding to the additional segment In order to determine the chromosomal origin of the extra material, the CISS hybridization technique was applied. X chromosome painting probe enabled us to exclude either a Xp duplication or the presence of X material on autosomes. Specific chromosome libraries for 14 autosomes, selected on the basis of high-resolution banding results, were used. This procedure enabled us to determine the 5 chromosome origin of the extra material on the tip of the X chromosome The combined application of high-resolution banding and CISS technique allowed us to define the karyotype of our patient as 46,X,der(X)t(X,5)(p22 3;q35 1) Previous reports on partial 5q trisomy showed a variety of clinical signs that were not present in our patient Thus the clinical phenotype may be less severe when an X chromosome is involved in an unbalanced translocation, compared to unequal autosomal interchanges.

3.029

Molecular characterization of a new type of chromosome 9 variant in two boys affected with a Bardet-Biedl syndrome.

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In the family we analyzed, a mother transmitted to her two affected sons an unusual pericentric inversion of chromosome 9, characterized by the presence of a darkly stained G-positive band on the short arm, immediately distal to the inverted heterochromatin The family of four sibs was referred because two boys are affected with Bardet-Biedl syndrome (BBS), an autosomal recessive disorder characterized by mental retardation, pigmentary retinopathy, postaxial polydactyly, obesity and hypogenitalism We investigated the nature of this additional band since the pathogenesis of this syndrome is unknown and genetic heterogeneity has been documented We showed by FISH analyses, that this additional band contains chromosome 9 specific sequences (WCP 9) located above an inverted heterochromatic region (D9Z1), but without duplication of alpha or beta-satellite sequences (D9Z5). We documented in mapping experiments using cosmids previously assigned to region 9q13-q21, that there was no inversion of proximal long arm sequences We determined by microsatellite analyses that this rearrangement was most likely unrelated to BBS since the affected children inherited different chromosome 9 from their father This new chromosome 9 variant is different from the three types of inversion described in the literature; the exact nature of the sequences involved is under further investigation

3.030

Prenatal detection of an unbalanced cryptic translocation in a fetus with intrauterine growth retardation.

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Cryptic translocations are subtle aberrations involving telomeric ends of chromosomes High resolution chromosome analysis and molecular cytogenetic techniques are mandatory to detect these events We report a case of an unbalalanced cryptic telomeric translocation in a child with mild dysmorphic features. Ultrasonography performed at 34 weeks of gestation showed intrauterine growth retardation, monolateral renal agenesis, interatrial defect and micrognathia Chromosome analysis on fetal blood and amniocytes by standard cytogenetic techniques revealed a potential abnormality of 13q terminal region Fluorescence in situ hybridization with a 13qter specific probe showed positive signals on telomere of the long arm of only one chromosome 13. The fetus was, therefore, monosomic for that region The same probe revealed, in the father, signals on 13q and 3p telomeres. These data suggested the presence of a cryptic translocation between 13q and 3p terminal regions. After delivery, the newborn presented also an intraventricular defect At nine months of age she showed moderate growth retardation This case, even if complicated by partial trisomy 3p, confirmed previous reports of a critical deletion region in band 13q32 which is deleted in all 13q deletion patients with severe malformations (expecially of the brain) but not in patients with minor malformations.

3.031

Monosomy 8q and features of Fryns' syndrome.

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De novo interstitial deletion of chromosome region 8q22-8q24.1 has been previously reported as being associated with Tricho-Rhino-Phalangeal syndrome Type II or Langer-Giedion Syndrome (LGS) We report the clinical history and the postmortem examination of a patient with a similar deletion and features of Fryns' syndrome, but without any clinical sign of LGS GR, 29 yrs, presented for IUGR and polydramnios at 37 weeks of gestation. Ultrasonography of the fetus showed bilateral cleft lip and cleft palate; diaphragmatic hernia, monolateral renal agenesis, clinodactyly Chromosome analysis on fetal blood revealed a de novo interstitial deletion of chromosome 8q Fluorescence in situ hybridization with a probe specific for the 8q21.3 region did not detect any deletion for that area Further analysis with total genomic library of chromosome 8 ruled out the presence of a translocation Therefore the karyotype was: 46,XY,del(8)(q22q24 1) The infant born at term lived few minutes Necroscopy showed bilateral cleft lip, cletf palate, left diaphragmatic hernia, secundary left ventricle hypoplasia, dextrocardia with inversion of the chambers and pulmonary hypoplasia; horseshoe kidneys and renal dysplastic disease, cryptorchid and small testes, cerebral frontal lobes hypoplasia, clinodactyly with hypoplasia of the first finger of the right hand At Rx examination, there was no evidence of bone defects

3.032
The association between sporadic somatic parental aneuploidy and placental aneuploidy in habitual abortions

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Some couples experiencing habitual abortion, reveal an increased proportion of aneuploid cells in their lymphocytes This study was aimed to determine whether a correlation exist between the proportion of aneuploid lymphocytes cells of these couples and that of the corresponding miscarried placentae 36 recurrently aborting couples and their miscarried placenta were cytogenetically analyzed 55 5% of the placentae, were chromosomally unbalanced, includind trisomies, mosaics, triploidies, and increased proportion (over 10%) of sporadic aneuploid cells. The couples were divided into two groups group no 1: couples with chromosomally unbalanced placentae, and group no. 2 couples which their miscarried placenta revealed a normal karyotype with no more than few sporadic aneuploid cells 70% of the couples in group no 1, revealed increased proportion of aneuploid cells in their lymphocytes, compared with 6 25% in group no 2 A significant correlation was found between the proportion of aneuploid cells in the placentae and the proportion of maternal (but not paternal) somatic aneuploidy The results indicates that recurrently aborting couples with chromosomally abnormal abortuses, have an increased proportion of aneuploid cells in their lymphocytes. This can reflect a tendency to nondisjunction in the somatic cells as well as in the gamets Therefore, these couples may be at risk for chromosomally unbalanced offsprings

3.033
Uniparental disomy (UPD) in Familial Mediterranean Fever (FýMF) patients , due to somatic recombination

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Uniparental disomy (UPD) is a genetic disorder leading to various deleterious effects The general view is that UPD arises from two errors in chromosomal segregation of the same chromosome Either two prezygotic nondisjunction (ND) incidents occurring independently, each in a different parent, or a prezygotic ND event occurring in one parent followed by a postzygotic one taking place in the early embryo In the present study we report two UPD cases (one of maternal origin and the other of paternal origin) suggesting that chromosomal mal-segregation is not necessarily the root of the processes leading to UPD in man Both UPD cases were Familial Mediterranean Fever (FMF) patients having one parent exhibiting the FMF phenotype (homozygote recessive) while the other was free of the mutant allele (as assumed from his ethnic background) As judged from five different markers along chromosome- 16, the two patients showed a uniparental inheritance of the distal region of the short arm of chromosome-16 (which carries the FMF locus) and a biparental inheritance of the rest of chromosome -16 Evidently, the process leading to UPD in each of the FýMF patients was a post-zygotic error caused by a single incident of somatic crossing over

3.034
Familial partial 7p monosomy ascertained by Chorionic villus sampling

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The patient was referred for chorionic villus sampling at 16 weeks gestation due to a cystic hygroma and omphalocoele detected on ultrasound scan A maternal blood sample was also taken for cytogenetic studies because the patient had dysmorphic features and possible craniosynostosis Analysis of both direct and long term CVS preparations revealed a 46,XY,del(7)(p15 or 21p21) karyotype Analysis of the maternal blood revealed the same unbalanced karyotype Fluorescent in situ hybridisation studies confirmed both karyotypes to be unbalanced, with no evidence of chromosome 7 material elsewhere in the genome We compare the clinical features of our patient with those of other similar cases in the literature The prevalence of craniosynostosis among patients with partial 7p monosomy is of particular interest We are unaware of any other familial cases with a similar deletion

3.035
A case study of 3:1 segregation resulting in partial monosomy 2 and 21 derived from a maternal balanced translocation

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Chromosome analysis of lymphocyte metaphase spreads from a 14 year old boy referred for moderate mental retardation, delayed puberty and obesity showed an abnormal karyotype, 45,XY,-2,-21,+der(2)t(2,21)(p25 1,q11 2or21) Parental follow up showed this to be an unbalanced form of a rearrangement carried by his mother (46,XX,t(2,21)(p25 1,q11 2or21). A predisposition to 3 1 segregation may be expected, due to the involvement of a small acrocentric chromosome A 3 1 segregation mechanism is suggested for the mode of inheritance in this case, resulting in a tertiary monosomy in the child The child is effectively partially monosomic for the terminal end of the short arm of chromosome 2 and the centromeric region of chromosome 21 The mother of this child carries a high risk of producing offspring with an unbalanced karyotype, including those with a Down syndrome phenotype This would occur if either an adjacent 1 or 3 1 segregation resulting in interchange trisomy occurred This critical region for Down syndrome appears to be intact on the derived chromosome 2 The mother will be offered prenatal diagnosis in any future pregnancy

3.036
Prenatal diagnosis of tetrasomy 9p: a case report

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A 37 year old women was referred for fetal blood sampling following observation of abnormalities on ultrasound scan at 24 weeks gestation Cytogenetic analysis revealed a karyotype of 47,XY,+dic(9)(q21) in all cells observed Parental karyotypes were normal The pregnancy was terminated at 26 weeks and post-mortem examinationshowed the fetus to have multiple external dysmorphic abnormalities including bilateral cleft lip and palate, hypertelorism and low set ears The hands were small with short digits and hypoplastic nails The legs were thin and tubular with hyper extended knees giving a "back to front" appearance The skull contained a posterior fossa cyst typical of a Dandy-Walker malformation The heart was small with a persistent (L) superior vena cava Cultures from fetal tissues and placenta revealed a karyotype of 47,XY,+dic(9)(q21) in all cells observed confirming the prenatal result Tetrasomy 9p is rare and has been reported in 13 previous cases The abnormalities observed in our case are consistent with other reported cases indicating a relatively distinct syndrome This case represents only the second report of prenatal diagnosis of tetrasomy 9p

3.037
Unexpected chromosomal rearrangements found in prenatal diagnosis.

Carrió, Ana[1], Soler, A[1], Margarit, E[1]; Costa, D[1], Balmes, I[1], Estivill, X[1], Farguell, T[2], Puerto, B[2]; Fortuny, A[2].
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At present, the greatest number of cytogenetic prenatal diagnoses are performed for the indication of advanced maternal age or positive biochemical screening for Down's syndrome, but in some of these pregnancies unexpected chromosomal rearrangements are detected We report here our data from 11,058 cytogenetic prenatal diagnoses carried out for an indication different to parental chromosome structural rearrangement. In 8,589 pregnancies an amniocentesis was performed and in 2,469 cases the diagnostic was carried out on chorionic villi samples From a total of 45 unexpected chromosomal rearrangements detected, 36 were familial, 9 were "de novo" and only in one case we couldn't know the origin of the rearrangement These rearrangements were 22 reciprocal translocations, 18 Robertsonian translocations, 5 inversions and 1 duplication. Among the familial rearrangements only 2 unbalanced foetuses were detected (1 in 5,500) From the 9 "de novo" rearrangements 4 were unbalanced foetuses (1 in 2,750). The outcome of balanced "de novo" rearrangements were normal newborns We conclude that the foetal karyotype should always be analysed whenever an invasive technique for prenatal diagnosis is performed Unexpected cytogenetic findings, when familial, allow the detection of other balanced members and the prevention of unbalanced offspring.

3.038
Identification of a de novo structural abnormal X chromosome by fluorescence in situ hybridization

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Fluorescence in Situ Hybridization (FISH) is a very useful tool for determining the origin of de novo structural chromosome anomalies We present the cytogenetic and FISH results of a girl with neuromotor retardation, facial dysmorphism and multiple congenital abnormalities. The chromosome analysis on lymphocytes by GTG banding revealed 46,X,Xq- The parental karyotypes were normal However the clinical findings of the patient was not compatible with a pure partial deletion of Xq The q arm of the derivative chromosome X was not fully painted by genomic library X Taking into consideration the clinical findings and the banding pattern of the derivative chromosome, genomic library 9 was assayed, it was noticed that the derivative X chromosome was painted distally on q arm. Based on GTG banding pattern the breakpoints for this rearrangement were identified as being Xq21 and 9q13 The identification of this rearrangement was only possible by the evaluation of clinical features, together with cytogenetic and FISH findings.

3.039
A structurally abnormal chromosome 22 characterized by fluorescence in situ hybridization

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High resolution banding and standard banding techniques may not always be helpful in the interpretation of de novo structural rearrangements Fluorescence in situ hybridization (FISH) with chromosome painting, centromeric alphoid and cosmid probes is a valuable method for the clarification of these rearrangements We present the results of FISH application in a case with a derivative chromosome 22 The patient is a 3-year-old boy with mental retardation, behavioral disorder and facial dysmorphism The chromosome analysis by GTG banding revealed 46,XY,22q+ The parental karyotypes were normal CBG banding and NOR staining showed a dicentric bisatellited chromosome 22 The satellites observed on both arms of the derivative 22 were different in size with NOR staining The genomic library 22 painted the whole derivative chromosome Only one signal was observed on the derivative by using the alphoid centromeric 14/22 (D14Z1/D22Z1) DGCR (D22S75 probe with D22S39 chromosome 22 control probe) probe was used to detect a possible duplication of 22q, only one Di George region was present Further studies will help to identify the origin of the inactive centromere of this chromosome

3.040
Three further cases of duplication 3q

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Duplication of the distal long arm of chromosome 3 causes a rather specific pattern of congenital anomalies, the dup(3q) syndrome Many of these patients in addition, have deletion of [0]a small segment from another chromosome, however, the findings of dup(3q) tend to overshadow the consequences of the additional deletion We present three further patients with dup(3q) Patient 1 is a 9 year-old boy with psycho-motor retardation, trigonocephaly with premature craniosynostosis, cranofacial asymmetry, facial dysmorphism and limb anomalies Chromosome analysis disclosed mosaicism both in lymphocytes and fibroblasts 46,XY/46,XY,invdup(3)(pter→q28 ·q28→q26.1 q28→qter)?. A whole chromosome 3 library painted the entire rearranged chromosome in addition to normal homologue Analysis of the clinical picture, the banded karyotypes and the FISH examination with probes from the presumably duplicated segment is planned to prove that the determination was correct Patient 2 is a 9 month-old boy with growth and psycho-motor retardation, agenesis of the corpus callosum, trigonocephaly, and facial dysmorphism. Banded chromosome analysis revealed a 46,XY,3p+ karyotype. The father's karyotype disclosed a pericentric inversion, inv(3)(p25q25) Thus, the child is trisomic for 3qter→3q25 and monosomic for 3pter→3p25 The same unbalanced karyotype as in patient two was determined from amniocytes in the last pregnancy of the mother Autopsy disclosed no findings other than the agenesis of the corpus callosum at the 18 week old fetus. These three patients contribute to the knowledge of the clinical picture of dup(3q) with or without concomitant deletion.

3.041
Stein-Leventhal syndrome (polycystic ovarian disease) with a balanced (2;10)(q13;q26) translocation

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Cytogenetic analysis of a 19 year old woman with hirsutism, obesity and secondary amenorrhea revealed a 46,XX,t(2,10)(q13,q26) chromosome constitution High levels of testosterone and LH/FSH ratio and enlarged polycystic ovaries led to the diagnosis of polycystic ovarian disease The cytogenetic investigation of the family showed that the translocation was inherited from the father who was the only living child of his parents who had known to have three spontaneous abortions One of the three sisters of the proband who had irregular menses also carried the translocation Since she got pregnant in the meanwhile, the hormone levels and ovaries could not be investigated. The female fetus who had also inherited the balanced translocation was lost at 26 weeks, six days after a traffic accident The breakpoint regions 2q13 and 10q26 are therefore candidate regions for the gene causing the polycystic ovarian disease Of candidate genes already mapped to these regions are inhibin-beta (INHBB) at 2cen-2q13 and the androgen sensitive fibroblast growth factor 8 (FGF8) at 10q25-26

3.042

A centric inversion of chromosome 12 detected by FISH

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Division of Biomedical Sciences, Cerrahpa¦a Medical Faculty & The Genetic and Teratology Research Center (GETAM), Istanbul University, Turkey

Cytogenetic analysis of a 39 year old male with hypoplasic testes and azospermia resulted in 47,XXY,mar(?12) chromosome constitution Fluorescence in situ hybridization (FISH) of the marker chromosome using a chromosome 12 specific centromeric probe revealed a break in the alphoid DNA followed by an inversion of the whole short arm resulting in an pseudodicentric inversion chromosome 12. The karyotype is then designated as 47,XXY,inv(12)(cent p13 3). The active centromere was randomly selected in each cell Inversions can effect fertility in heterozygote carriers but since the proband had also Kleinfelter syndrome it was not possible to correlate the infertility problem of the proband with the inversion. To our knowledge this is the first report of an inversion involving the centromeric alphoid DNA

3.044

Fetal cells in maternal blood : PRINS technique for efficient detection.

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To detect fetal cells using PRimed IN Situ (PRINS) labeling on nucleated cells separated from a maternal blood sample Ten ml peripheral venous blood was drawn from 10 pregnant women (12 to 35 WG) before amniocentesis and 3 controls Nucleated cells were separated using a double density gradient centrifugation Mononucleated and polynucleated cells were recovered by aspiration, submitted to hypotonic treatment, fixed and stored at -20°C The slides prepared with these cell suspensions were denatured and dehydrated The single target PRINS technique was then performed using chromosome 9, X and Y specific primers and double target PRINS using a combination of X and Y specific primers Control labeling efficiency was 99 8% for 9 and Y chromosome specific primers Spots corresponding to Y chromosomes were observed for 7 of the 10 maternal blood samples tested, fetal sex was in accord with the fetal karyotype The sample studied by double PRINS showed fetal nuclei with spots of two different colors The morphological aspect of labeled nuclei suggested they belonged to fetal granulocyte Non invasive fetal cell detection was successful in maternal blood by simple or double PRINS technique after a simple cell enrichment procedure in pregnancies as early as 12 WG This is a fast, simple, efficient method for determining fetal sex which could be applied to prenatal diagnosis of aneuploidies

3.046

Submicroscopic rearrangements of 16p13.3 in two families with alpha thalassaemia mental retardation syndrome (ATR-16)

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Submicroscopic chromosome rearrangements, particularly involving telomeres, have been associated with mental retardation in several clinical syndromes Previous studies of patients with a thalassaemia and mental retardation associated with deletions of the a globin locus (ATR-16 syndrome) have revealed deletions, truncations and translocations involving 16p13 3 We report here two families having members with the ATR-16 syndrome and rearrangements of chromosome 16 not detectable by G-banding analysis Both probands were shown to have a deletion of the terminal region of 16p by fluorescence in situ hybridisation (FISH) and molecular analysis Further FISH studies revealed that case 1 had an unusual rearrangement of chromosome 16 with 16q terminal sequences present on the shortened 16p13 3, presumably resulting from an earlier inversion and crossing over during meiosis. This abnormality was present in three family members, and therefore appears to be a stably inherited recombinant chromosome In the second case, FISH revealed that the deletion of 16p13.3 was due to inheritance of the unbalanced chromosome 16 derived from a maternal t(16,20)(p13 3,q13.3) The two family members who had the unbalanced form of the translocation were both mentally retarded, whereas those who had inherited the balanced form appeared phenotypically normal These two cases highlight the importance of looking for submicroscopic telomeric rearrangements in all patients with unexplained mental retardation and illustrate the value of FISH in the analysis of such patients

3.047

Recombinant chromosome 18 in monozygotic twins resulting from a maternal paracentric inversion inv(18)(q21.1 q22)

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Male monozygotic twins, first born to young healthy parents, presented at birth with prenatal growth retardation and dysmorphic features Both had high forehead, micrognathia, high arched palate, short neck, clenched hands with malposition of the thumbs, prominent heels, skeletal anomalies and disturbed auditory evoked potentials They were discordant for cardiac anomalies (ASD in one, multiple VSD in the other) as well as for pterygium colli and malposition of the feet, present in one twin Prometaphase chromosome analyses revealed, in both twins, a recombinant chromosome 18 with apparent duplication (q21 3q23), resulting from a paracentric inversion (q21.1q23) in the mother. FISH studies showed in addition a deletion involving 18q23-qter. Liveborn unbalanced progeny resulting from a parental paracentric inversion has been only rarely reported The discordant phenotypic expression of such chromosomal imbalance in monozygotic twins needs to be emphasized

3.048

Analysis of uncultured and cultured amniotic fluid cells with specific probes for chromosome X, Y, 13, 18 and 21.

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[1] *Institute of Medical Genetics and* [2] *Obsterican Hospital, Med Fac (Charité), Humboldt-University, Luisenstr 13a, D-10098 Berlin, Germany*

Cytogenetically different cell-lines are relatively frequent not only between different tissues of an organism but also in different cell populations of one and the same tissue The proportion of these mosaic cell lines can changes both during long time cell culture and in vivo over the years Because of cell selection in vitro cells used for direct preparation of chromosomes can represent another population than cells grown in culture This phenomenon was already shown in neonatal blood cells In amniotic fluid cell culture not all cells that were received by sampling are able to develop in the same way Therefore and because of artificial changes during the culture period the results after culture do not always represent the exact original state To demonstrate these differences we compare cytogenetic results in uncultured and in cultured amniotic fluid cells We have used fluorescence in-situ hybridisation (FISH) in interphase nuclei by applying α-satellite probes (tricolour probe mixture) which hybridise to the centromeric region of each of the chromosomes X (Xp11 1→q11 1), Y (Yp11.1→q11 1) and 18 (18p11 1→q11.1) and locus specific probes (dual colour probe mixture) for chromosome 13 (13q14) and 21 (21q22 13→22 2)

3.049

Unexpected chromosomal rearrangements found in prenatal diagnosis.

Carrió, Ana[1]; Soler, A[1], Margarit, E[1], Costa, D[1], Balmes, I[1], Estivill, X[1], Farguell, T[2], Puerto, B[2], Fortuny, A[2].
Prenatal Diagnosis Unit [1]Genetics Service and [2]Department Obstetrics and Gynecology, Hospital Clínic, Barcelona, Spain

At present, the greatest number of cytogenetic prenatal diagnoses are performed for the indication of advanced maternal age or positive biochemical screening for Down's syndrome, but in some of these pregnancies unexpected chromosomal rearrangements are detected We report here our data from 11,058 cytogenetic prenatal diagnoses carried out for an indication different to parental chromosome structural rearrangement In 8,589 pregnancies an amniocentesis was performed and in 2,469 cases the diagnostic was carried out on chorionic villi samples From a total of 45 unexpected chromosomal rearrangements detected, 36 were familial, 9 were "de novo" and only in one case we couldn't know the origin of the rearrangement These rearrangements were 22 reciprocal translocations, 18 Robertsonian translocations, 5 inversions and 1 duplication Among the familial rearrangements only 2 unbalanced foetuses were detected (1 in 5,500) From the 9 "de novo" rearrangements 4 were unbalanced foetuses (1 in 2,750) The outcome of balanced "de novo" rearrangements were normal newborns We conclude that the foetal karyotype should always be analysed whenever an invasive technique for prenatal diagnosis is performed Unexpected cytogenetic findings, when familial, allow the detection of other balanced members and the prevention of unbalanced offspring

3.050

Down Syndrome and aging: evaluation of 21 chromosome loss.

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The object of the study is to verify if the aging in Down subjects produces the preferential loss of chromosome 21 To test this hypothesis we set up PHA stimulated peripheral blood cultures from DS subjects of different ages and analysed more than 50 QFQ banded metaphases and checked the presence of mosaicism in at least 100 interphasic nuclei by FISH with 21 library We performed the study on 69 persons which show full DS phenotype, aged from 1 to 55 years and with primary trisomy. At the same time we evaluated a sample of 31 normal subjects, aged from 17 to 66 years On the whole a preferential loss of 21 chromosome was observed in 61 out of 69 DS persons, distributed by ages as follow. 14 cases <15 years % mean 2 50, range 0-9 72, 26 cases from 16 to 20 years % mean 4 00, range 0-8 82, 29 cases >20 years: % mean 5 13, range 0-22 91 In the control group the 21 loss was observed to range from 0 to 2 27 (% mean 0 66) All the other chromosomes were lost with similar frequency for both groups The presence of mosaicism was confirmed in nuclei after FISH

3.052

Uniparental disomy for chromosome 14 in robertsonian translocation 13-14

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Uniparental disomy (UPD) is caused primarily by meiotic non disjunction events, followed by trisomy rescue. Balanced Robertsonian translocations appear to be associated with an increased risk of UPD This condition is characterized by the inheritance of two homologous chromosomes from one parent and none from the other The consequence is the loss of function of imprinted genes or the expression of a recessive disease due to reduction to homozygosity Certain imprinting effects are known for chromosomes 7, 11, 14, and 15 Genetic counselling in subjects carrying a robertsonian translocation involving these chromosomes needs molecular studies to evaluate the presence of UPD We evaluated UPD in 13 subjects carrying a 13-14 robertsonian translocation Five cases were evaluated during prenatal diagnosis Molecular analysis performed using microsatellite markers, D13S174, D13S153, D14S64, D14S43 D14S51, D14S49, D14S52, D14S63 and MYH7 locus markers MYOI and MYOII UPD was detected in one case the molecular analysis showed the presence in the propositus of two chromosomes 14 of maternal origin and no chromosome 14 from the father indicating a maternal uniparental disomy for chromosome 14 (mUPD14) Our patient shows several similarities with other reported cases of mUPD14, suggesting imprinting of a region(s) of chromosome 14 and defining a possible mUPD14 Syndrome

3.053

Evidence by FISH for an intrachromosomal amplification of the hsp90β gene and for transcriptional activity within the site of amplification in the A431 tumor cell line.

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Numerous observations have led to the hypothesis that heat shock proteins (hsps) may be involved in some steps of tumorigenesis While an overexpression of hsps has been reported in many tumors, specific amplification involving these genes has never been reported so far In this context, the amplification of the hsp70 and hsp90 β heat-shock genes was investigated by FISH in three human tumor cell lines In the A431 cell line derived from a vulvar carcinoma, an amplification of the hsp90β gene - mapping the 6p12 locus - was evidenciated This amplification is restricted to one of the four chromosomes 6 present in this cell line, and is accompanied by a deletion in the 6p21 3 region spanning at least the histone H1 1 gene and a portion of the hsp70 gene Moreover, a detection of the nuclear hsp transcripts by FISH clearly demonstrates a transcriptional activity of the extra-copies of the hsp90β gene within the site of amplification Indeed, on the four foci corresponding to the accumulation of the nuclear hsp90β transcripts at the site of transcription, one appears as a cluster of fluorescent signals These results reinforce the hypothesis that hsps may play an essential role in the tumor process

3.054

Altered apolipoprotein E allele distribution in parents of Down syndrome probands.

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An increased risk of Alzheimer disease (AD) was recently reported in young mothers of Down syndrome (DS) individuals Allele e4 of the apolipoprotein E (apoE) gene is a genetic susceptibility factor for AD We decided therefore to examine the distribution of apoE alleles in probands with DS and their parents The material consisted of 188 Danish cases of non-mosaic, free trisomy 21 of known parental origin determined by DNA polymorphism analysis from a population-based study of DS We compared the frequency of apoE alleles in DS probands and their parents with a previously published Danish control sample In the DS probands there was no significant difference in the apoE allele distribution compared to controls The frequency of allele e4 in the fathers (11 8%) was significantly lower than in controls (17 4%, p=0 02). The frequency of allele e4 in the mothers (19 4%) was not significantly different from that of controls Nevertheless, in young mothers with a meiosis II error the e4 frequency was 30 0% significantly higher than in older mothers with a meiosis II error (13 0%, p=0 03) We hypothesise that apoE allele e4 is a risk factor for meiosis II nondisjunction in young mothers

3.055
Single cells translocations

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The number of chromosome aberrations in single cells of lymphocyte cultures of individuals with normal chromosome constitution was significantly greater in our routine cytogenetic practice after 1986 Standart blood cultures of 2657 patients were studied since November 1987 because of routine cytogenetic reasons Patients with radiation or chemical exposure and blood disorders were excluded A total of 16090 cells were analyzed and 27 sporadic aberrations were found 24 translocations, 1 invertion, 1 ring and 1 extra bisatelite chromosome Nine of the translocations were t(7,14), 2 - t(7,7), 4 - with affected 7 or 14. The distribution in each year of study was 1987 - 2, 1988 - 4, 1989 - 6, 1990 - 10, 1991 - 5 Single cell aberrations were not found in 1133 controls with 7097 cells referred for the same cytogenetic reasons before November 1987 We compare our results with cases in patients with ataxia-telangiectasia and with the increased incidents of malignancy in them The increase of single cell rearrangements in our routine practice without any change of our laboratory methods allows us to offer for discussion the eventual connection between this phenomenon and the Chernobyl catastrophe As it is well known, Bulgaria was one of the most affected areas

3.056
Atypical phenotype in Prader - Willi syndrome

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15q11-13 microdeletion is usually associated with the well known phenotypes of Prader-Willi and Angelman syndromes, but atypical clinical presentations have been also described Four out of more than 30 patients with deletion , we have seen in recent years, presented clinical symptoms, quite unusual for PWS. All of them had obesity and mental retardation, but their faces, growth and sexual development differed too much of the classical clinical picture The diagnostic significance of the cytogenetically detected 15q12 microdeletion is discussed from the point of view of genetic counseling in similar cases No doubt, final decision about diagnosis could be made only after DNA testing

3.057
Cross-hybridization of 13/21 alpha-satellite DNA probe to chromosome 22 in a family.

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Repetitive DNA probes are the most frequenly used in interphase cytogenetics to count the number of copies of a chromosome in interphasic cells Alpha-satellite DNA is a repetitive DNA located at the pericentromeric region of all chromosomes Every chromosome has its own specific alpha-satellite sequence, but chromosomes 13 and 21 as well as 14 and 22, share the same sequence Is well known than 13/21 alpha-satellite DNA probe is very polymorphic, and in many cases one of the chromosome signals is not obseved in interphase nuclei, it is because the alpha-satellite region of the chromosome doesn't hybridize due to the low number of copies of the alphoid sequence (Verma and Luke, 1992, Genomics 14, 113-116) This probe is not used regularly for prenatal studies by FISH, normally laboratories use specific probes to study chromosomes 21 and 13 In a prenatal study of amniotic fluid we had an non informative result using probes LSI21(Vysis) and 13/21(Oncor), the couple was studied cytogenetically and by FISH techniques co-hybridizing both probes Both had normal chromosome complement but in the woman we observed 5 signals with 13/21 probe and 2 signals of LSI21 in interphasic cells In metaphases, the extra signal were localized in a small acrocentric chromosome without LSI21 signal, identified as a chromosome 22 by cytogenetics We carried out a familiar study,and the same cross-hybridization was observed in her sister, her mother and in two sisters of the mother, but not in her maternal uncle This cross-hybridization between 13/21 probe and chromosome 22 has been observed by us in two more cases, and another case has been described (Verlinsky et al, 1995, Prenat Diagn 15, 831-834) It is important to know than the 13/21 probe used in interphase cytogenetics can produce false negative diagnosis due to the polymorphism of the sequence number of copies and false positive results in families with cross-hybridization with chromosome 22 Acknowledgments To Dr A Vergés, Centre Mèdic Teknon (Barcelona) This work was financed partially by Fundació Catalana Síndrome de Down (Barcelona).

3.58
Tissular mosaicism for partial trisomy

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Duplication of 5p whether partial or complete, has been described in over 50 patients with multiple congenital anomalies and mental retardation. The most common features reported include minor facial anomalies, cardiac defects, intestinal and renal malformations, limb abnormalities and hydrocephaly. Clinical report This 2880g male newborn was admitted to the neonatal intensive care unit for cyanosis and respiratory distress Physical examination demonstrated macrocephaly and dysmorphism with prominent forehead, frontal bossing, bilateral temporal hollowing, low set ears with unfolded helices, microretrognathia, broad thumbs, pectus excavatum, wide spaced nipples, hypotonia and muscular hypotrophy Cardiac investigation revealed a large ventricular septal defect and a severe aortic coarctation Cerebral ultrasound showed bilateral ventriculomegaly At day 3, the patient died and autopsy examination revealed hypoplasia of the corpus callosum and neurogenic myopathy Karyotype from peripheral leukocytes was normal Skin fibroblast culture revealed in all the metaphases a de novo marker chromosome, subsequently identified by FISH as a derived chromosome 5 Additional FISH analyses are underway to precisely map the segments involved in this partial trisomy 5 The report of this "new" chromosomal rearrangement highlights the need to study several tissues when a normal blood karyotype is found in an unusual or multisystemic clinical presentation

3.059
Mosaic "pure" interstitial deletion del(7)(q32q34)

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Several reports (90) have been published describing the clinical features associated with deletions of various segments of 7q But we haven't found any cases of the mosaic "pure" distal 7q deletion in the literature We report the first patient, a 11 years old girl with mosaic interstitial distal 7q deletion She was born with birthweight 2100 g in severe asphyxia, resulting afterwards in tetraparesis spastica On examination at the age of 11 years she was with short stature, several mental retardation and had microcephaly, microphthalmia and right sided coloboma of the iris Cytogenetic studies on cultured lymphocytes using GTG and R banding revealed a deleted marker chromosome 7 in 30% of cells Her karyotype was interpreted as 46,XX,del(7)(q32q34)/46,XX Up till now the majority of cases of distal 7q deletions were associated with duplications of other chromosomes giving quite severe clinical features holoprosencephaly, ectrodactyly and short life-time In our "pure" case the segment 7q32-q34 was very small and mosaic giving milder clinical features and long span of life It is also possible, that in our patient the main clinical feature - serious ocular defect (microphthalmia, coloboma of the iris) is caused by the gene located in chromosomal segment 7q32-q34

3.060

Association of sex chromosome abnormalities, extra bands in chromosomes 1 and 9, and rare fragile sites in infertile men

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We have examined constitutional chromosome abnormalities and rare fragile sites in 20 men with azospermia (5 cases)/oligospermia (15 cases) Chromosome abnormalities have found in two cases One of these have had a deletion of the long arm of the Y chromosome, 46,X,del (Y)(q12 qter), and the other case had 47,XXY karyotype Moreover, we detected extra G bands, especially in heterochromatin regions of chromosomes 1 and 9 result from some unexpected relations between euchromatin and heterochromatin regions during the meiotic divisions In the rare fragile site examinations, three fragile site carriers were detected and the regions of these were identified as fra(10)(25 2) fra(11)(q13 3), and fra(12)(q13 1). The overall frequencies of folate sensitive, FudR, and BrdU inducible fragile sites in oligo/azopermic cases were significantly higher than the control groups However, up to now, no any relation among the rare fragile site and infertility has been defined, therefore, it would be useful if large-scale studies on infertile cases are performed.

3.061

Association between unexplained mental retardation and pericentric inversion of chromosome 12

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A pericentric inversion in one homologue of chromosome 12, was seen in mother and also in her son living in the same chromosomal region is described The breakpoints of both cases were at 12p11 and 12p13 Both of the cases were also heterozygotic carriers of the inversion. This inversion was detected by the cytogenetic analyses (GTG, C- and HRB banding techniques) carried out in 30-years-old male patient refered to our center because of the unexplained mental retardation The proband's clinical features were mental retardation (IQ 60), being aggressive, schizophrenia, obesity and his initial speaking age was 12 The proband karyotype was detected as 46,XY,inv(12)(p11q13) His father's karyotype revealed as 46,XY and he was described phenotypically normal with physical examination Although no phenotypical features and mental retardation was seen in the mother, the chromosomal constitution of hers was also determined as 46,XX,inv(12)(p11q13) Thus, it was concluded that, the physical findings of the proband are the consequence of this inversion. The phenotype of the proband could be the result from the submicroscobic deletion/duplication on each chromatids, occured during the uneven number of crossover of inversion loop (mother's)

3.062

Improved prenatal diagnosis by FISH multiprobe and immunocytochemistry using a monoclonal antibody against 5-methylcytosine

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De novo structural chromosome abnormalities present special problems in prenatal diagnosis, especially with cases ascertained for routine cytogenetic screening for fetal Down Syndrome We describe a Case showing substantial extra chromosome material on 22p, where FISH analysis using a Multiprobe system (Cytocell Ltd) and immunocytochemistry with a monoclonal antibody against 5-methylcytosine together with traditional cytogenetic technology indicated the extra 22 p material to be composed of heterochromatin and repeated DNA sequences which were selectively methylated The additional 22p material made the aberrant chromosome the size of a 16 Traditional cytogenetic analysis to include G-, C-, DAPI and Ag techniques showed pale G-banding, negatice C- and DAPI staining, but some Ag deposition at 22pter FISH by the Multiprobe system indicated absence of extra 22p euchromatin derived from any of the 23 chromosomes analysed this way On the other hand, immunocytochemistry using the monoclonal antibody against 5-methylcytosine demonstrated a selective strong labelling, indicating the extra material to be composed of methylated repeated DNA sequences We conclude that this extra chromosome material, although substantial, is unlikely to be associated with any phenotypical somatic abnormality or mental disability of the child Parents elected to continue the pregnancy, and the child appeared normal at birth We further suggest that the combination of the new molecular and immuno-cytogenetics technology used in this Case will provide much improved diagnostic capability for Clinical Cytogenetics in general

3.063

FISH analysis of blood lymphocytes demonstrates increased frequency of chromosome aberrations in smokers

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It is well known that smoking is associated with an increased frequency of SCE s in blood lymphocytes Using FISH with composite probes for chromosomes 1, 2 and 4 or 1, 3 and 4 together with a pancentromeric probe we have now demonstrated an increase in chromosome aberrations of blood lymphocytes (trisomy, translocations, dicentrics, chromosome/chromatid breaks, centric rings, inversions and complex configurations) in 5 male smokers in comparison to that in 4 non-smoking controls Scoring approximately 2,000 metaphases persubject the frequency of chromosome aberrations in smokers was found to be highly statistically raised (p=0 001) in comparison to controls These results are of interest with respect to the recent observation of raised levels of chromosome aneuploidy in sperm of smokers (Wyrobek et al 1995, Amer J Hum Genet 57, 4, A131 (Abstr No 737)), which may be the result of 3·1 segregation of germ-lin translocations, induced in spermatogonial stem cells In a retrospective epidemiological study it has recently been shown that smoking in fathers is associated with an increased risk for cancer in their children (Sorahan et al 1995, Ann Epidemiol 5, 5, 354-359) It seems reasonable to conclude that the increased risk for childhood cancer may have been mediated via the smoking-induced genetic load in fathers' sperm Further research is required to identify precisely the alleged paternal germ-line mutations predisposing to cancer in offspring

3.064

Repeated fetal loss in two related first cousin marriages with couples all carrying the same translocation t(13q;14q)

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Cytogenetic and phenotypic findings and reproductive history of two related families in which both parents carried the same chromosomal translocation involving the long arms of chromosomes 13 and 14, are reported Family 1, couples being first cousins, was referred to our department because of having three first trimester spontaneous abortions in their reproductive history Cytogenetic analysis from the peripheral blood of the mother revealed a reciprocal translocation· 45,XX, t(13q;14q) Her husband also showed the same translocation Brother of the index patient was married to the sister of the index patient's husband (Family 2) Couples of family 2 were also first cousins and had experienced three first trimester spontaneous abortions Chromosomal analysis of these couples revealed the same chromosomal aberration, t(13q,14q) Cytogenetic studies of the mothers of these

two families, the mothers naturally being the "sisters", yielded the same robertsonian translocation, as expected No phenotypic abnormalities were found in any of these six translocation carriers Both families had no child until recently From the fourth pregnancy of "Family 2", a dizygotic twin was born a female with a normal karyotype but a congenital heart defect and a male with the reciprocal balanced translocation but also a right inguinal hernia and bilateral cryptorchidism in association with a hypospadias

3.065

A further prenatal diagnosis of mosaic tetrasomy 12p confirmed by in situ hybridization

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A further case of mosaic tetrasomy 12p detected prenatally is reported The 38 year-old mother in her third pregnancy was referred to our prenatal diagnosis center at 15 weeks' gestation because of advanced maternal age An amniocentesis was performed and amniotic fluid cell cultures yielded a 46,XX / 47,XX,+i(12)p? karyotype The fetus was suspected to have a Pallister-Killian syndrome The diagnosis was confirmed by FISH performed on amniotic fluid cell cultures An ultrasound examination showed the fetus to have a diaphragmatic hernia The pregnancy was terminated with the consent of the family The fetus showed multiple congenital abnormalities including a large and coarse face, frontal bossing, a high frontal hairline, hypertelorism, wide-flat nasal bridge, small, upturned nose, full cheecks, large mouth, short mandible, short neck with excess nuchal skin, and low set ears Fetal skin fibroblast cultures confirmed the cytogenetic diagnosis of tetrasomy 12p whereas the fetal blood yielded only normal karyotype Karyotypes of the parents were also normal Autopsy findings of the fetus confirmed the diagnosis of congenital diaphragmatic hernia

3.066

Turners syndrome and hypoparathyroidism in a female with a mosaicism for a rearranged X chromosome

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We have identified a female with Turner syndrome and hypoparathyroidism (HPT) with a 45X/46X,t(X,Xq,Xp) mosaicism The 46X,t(X,Xq,Xp) cells were observed in about 30% of blood lymphocytes in three consecutive analyses Prophase banding and FISH analyses showed that the rearranged X chromosome consists of virtually two complete X chromosomes The karyotype of the rearranged X chromosome is (XptelÆXq28 Xq11ÆXq28 Xp22ÆXp11)
Chromosome analysis of the mother showed a normal karyotype The female has a short stature, coarctation of the aorta and in addition, idiopathic HPT with low serum level of parathyroid hormone Molecular investigation revealed homozygosity for 17 polymorphic microsatellite markers along the Xp and Xq This suggests isotrisomi for a major part of the X chromosome in the 46X,t(X,Xq,Xp) cells X linked recessive hypoparathyroidism has been mapped to Xq26-q27 (Thakker R V et al, 1990) We hypothesize that the rearranged X chromosome is involved in the etiology of HPT in this patient, either by a direct disruption or by a positional effect Further FISH analysis is in progress in order to refine the breakpoints and to determine loss of chromosomal material from the translocated X chromosome We are also investigating the X inactivation pattern in the 46X,t(X,Xq,Xp) cell line using BrdU incorporation and antibodies against acetylated isoforms of histon H4 in order to characterize the distribution of inactive and active regions

3.067

Investigation of sex chromosomes spermatozoa from 47,XYY males using a three colour FISH procedure

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Objective As little is known about the meiotic behaviour of the extra Y chromosome in 47,XYY males, spermatozoa, final products of the meiosis, from two 47,XYY males were analysed by a three colour FISH procedure Materials and methods Semen samples analysis demonstrated normal parameters according to WHO standards Labelled probes specific for the centromere of chromosome 1,X and a highly repeat sequence on the long arm of chromosome Y were cohybridisex on interphase decondensed sperm nuclei This set of probes allows the identification of hyperhaploidies (spermatozoa with 24 chromosomes), diploidies (spermatozoa with 46 chromosomes) and their meiotic origin (meiosis I or II) After detection yellow, red and green signals were scored using a triple band pass filter FITC/rhodamine/DAPI To compare results from 47,XYY males and control men a Student test was performed A difference was considered significant at p=0 05 Results: For both 47,XYY men (24,315 sperm nuclei analysed from one male and 10,827 from the other one) sex-ratios differ to 1 1 proportion Compared to control sperm (142,050 sperm nuclei analysed), an excess of Y-bearing spermatozoa (23,Y, 24YY and 46,YY) was observed Conclusion Our observations suggest that a small proportion of primary spermatocytes with two Y chromosomes should be able to progress through meiosis and to produce Y-bearing sperm cells

3.068

Study of families with recurrence of free trisomy.

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Recurrence of free trisomy has so far been studied in a limited number of families We have studied 5 nuclear families with recurrence of free trisomy: 3 with recurrence of trisomy 21, 1 with trisomy 21 and 46,XX/47,XXY and 1 with trisomy 13 and trisomy 18 The karyotypes of the parents were normal in all the families The maternal age ranged from 21 to 43 years and the paternal age ranged from 32 to 49 years The origin of nondisjunction was studied by DNA polymorphism analysis using microsatellites spanning the length of chromosomes 21q, 18 and 13q, and an RFLP on the X chromosome. In 2 families the extra chromosome originated from different parents (1 family with recurrence of trisomy 21 and 1 family with trisomy 21 and 46,XX/47,XXY) In 2 families the extra chromosomes were of maternal origin (1 family with recurrence of trisomy 21 and 1 family with trisomy 13 and trisomy 18), but in the one sibling probably by mitotic, somatic origin (trisomy 18) One family is still under investigation Chance alone may explain the recurrence of several of the families However, more families are needed to investigate any possible genetic predisposing factor

3.069

Cytogenetic investigations in children with mental retardation and congenital malformations; the increase frequency of chromosomal variants 1qh+, 9qh+, 16qh+ among 1999 patients.

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Cytogenetic investigation of a group of children with nondifferential mental retardation and congenital malformations was performed Chromosome variants (1qh+, 9qh+, 16qh+) have been detected among 1999 children in 201 (10,1%) analysed cases 1qh+ -45 (2,3%), 9qh+ -132 (6,6%), 16qh+ -24 (1,2%) cases, respectively In the contrary, the frequency of chromosomal variants in control group of 200 healthy children was significantly lower (2,6%) It is possible that children with chromosomal variants can be considered as a group of risk for congenital abnormalities and mental retardation Hybridization in situ (ISH,FISH with chromosome specific DNA, complex Cot1-DNA probes for all centromeric regions) have been used for detalization of molecular structure of chromosomal variants (Vorsanova, Yurov, Demidova, Vechova, 1993, Soloviev et all, 1995) Independent variations of copy number of "classical" and alpha-satellite DNA sequences, forming heterochromatic chromosomal regions (1qh+, 9qh+, 16qh+) has been detected in patients with chromosomal variants Ref 1 Vorsanova S G , 1993, Cytology and Genetics, 27, 3, 72-78 2 Soloviev 1995, Prenatal Diagnosis, 15,2370248

3.070

Incidence of chromosome 21 disomy in human spermatozoa as determined by fluorescent in situ hybridization.

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Trisomy 21 is, by far, the most common type of trisomy in humans, about 95% of cases are originate by non-disjunction at meiosis (I or II) Analysis of the chromosomal constitution of human gametes will allow to determinate the incidence of non-disjunction in the parents, as opposed to the incidence of trisomy in their offspring Using fluorescent in situ hybridization (FISH), we have evaluated the incidence of chromosome 21 disomy in decondensed sperm heads Sperm samples were obtained from nine phenotypically normal healthy males. The samples were fixed in methanol · acetic acid (3 1) Sperm nuclei were decondensed by slide incubation in 5mM dithiothreitol (DTT) and 1% Triton X-100 A locus specific probe for chromosome 21 (Spectrum Orange,Vysis Inc) and a centromeric probe for chromosome 6 (Spectrum Green, Vysis Inc) were used for the dual FISH study The results show no interindividual differences among the individuals analysed and point out that the incidence of chromosome 21 disomy (0 38%) is significantly higher ($p<0.05$) than disomy for chromosome 6 (0.14%) This is in good agreement with the present knowledge on the tendency of some chromosome pairs to non-disjunction and supports the existence of either chromosome-specific mechanisms of non-disjunction, or of a differential effect of general non-disjunction mechanisms on some chromosome pairs

3.071

Extremely increased radiosensitivity of progeria cell chromosomes.

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Several cases of human inherited accelerated ageing, which cannot be refered to as Hutchinson-Gilford progeria or Werner's syndrome, have been described in Russia The patients demonstrate highly accelerated ageing, wrinkled skin, long lobes of the ears Mental development and fertility are normal The work was carried out on periferal blood lymphocytes of the patients and immortalised lymphoblastoid cells derived from the same patients using Epstein- Barr virus treatment The cells were X-irradiated at doses 0 5, 1 and 1 5 Gy Both, blood lymphocytes and lymphoblastoid cells of the progeria patient (PR3SP) showed 2-fold increase in the rate of X-ray induced chromosome aberrations compared to that in normal cells Frequency of sister chromatid exchanges after X-irradiation showed no essential changes It is known, and we also showed it for PR3SP skin fibroblasts, that progeria cells have sharply limited proliferative lifespan in vitro (no more than 10 population doublings) - it is the main cytological feature of progeria Immortalisation eliminates this phenomena However, the increased sensitivity of chromosomes to ionizing radiation remains, indicating that it rather has any other reason than the accelerated ageing

3.072

Acrocentric chromosomes without short arms: is there an increased risk for an autosomal trisomy due to isochromosome formation ?

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We report on two patients with a mosaic trisomy 21 and one patient with a mosaic trisomy 13 based on the presence of an isochromosome 21 and 13, respectively The first patient, a girl, was born with multiple congenital anomalies suggestive for Down syndrome Cytogenetic analysis of her lymphocytes revealed a 46,XX,del(21)(p11 1)/46,XX,i(21q) (18/31) karyotype. Both parents had normal karyotypes without a deletion 21p The second patient is a 58 years old female who lives at an institution for the mentally retarded She has Down syndrome Cytogenetic investigation of her lymphocytes revealed a 46,XX,del(21)(p11 1)/46,XX,i(21q) (7/25) karyotype The parents were not available for investigation. The patient has several normal sibs. The third patient was a newborn girl with multiple congenital anomalies suggestive for Patau syndrome (trisomy 13). A 46,XX,del(13)(p11 1)/46,XX,i(13q) (41/9) karyotype was found in a blood culture Chromosome studies of the parents were rejected The patient has one healthy brother In conclusion, all three patients showed two cell lines, one with an acrocentric chromosome without a short arm and a second cell line with an isochromosome and its normal homologue. This observation might implicate that acrocentric chromosomes with a deleted short arm predestine to isochromosome formation and thus give an increased risk for aneuploidy

3.073

Loss of the region 16qter in a child with an unbalanced cryptic translocation 46, XX, -5, +der(5) t(5;16) (p15;q24).

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We reported the case of an unbalanced cryptic translocation 46, XX, -5, +der(5) t(5,16) (p15;q24) in a girl born from phenotypically normal parents, presenting facial dysmorphic features, respiratory problems, a vesico-ureteral reflux of low grade, and mental retardation Initially, cytogenetic analysis of the proband's peripheral blood showed no chromosomal abnormality Later on a doubt for the integrity of the chromosome 5 in the mother, followed by a FISH analysis using chromosome 5 painting in her metaphase chromosome spreads, showed an apparent balanced

cryptic translocation t(5,16) (p15,q24), which was subsequently confirmed by a chromosome 16 specific painting The same probes revealed a partial monosomy of 5p$^{15}_{\rightarrow pter}$ and a trisomy of 16q$^{24}_{\rightarrow qter}$ on the proband The extend of the trisomy 16q$^{24}_{\rightarrow qter}$ on the proband was further analysed using the microsatellites AFM031xa5 (D16S402) and AFM196xg1 (D16S413) known to map to 16qter. The amplification profiles were clearly indicated inheritance of unique alleles from both parents in the child, excluding a trisomy of 16qter in the proband Thus, using these microsatellites a more complex chromosomal rearrangement was found. The presented family was a branch of a large family where three brothers of the proband's mother were also found to carry the apparently balanced cryptic translocation t(5,16) (p15,q24), while their children all phenotypically abnormal, were determined to have the unbalanced form of the translocation In the current case, for which a G syndrome was proposed in 1984, different techniques of molecular biology were necessary to finally unveil in details the chromosomal abnormality causing the abnormal phenotype

3.074
Molecular-cytogenetic diagnosis of chromosomal abnormalities using uni and multicolor FISH.

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Uni- and multicolor fluorescence in situ hybridization (FISH) with an original collection of centromeric, telomeric, region-specific DNA probes (plasmids, cosmids, PACs and YACs) were used for identification of chromosomal abnormalities in clinical cytogenetics The set of DNA probes was developed for rapid detection of more common chromosomal diseases - trisomy 21 (Down syndrome), trisomy 13 (Patau syndrome), trisomy 18 (Edwards syndrome), structural chromosomal abnormalities, fragile X syndrome, aneuploidies of sex (X and Y) chromosomes, including mosaic forms, marker chromosomes A novel in situ hybridization protocol and directly fluoresceinated DNA probes were used for rapid detection of chromosomal anomalies (during 15-30 min) In general, 5176 cytogenetic studies were performed, including 3208 postnatal and 1968 prenatal analyses Chromosomal aberrations were detected in 10 6% cases Molecular-cytogenetics was used in 164 cases with different chromosomal aberrations for detalization and confirmation of diagnosis Our experience showed that (1) FISH should be utilized only as an adjunctive test for classical cytogenetic studies; (2) FISH should be used for restricted number of cases when banding techniques are ineffective, (3) FISH allows to confirm the cytogenetic diagnosis, to determine the percentage of mosaicism or contamination by maternal cells in CVS, (4) multicolor probe detection gives an additional possibility in FISH analysis Our results demonstrate that new molecular-cytogenetic methods provides an accurate and simple approach for pre- and postnatal diagnosis of common chromosomal diseases

3.075
FISH mapping of centromeric and telmeric PAC clones

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We used P1-derived artificial chromosomes (PACs) clones with large insertions of human DNA (Ioannou et al , 1994) for isolation of centromeric and telomeric DNA probes No chimaerism and no insert instability have been observed for PACs with average insert size of 130-150 kb (Ioannou et al ,1994) More than 1500 alphoid and 600 telomeric clones have been detected in whole genomic PAC library, covering most part of centromeric and telomeric chromosomal regions in the human chromosomes FISH mapping of selected PAC clones was performed using different labeling and hybridization protocols. Alphoid PAC clones produce strong centromeric hybridization signals, but they usually need prehybridization with total human DNA to suppress interspersed repetitive sequences presented in most part of centromeric alphoid clones Telomeric PAC clones produce excellent signals and can successfully substitute cosmid or YAC telomeric probes In general, collection of PAC clones can be useful in detailed mapping of special chromosomal regions, containing unstable in others cloning vectors insertions of repetitive DNA, and for identification of heterochromatin or telomere associated encoding sequences Collection of PAC clones, consisting of stable and large insertions of human DNA, gives an rich source for detection of new DNA probes for human molecular-cytogenetics

3.076
Follow-up of human sperm aneuploidy rates after irradiation, using multicolour FISH

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Multiple structural chromosome abnormalities had been previously detected in the spermatozoa from a patient undergoing radiotherapy for a Hodgkin's lymphoma, using sperm fusion with hamster oocytes (Rousseaux et al. 1993, Hum Reprod, 8:903-907) The aim of the present study is to detect chromosome X, Y and 1 aneuploidies by fluorescence in situ hybridisation (FISH) on the same sperm samples Spermatozoa were collected, once immediately after the last irradiation session (day0) and a second time 38 days after the end of the treatment (day38). The centromeric probes specific for chromosomes X, Y and 1 were labelled with digoxigenin11dUTP, biotin16dUTP, or a mix of digoxigenin and biotin, co-hybridised on the interphase decondensed spermatozoa, and respectively detected as red, green and yellow signals Immediately after irradiation, the aneuploidy rates were high, most of them involving the X chromosome Indeed, the frequencies of 24,XY and 24,XX sperm cells, respectively 8 7% and 1.7%, were more than ten times those observed in controls These results suggest that irradiation would affect the X chromosome segregation during first and second meiotic division It would be of interest to study the segregation pattern of other chromosomes in this patient as well as in other patients undergoing radiotherapy.

3.077
A case of partial trisomies 10 and 21 detected by FISH analysis.

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Fundació Catalan Síndrome de Down Bacelona Spain

A peripheric blood sample from a 8 years old boy with dysmorphic features, diagnosticated at birth as having Down syndrome, was studied cytogenetically The result was ahigh mosaic trisomy 21 As the phenotype did not correspond to the laboratory findings, the study was repated in the proband and in his parents. This time the standard G banding lymphocyte study was completed by FISH For this purpose we used an specific alphoid probe for chromosomes 13 and 21 (D13Z1/D21Z1)(Oncor), an specific cosmid 21 probe (21q22 3, Oncor) and a direct labelled locus specific 21 probe (LSI 21, Spectrum Orange, Vysis) The cytogenetic study in the proband confirmed a 46,XY,Yqh+/47,XY,Yqh+,+21 (4%/96%) karyotype FISH analisys showed 4 hybridization signals when the 13/21 probe was used and only 2 signals for cosmid 21 probe, being one of the chromosome 21 polymorphic for the centromeric probe Co-hybridization with centromeric and LSI 21 proble was also carried out giving the same result Cytogenetic and FISH studies in the father were normal The cytogenetic result in the mother was 46,XX,t(10,21)(q25,q22) FISH analisys showed 3 hybridization spots for centromeric probe and 2 for the cosmid and LSI probels, but the cosmid and LSI were localized in a chromosome from the C group, identified as number 10 by cytogenetics The translocated chromosome 21 was also polymorphic for 13/21 probe This patient carries two different chromosome abnormalities, a small trisomy 10 due to an unbalanced translocation between chromosomes 10 and 21, and a partial trisomy 21 due to a chromosomic non-disjuntion The dysmorphic traits and the moderate mental retardation present in the proband is probably because of the

partial trisomies 10 and 21. The region lost in the chromosome 21 is the responsable of Down syndrome phenotype, as defined by Epstein et al (J Hum Genet 49:207-235, 1991) We thank Dr. V Català, E Cuatrecasas, MJ Acevedo and M. Crespo for their help

3.078
Searching for imprinted regions in the human genome.

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Genomic imprinting can be defined as the differential expression of a gene depending on the parent of origin. Our strategy for identifying new imprinted sequences in the human genome is based on three stages identify a chromosome potentially involved in genomic imprinting (1) Uniparental disomy (UPD) may affect development if the chromosome involved bears imprinted regions Structural abnormalities being associated with an increased risk of UPD, we systematically studied patients with anormal phenotypes and balanced translocations or inversions. Searching for UPD is performed by amplification of polymorphic microsatellite markers using standard PCR conditions (2) Delineate a potentially imprinted region on a selected chromosome Clinical data from patients with UPD for various chromosomes suggest that some of them, chromosomes 11, 15, 7, 14 , bear imprinted genes. In cases where imprinted genes have not been localized, comparative mapping with the murine genome allows the delineation of a smaller region, potentially imprinted, on these chromosomes (3) Precize the imprinted loci Recent investigations of DNA replication have demonstrated asynchronous replication of the two alleles of genes within imprinted domains We studied replication timing of specific genomic sequences on selected region of chromosome 14 by FISH analysis on interphase nuclei

3.079
Identification of two of XX male syndrom using fourescent in situ hybridization

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This poster describe two caes of XX male syndrom. One is 46, XX male and other with the mosaic karotype 46, XX/47, XX, + I X-Y interchange is not visable with conventional cytogenetic analysis Using flourescent in situ hybridization of WCP-DNA probe specific for Y chromosome, we demonstrated the result of abnormal crossing-over during the paternal meiotic cell division, which cause structural chromosome abnormality of X and Y chromsome A small part of Yp chromsome is translocated to Xp chromsome. We have used also WCP-DNA probe specific for X chromosome and Y chromsome for identification of an additional ring chromsome in the mosiac caes

3.080
Detection of X chromosome aneuploidies comparative genomic hybridization (CGH)

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Comparative Genomic Hybridization (CGH) is a new method for detection of unbalanced chromosome aberrations. CGH is based on a cohybridization of normal DNA with DNA to be analyzed, differently labelled, on normal metaphase spreads Chromosomeimbalances of the test DNA is detected by the measurement of the fluorescence intensities ratio CGH has been used as an adjunctive method to conventionnal cytogenetics in tumor cells and recently in clinical cytogenetic cases However, this method needs an image ananlysis equipment for detecting small deletions or amplifications For this reason, CGH has been developed and mostly used by research laboratories We have developed CGH method in our routine cytogenetic laboratory to evaluate its using without any analysis equipment We used DNA from patients with X variation number (46,XY, 48,XXXX, 46,Xi(Xq) for a CGH analysis An equimolecular mixture of test DNA and nromal DNA differently labelled were prennealed in a n excess of human Cot-1 DNA and then hybridized to normal metaphase spreads during 3 days Chromosome X copies variations were directly idetify on microscope by a different flurescence intensity of the X chromosome DAPI identified We demonstrate that CGH approach should be used to detect chromosome aneuploidiesby any routine clinical cytogenetic laboratory

3.081
A pattern of congenital anomalies including the Rieger eye malformation in a boy with deletion of chromosome 4q25->q27 following paternal insertional translocation ins(6;4)(q25;q25q27)

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The Rieger eye malformation is characterized by hypoplasia of the anterior part of the iris, ectopic pupil and/or coloboma, corneal clouding, hypoplasia of the anterior chamber, secondary glaucoma and variably other eye anomalies including cataract and microphthalmia It mostly occurs as the major feature of the dominantly inherited Rieger syndrome (RGS) in which it is combined with anomalies of facial and oral structures, umbilicus and sometimes with mild mental retardation Several patients in whom the Rieger eye malformation was part of a malformation syndrome showed an interstitial deletion in the long arm of chromosome 4, and in larger families with several affected members linkage to the epidermal growth factor (EGF) on 4q25 could be demonstrated. In addition, the Rieger syndrome was also found in a patient with a presumably balanced translocation rcp (1,4), with the 4q breakpoint disrupting the RGS gene A 6 year-old boy with severe to profound mental retardation displayed the following congenital anomalies: the complete Rieger eye malformation including microphthalmia and ectopic pupils, a round and bulbous nasal tip, misshapened ears with prominent anthelices, high-arched palate, a cardiac defect and the Wolf-Parkinson-White syndrome Growth and head circumference were normal GTG banded karyotypes revealed an interstitial deletion in 4q, the healthy father 's karyotype showed an insertional translocation ins(6;4)(q25;q25q27) Thus, the Rieger eye malformation in the deleted boy results from deletion of the entire RGS gene and not from disruption. As the deletion involves the entire band 4q26 and parts of the adjacent bands 4q25 and 4q27, the RGS gene must map to 4q25-q27 and, combining the cytogenetic data from previous similar cases, all with de novo deletions, probably maps to distal 4q25

3.082
Intrachromosomal triplication of 13q14

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A 4 year-old boy with short stature, microcephaly and developmental delay displayed the following minor anomalies low frontal hairline, deep-set eyes, upslanting palpebral fissures, excessive dental caries, pointed chin, extension contractures of the thumbs, single transverse palmar crease on the right, and hypertrichosis of the legs Banded chromosome examination revealed a 13q+ chromosome with an additional segment in the proximal long arm FISH examination with the retinoblastoma gene (RB1) probe (Oncor®, Inc) demonstrated three copies of the signal in the rearranged chromosome 13 and one in the normal homologue. The distance between the 3 signals was unequal, indicating that the middle repeat probably is inverted in orientation PCR analysis with markers D13S284 and D13S155 mapping to 13q14 3 disclosed paternal origin of the additional alleles. There are possibly non-furtuitous parallels between this case and another with interstitial triplication of 15q12 we recently observed (Schinzel et al 1995, J Med Genet 31 798-803) In both cases, the middle repeat was inverted,

indication the same mechanism of formation, The 15q12 triplication patient displayed findings similar to those common in Angelman syndrome which is caused by deletion or mutation of a maternally imprinted gene or genes, and the abnormal chromosome was of maternal origin while in this case the origin is paternal and the Rb1 gene which maps within the triplicated region is probably paternally imprinted

3.084

Rapid prenatal diagnosis in uncultured amniotic fluid using Fluorescence in-situ hybridization with directly labeled X and Y probes.

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In the last years, FISH (Fluorescence In-Situ Hybridization) has gained a significant role in cytogenetic diagnosis Chromosomal specific probes have been used to detect the number of copies in both interphase and metaphase nuclei FISH analysis in interphase nuclei enables detection of chromosomes abnormalities in situations where metaphase spreads are impossible to be analysed Particular attention has been focused on the application of the FISH technique for prenatal diagnosis in interphase nuclei Using X and Y chromosome specific directly labeled DNA probes (VYSIS), we have identified the sex of 25 fetuses in a minimal sample (3-5ml) of uncultured amniotic fluid, four hours only after amniocentesis procedure was carried out We confirmed the FISH results two weeks later, with the karyotyping analysis. The determination of sex was correctly identified for all the samples The FISH technology for prenatal diagnosis with direct labeling X and Y DNA probes is of emerging value as a rapid and accurate tool for the identification of fetal sex in X linked diseases and aneuploidies of the sex chromosomes and in cases where fetal anomalies are ultrasonically diagnosed in a relatively advanced gestation age

3.085

Prenatal diagnosis of a balanced whole-arm reciprocal translocation: cytogenetic and molecular characterization.

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Relatively few examples of balanced whole-arm reciprocal translocations have been described up to now, and this is, to our knowledge, the second described in prenatal diagnosis Fetal chromosome analysis was undertaken by amniocentesis for maternal anxiety and a balanced translocation was found The proposed karyotype was 46,XY,t(17,18)(17q18q,17p18p). His mother had normal chromosomes, but the same reciprocal translocation was found in the father, who was also affected by retinitis pigmentosa The fetal ultrasound findings were normal Since balanced non-acrocentric whole-arm reciprocal translocations are rare, and in order to confirm cytogenetic interpretation, fluorescence in situ hybridisation (FISH) was used to characterize accurately the breakpoints and involvement of centromeric DNA sequences in the chromosome rearrangements FISH was performed using whole chromosome paint probe and a satellite probe of chromosome 18 This study showed that der(17q18q) retained the centromeric region of chromosome 18, which was absent in der(17p18p) This information allowed to specify that the karyotypes were 46,XY,t(17,18)(17pter cen17 18pter,18qter cen18 17qter)

3.086

Incidence of disomic sperm nuclei in a 47, XYY male assessed by fluorescence in situ hybridization (FISH).

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Meiotic studies in 47, XYY males resulted in controversial hypothesis of the behaviour of the extra Y chromosome during spermatogenesis. Using fluorescent in situ hybridization (FISH) in decondensed sperm nuclei, we have assessed the sex-chromosome distribution in spermatozoa from a 47, XYY male compared to controls Semen samples were fixed in methanol · acetic acid (3 1) Sperm nuclei were decondensed by slide incubation in 5mM dithiothreitol (DTT) and 1% Triton X-100 Triple colour FISH with DNA probes for chromosomes X, Y and 18 was used for the study Statistical analysis revealed significant differences between the ratio of disomic spermatozoa present in the problem sample (0 39% XY, 0 13% XX, 1 31% YY), and our control series These results point out that, although the extra Y chromosome is usually eliminated during spermatogenesis, some germ cells are able to complete meiosis and, as a result, give rise to disomic spermatozoa Sex chromosome pairing and meiotic behaviour in this patient will be discussed

3.087

Prenatal Diagnosis of a X;Y translocation (q28;q11).

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Amniocentesis was carried out in view of an increased risk for Down syndrome according to maternal serum screening The foetal karyotype was 46,X,der(X)t(X,Y)(q28,q11) Karyotypes of both parents were normal Repeated ultrasound scans showed a foetus of apparently normal female anatomy Additional molecular, cytogenetic and FISH studies demonstrated absence of SRY and Yp material and non random inactivation of the der (X) The pregnancy is proceeding uneventfully so far X,Y translocations involving Xq are very rare The 3 cases reported thus far had breakpoints on Xq22 and presented with a female phenotype with Turner stigmata including ovarian dysgenesis To our knowledge our case is the first instance of X;Y translocation with breakpoint on Xq28 The presently available data enable to predict an unambiguous female phenotype and probably normal height and absence of Turner stigmata The question as to whether the child will present gonadal dysgenesis must await the results of gonadotropic response tests (planned after birth) as well as those of further FISH studies aiming at assessing the presence of Yq euchromatin

3.088

Partial monosomy 8p in an infant.

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A M , the first child of a 31 years old father and 25 years old mother was born at term after an uneventful pregnancy Her birth weight is 3210 g and her size 49 cm Her face is round with minor anomalies slight palpebral fissures, high nasal bridge, short nose, cleft palate, retrognatia and low set small malformed ears She has nor congenital heart defect nor hand and feet anomalies Her EEG and cerebral ultrasounds is normal Chromosome studies was performed on PHA stimulated peripheral blood using R banding technique Careful scruting revealed a deletion of the short arm of chromosome 8 in all metaphases examined and the karyotype was interpreted as being 46, XX, del(8)(p21?3) Fluorescence in situ hybridization (FISH), using a chromosome 8 paint confirmed the deletion, without translocation Molecular and classical cytogenetic studies showed a normal karyotype in the both parents Furthermore YACs from the CEPH library mapped to the short arm of

chromosome 8 have been used for FISH to specify the size of the deletion and the breakpoints This study was helpful in comparing the clinical features of this patient with those of the cases previously reported

3.089
Development of Primed IN Situ labelling (PRINS) for cytogenetic analysis of somatic hybrids, cell lines and tissue sections.

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The primed *in situ* (PRINS) labelling method was developed as an alternative to classical cytogenetics and fluorescence *in situ* hybridization (FISH) for the characterization of interspecific somatic hybrids Full karyotypes were performed by PRINS using Alu specific primers generating the painting of all human material associated with R-like banding The representativity of individual human chromosome was established using primers specific for discriminent α-satellite DNA sequences providing specific signal on the centromeres of the targeted chromosome and corresponding spots in interphase nuclei The methodology was tested with several classical and irradiated somatic hybrid lines Thus, a clone was demonstrated monochromosomal for the der(11) from a t(11,22) patient Tne use of directly labelled hapten such as fluorescein-12 dUTP allows that the whole process can be performed under a timespan of 2 hours Primers specific for the centromeres were also developed for cytogenetic characterization of colon cancer cell lines like Caco2 and HT29 The methodology was adapted to frozen tissue sections which may be useful for cytogenetical analysis in pathologies particularly in cancers

CANCER GENETICS

4.001
Familial Hodgkin's Disease: epidemiological characteristics suggest a genetic aetiology.

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Aetiology of Hodgkin's Disease (HD) is still poorly understood, with putative involvement of genetic and/or environmental factors However, it is well documented the existence of a familiar form of the disease (FHD), with a 3 to 7-fold increased risk for developing HD in relatives of patients Following two reports of FHD in which a net prevalence of male cases was incidentally inferred, in order tbeo tter investigate this aspect we compared the sex prevalence of all FHD patients retrieved in a comprehensive literature search with that of sporadic cases 328 patients with FHD were considered, and in this group the M/F ratio was 1 5/1, identical to that of sporadic HD When other parameters such as age at onset and histological type of the lymphoma were considered, again no difference was found between familiar and sporadic cases An unexpected finding was the absence of the second peak of incidence, corresponding to subjects older than 50, when age of onset of patients with FHD was compared to that of sporadic cases This notion raises challenging speculations as to the role of genetic factors acting predominantly early in life in the development of FHD

4.003
Bilateral sporadic retinoblastoma in monozygotic twins an unusual and unexpected observation

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An 18 months old girl was diagnosed as bilateral retinoblastoma in the Eye Department of Ataturk University Medical Faculty She had a huge tumor 7x4x5 cm in sizes in the right orbit and leucocoria in her left eye The diagnosis of retinoblastoma was established pathologically after enucleation of both eyes While trying to make the pedigree analysis we learned that our patient was a pair of monozygot twins In the clinical examination of second girl, bilateral leucocoria was observed and following enucleation of her both eyes, our results were confirmed histopathologically as retinoblastoma Except these twin siblings parents have three healthy sons and one more healthy daughter older than our patients All their eye examinations showed no abnormality Cytogenetic analyses of parents, monozygot twins and other children were performed and no numerical or structural anomalies were observed on pas irof 13 chromosomes Their pedigree analysis had no positive sign that it was inherited Even though no family history of retinoblastoma and intact chromosomes these monozygot twins have bilateral multifocal retinoblastoma Consequently, it is an interesting event of a probable de novo germ-line mutation of one of their parents

4.004
Chromosome analyses of malignant pleural effusions

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Histopathologic examination of malignant pleural effusions by standard cytologic techniques result enough or false negative identifications in about 50% case examined Cytogenetic analysis of the malignant pleural effusions demonstrate chromosome abnormalities both numerical and structural including marker chromosomes Cytogenetic diagnosis still is not included these routine techniques The present study of 25 malign pleural effusions were analyzed through direct chromosome analysis method to determine the role of cytogenetic diagnosis Slides were stained conventional Giemsa and GTG-banding techniques The slides were counted and evaluated until 20 suitable metaphases per patient According to the our findings in this study, 94% (23/25) of the patients were numerical and/or structural anomalies, 6% (2/25) of the cases with primer lung cancers were normal karyotyping According to the malignant pleural effusions etiology, 60% (15/25) of the patients have primary lung cancers, 24% (6/25) of the patients have metastatic breast cancer, 16% (4/25) patients have lymphoma Our study goes on We conclude chromosome analysis is useful diagnostic criteria in malignant pleural effusions

4.005
Genetic screening in retinoblastoma.

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Retinoblastoma (Rb) is the most common intraocular malignancy of childhood comprising 2-3% of all childhood cancers It has both hereditary (just over 40%) and non-hereditary forms Inheritance is autosomal dominant with high but incomplete penetrance (90%) Rb formation requires loss of both alleles of a tumour suppressor gene, RB1 Germline RB1 mutation carriers are also at high risk for development of a number of second cancers In cases with positive family history (15%) genetic screening for mutant gene carrier status is possible through linkage analysis In the absence of family history, identification of causative mutation is necessary for predictive testing in sporadic genetic cases (all bilateral sporadics, 2-5% of unilateral sporadics) In our Rb unit at St Bart's, using intragenic RB1 polymorphism we performed linkage analysis in 30 Rb families and carried out 5 pre-natal and numerous pre-symptomatic screenings in the last 2 years We now have genetic data on 96 Rb families together with tumour and constitutional DNA banked from both familial and sporadic cases With our successful RB1 mutation analysis programme predictive testing is becoming possible in an increasing number of sporadic genetic Rb cases including unilateral sporadics whose mutations were found to be germline following identification in tumour tissue

4.006
High frequency of loss of heterozygosity defines a subtype of renal cell carcinomas: specific loss at chromosomal regions 1p, 2p, 6p, 10p, 13q, 17p, and 21q characterizes the chromophobe cell carcinoma subtype

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Renal cell carinoma (RCC) comprises a heterogeneous group of kidney tumors The classification system currently used by the WHO does not include any subclassification Recently, a new classification system has been developed, primarily based on immunohistogenetic differences, postulating at least 7 subtypes of RCC Applying minisatellite analysis with highly polymorphic VNTR-markers, we studied more than 50 RCCs of different subtypes for loss of heterozygosity (LOH) at the chromosomal regions 1p, 2p, 6p, 7q, 10p, 11p, 13q, 14q, 17p, 21q and 22q, 3p probes were excluded, as LOH at 3p and mutations in the von Hippel-Lindau tumor suppressor gene are already established as specific events for the clear-cell subtype Our molecular findings were correlated with clinical and histopathological data Clear-cell and chromophilic cell tumors showed low levels of LOH at the sites tested, except for 14q Loss at 14q was not tumor-type specific, but correlates with vaadnced tumor stage Chromophobe cell tumors, however showed specific LOHs in the majority (60-90 %, i e 9/10) of the 10 cases studied at 1p, 2p, 6p, 10p,

13q, 17p and 21q Our findings strongly indicate that a specific pattern of LOH defines the chromophobe cell type as a moleculargenetic entity of RCCs

4.007
Gains and losses of DNA sequences in liposarcomas evaluated by comparative genomic hybridization

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Comparative genomic hybridization (CGH) was used to detect and map the regions of gain, amplification and loss of DNA sequences in 14 liposarcomas Thirteen tumors showed DNA sequence copy number changes of one or more genomic regions (mean six aberrations/tumor; range 0-17) The most common gains were seen at 12q14-21 (50% of tumors) Other frequent gains (29%) were of 1q21-24, 8cen-q21 2, 19q, and 20q Amplification was observed in six (43%) tumors and included as minimal common segments bands 12q15, 1q24 In five (36%) tumors, sequences at 1q21-24 and 1q32 were found to be gained simultaneously with 12q14-21 The most common losses of DNA sequences (21%) involved 9p21-pter and 13q21-qter The detection of a novel recurring amplicon at 1q21-24 and frequent simultaneous DNA sequence gains at 12q14-21 suggest that genes linked to both these regions may play a significant role in the development and progression of liposarcoms

4.008
Screening for breast cancer in women with a family history.

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The family history clinic in Manchester was established in 1987 and to 30/9/95 has seen and counselled 2,408 women Those women at twice the population risk or greater of breast cancer have been offered mammography at yearly intervals if under the age of fifty, and at two yearly intervals if aged between fifty and sixty During this time, 13 cancers have been detected, 5 prevelance cancers, 4 incidence cancers and 4 interval cancers This gives a detection rate of 3 86 cancers per 1,000 prevelance mammograms, a figure which is comparable to that of the National Breast Screening programme in women who on average are 10- 20 years older that our population It also gives a detection rate of 1 85 cancers per 1,000 incidence mammograms This study therefore provides preliminary evidence that screening of a high risk population (due to a family history of breast cancer) is likely to be an effective and necessary service

4.009
Investigation of the role of the DCC gene in colorectal cancer

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A complete or partial loss of chromosome 18 is thought to be an important event in the adenoma to carcinoma sequence of colorectal tumours The most common region of loss on chromosome 18 has been identified to be around the tumour suppressor gene, DCC (Deleted in Colorectal Carcinoma) localised within 18q21 In the present study we are determining the status of the DCC gene in several cell lines a transformed adenoma line (APC), adenocarcinoma (HNPCC), and two carcinomas (APC & sporadic colon cancer) by various techniques Conventional cytogenetic analysis showed chromosome rearrangement and loss involving chromosomes 1,2,8,13,14,17,18 and X. This was further confirmed by fluorescent in situ hybridisation (FISH) with chromosome paints and alpha-satellite probes Using seven yeast artificial chromosomes (YACs) as probes from a contig spanning the DCC locus, DCC was found to be present as 2 copies in all the colon cancer cell lines Evidence for intragenic DCC deletion was found in one cell line Amplification of markers within the gene by polymerase chain reaction (PCR) and analysis by single strand conformation polymorphism (SSCP) demonstrates rearrangements and deletions in the gene sequence Our results suggest the possibility of using chromosome 18q (DCC gene) loss as a prognostic marker in patients with Duke's B (non-metastatic) colorectal cancer

4.010
Molecular genetic analysis of gastric cancer

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Little is known about which genetic changes are important in gastric carcinogenesis We examined twenty-six gastric cancers for mutations of the APC and TP53 tumour suppressor genes by SSCP and heteroduplex analysis We also employed the protein truncation test (PTT) to screen the mutation cluster region (MCR) of APC LOH of the APC, TP53, MCC and DCC genes, and microsatellite instability were additionally investigated Mutations of the APC gene were detected in only 4% (1/26) gastric cancers whereas mutations of TP53 were found in 31% (8/26) tumours Fifteen microsatellite repeat loci were analysed for instability However this was an infrequent event occurring in just one tumour LOH was detected at microsatellite loci on chromosomes 14q, 22q, and 2p in the region of the hMSH2 gene LOH at 22q was most frequent (22% of informative cases) LOH of APC was observed in just 8% of informative cases, of MCC in 10% of cases, of DCC in 4 6% of cases, but of TP53 in 29% of cases We thus find alteration of the TP53 gene to be the most significant of these genetic changes in gastric cancer

4.011
Isolation of candidate BCLL suppressor genes from the chromosomal region 13q14.

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B-cell chronic lymphocytic leukaemia (BCLL) is associated with translocations and deletions, including homozygous deletions, involving the region between D13S273 and D13S284 in chromosome band 13q14. D13S25 is in the minimal region of overlap of the deletions This suggests the presence of a tumour suppressor gene in the proximity of this marker Seven YACs containing D13S25 were tested for their content of adjacent 13q14 markers Three of the smaller (400 kb) YACs mapped entirely within the region between D13S273 and D13S284 These were used as probes for the direct screening of cDNA libraries In this way, three cDNA clones of 1-2 kb were isolated, two of which hybridized to mRNAs of 2 3 kb, whilst the third hybridized to mRNAs of 3 5, 2 9, 1 7, and 1 3kb These clones are presently being analyzed Screening of databanks (EMBL/GDB) did not reveal similarity to other genes We are currently applying a 3' terminal exon trapping system on cosmids positive for at least one of the interjacent markers in order to find out whether more genes can be identified in the region

4.013

Microsatellite stability in heterozygous extratumoral tissues of hereditary non polyposis colorectal cancer (HNPCC) patients.

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It is now established that Hereditary Non Polyposis Colorectal Cancer (HNPCC) is due to mutatio insn a mismatch repair (MMR) gene Presently four human MMR genes, hMLH1, hMSH2, hPMS1 and hPMS2 have been identified as homologs of yeast MMR genes In most of the families the replication errors are revealed in tumors by allelic variation or instability in microsatellites as (CA)n repeats (replication error phenotype [RER+]) It has been established also that both copies of a MMR gene were mutated or deleted in tumors, which could result in this [RER+] phenotype But it is not clear whether extratumoral tissues exhibit a smooth [RER+/-] phenotype though both a germinal mutated and a functional wild type allele of the MMR gene are present Such a [RER+/-] phenotype has been advocated in order to account either for tumorigenesis or variation of triplet arrays responsible for several diseases like Fragile-X syndrome An extensive study of intergenerational variation of 80 microsatellites in HNPCC patients' offspring, in two families with hMLH1 or hMSH2 mutations, revealed no significant increase in allelic variation when compared to controls Such a result is corroborated by a recent study of Parson et al (Science, 1995, 268 738) who reported that only a small set of dominant mutations of hMLH1 or hPMS2 MMR genes could lead to microsatellite instability in extratumoral tissues The CGG triplet array stability of the FMR1 gene (fragile-X syndrome) in 10 HNPCC patients is now under progress through small-pool PCR analysis

4.014

Epithelial renal cell tumors: a cytogenetic and molecular model

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Renal cell carcinoma(RCC) accounts for 85-90% of all renal cancers in adults and represents 3% of all adult malignancies RCCs are a heterogeneous group with quite variable presentation and thus different prognosis WHO classification does not distinguish adequately between the various histological subtypes of RCC and therefore correlation of cytogenetic and molecular findings are reported rarely In 1986 Thoenes and Storkel proposed a classification based on cytomorphological, electron microscopical and histochemical observations We present data of more than 100 sporadic RCCs of four different subtypes of this classification system We applied Cytogenetics, FISH, PCR/SSCP- and microsatellite-analysis and correlated these findings with the clinical course of the patients Our findings support data from the literature, that there is a close histogenetic, cytogenetic and molecular genetic relationship between clear cell carcinoma and chromophilic carcinoma as well as between oncocytoma and chromophobe RCC

4.015

Germinal and somatic instability of a STR polymorphism of intron 1 of tumor suppressor gene TP53: loss of heterozygosity in adenocarcinomas.

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Expansion and/or contraction of repetitive DNA sequences have been associated with cancer and several humanen getic diseases. Mutations in TP53 locus are reported to be the most common single somatic genomic alteration in human cancer Therefore we have studied the pentameric short tandem repeat polymorphism (STR) of intron 1 of TP53 gene in terms of somatic and meiotic stability Genotyping of this STR was performed in PCR amplification products using polyacrylamide gel electrophoresis followed by silver staining The somatic instability of this STR was assayed in a sample of 21 gastric and colonic adenocarcinomas by comparison with DNA from paired normal cells Up to now we have not found mutations giving rise to alleles absent in paired normal cells, but we detected loss of heterozygosity in 1 out of 7 informative cases in gastric adenocarcinomas and 2 out of 5 informative cases of colonic adenocarcinomas These findings confirm the involvement of TP53 in these cancers and suggest that heterozygosity losses are more frequent in colonic than in gastric tumorigenesis Concerning meiotic stability, we have studied 117 nuclear families with 299 offspring and 238 mother/child pairs. Both analyses confirmed the regular mendelian patterns of inheritance, no cases of exclusions being found We conclude that this STR seems more unstable at the somatic level than in meiotic transmission Gene frequencies estimated from a random population sample from North of Portugal are also reported

4.016

Detection of trisomy 8 by conventional cytogenetic techniques and interphase fish analysis in 35 myeloprolyferative disorders: a comparative study.

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Trisomy 8 is a common finding in patients with Myeloprolyferative Disorders (MPD) Its role in the pathogenesis of these diseases is unclear To better understand the role of trisomy 8 in the biology of MPD, some studies have recently been performed using interphase Fluorescence In Situ Hybrydization (FISH) with unclear results In order to know the incidence of masked trisomy 8 in MPD, we performed an interphase FISH analysis in which the chromosome 8-specific centromeric probe (Oncor Inc , Gaithersburg) was used in 15 chronic myeloprolyferative disorders and 20 myelodysplastic syndromes with normal karyotypes in which an elevated number of metaphases were analyzed Three cases with trisomy 8 detected by Conventional Cytogenetic (CC) techniques (GTG banding) and three cases without hematological pathologies were included as positive and negative controls, respectively A statistical study was carried out in which percentages of cells bearing three fluorescent signals obtained in the control specimens and in the 35 MPD were compared Trisomy 8 was only detected by interphase FISH in positive controls, but we failed to detect masked trisomy 8 in the 35 MPD analyzed Some conclusions arise from our study 1 -Masked trisomy 8 is a rare event in MPD with normal karyotypes in which a high number of metaphases have been screened by CC techniques 2 -CC techniques (GTG banding) is a very reliable method for the detection of trisomy 8 in MPD The fact that we have detected trisomy 8 by FISH analysis only in the cases in which it was detected by CC techniques, suggests that trisomy 8, in the majority of cases, is beared by cells that are actively dividing

4.017

Breast cancer detection among women followed up or assessed because of their family history.

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Over a period of 16 years all women attending a symptomatic breast clinic have been asked if anyone in their family has had breast cancer Women who were found not to have breast disease, but reported a family history of breast cancer, have been invited back on an annual basis for evaluation. A specific clinic jointly run by surgeons and geneticists for women referred with a family history of breast cancer has also recently been established Approximately 600 women have been identified over the period and 12 have subsequently been found to have breast cancer The family and clinical histories of these 12 have been collected Four of the women lacked a family history sufficient to reach usually accepted criteria of significance Four women were over the age of 50 at diagnosis All the cancers were detected early, were operable and the women are doing well Identification and treatment would have been delayed if strict criteria for significant family histories had been applied to 4 of these cases

4 018 (Blank)

4.019

Identification of germline mutations in exon 11 of the BRCA1 gene in breast and ovary carcinoma patients.

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The occurrence of germline mutations in exon 11 of the BRCA1 gene has been investigated by the Protein Truncation Test (PTT) in three different groups of patients The first group included 21 women with either breast cancer diagnosed before 50 years or ovarian cancer and one or more first degree relatives with the same phenotype In this group mutations were found in 8 cases in 1 of 6 families (17%) with breast carcinomas only, in 2 of 6 families (33%) with ovary carcinomas only and in 5 of 9 families (56%) with both breast and ovary carcinomas The second group included 8 patients with breast or ovarian cancer in association with a second primary tumor and/or several cases of cancers of different histotypes in the family Only one individual, a woman who developed both breast and ovarian cancers, was found to carry a BRCA1 mutation Finally BRCA1 germline mutations were detected in 3 out of 43 women with early onset breast carcinomas, i e diagnosed before 36 years Only one of the three positive cases reported a family history of cancer These results indicate that, beside family history, the occurrence of both breast and ovarian cancers in the same individual and the early age of onset may be selection criteria for BRCA1 mutation screening

4.020

Familial risks of squamous cell carcinoma of the head and neck.

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We set out to determine the contribution of inheritance to the incidence of squamous cell carcinoma of the head and neck (SCCHN) We constructed two historical cohorts from the the FDRs (firs-degree relatives) of cases with SCCHN and the FDRs of spouses of cases. There were 1429 FDRs of 241 index cases of SCCHN and 934 FDRs of 156 index spouse controls We compared the relative risk (RR) of developing SCCHN in the FDRs of cases compared with the FDRs of spouse controls The adjusted RR for developing SCCHN if the index case had SCCHN was 3 79 (95%CI 1.11-13 0) There were no significantly increased risks associated with a family history of cancer at other sites The adjusted RR for SCCHN was 7 89 (95%CI: 1 50-41 6) in the FDRs of those with multiple SCCHN These data strongly suggest that genetic factors are important in the etiology of SCCHN, in particular for cases with multiple primary cancers Given the prolonged carcinogen exposure of these subjects, these genetic factors may have a role in modifying carcinogen activity or in host resistance to carcinogens Understanding this susceptibility may be of importance in the preventive management of those at high risk

4.022

Disturbances of APC gene mRNA and protein isoform levels due to an exon 14 splice acceptor mutation suggest a novel pathogenetic mechanism in FAP

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The protein truncation test (PTT) on APC transcripts reveals a complex pattern of in vitro-translated polypeptide chains This variety of proteins is due to multiple APC mRNA isoforms arising from alternative splicing of the exons 9, 10A and 14 as recently reported by us (Sulekova et al , Int J Cancer, 1995) Since these APC mRNA variants constitute transcripts derived from physiological splice events, i e skipping of the exons 9, 10A and 14, in vitro-translated proteins of reduced m w are also detectable in healthy individuals Having this in mind, we identified a novel mutation by PTT, which resulted in the constitutive skipping of exon 14 on one allele of the APC gene due to an inherited splice acceptor mutation Although, truncated proteins below 100 kDa are degraded intracellularly, a stable APC protein of 65 kDa was detected in cellular extracts of the patient's lymphocytes We have observed, that APC protein isoforms resulting from skipping of exon 14 exist, although at moderate levels, in the cell as physiological molecules, which are protected by an unique C-terminus of 19 aminoacids We suggest, that steady state level increase of the endogenous 65 kDa APC isoform at the expense of the classic APC protein of 300 kDa represents a pathogenetic mechanism leading to FAP Funded by Deutsche Krebshilfe, W88/93/Ba2

4.023

A leucine-zipper-like motif is encoded by APC gene transcripts derived from the brain-specific exon BS

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We have analyzed the expression of APC exon BS derived transcripts In contrast to reports published earlier APC mRNAs harbouring exon BS sequences are detectable in all cell lines analyzed so far including colonic cells and B-cells However, the major transcription start site is not in APC exon A, but rather at an as yet unidentified position in the 5' flanking region Such BS-specific transcripts consist of the traditional APC exon connections from 1 to 15 A second, much more interesting type of BS-derived mRNA molecules was identified to skip the first coding exon Most importantly a novel open reading frame of 64 aminoacids starts with an ATG in an optimal nucleotide context according to the Kozak-rules in exon BS and continues through the downstream located exons of the APC gene In vitro transcription/translation of cloned cDNAs specific for this type of transcript confirmed the synthesis of the deduced open reading frame, which was corroborated by immunochemical means using epitope-mapped mAbs The leucine-zipper-like structure of the exon BS-specific N-terminus of 64 aminoacids predicts this domain to function in the formation of coiled-coils Our preliminary analyses, however, do not support the notion that this exon BS-encoded domain mediates APC homo-oligomerization as the exon 1-encoded heptad repeat motif does Funded by Deutsche Krebshilfe, W88/93/Ba2 and Wilhelm Sander Stiftung, 094 001Ba

4.024

An evaluation of direct mutation screening in MEN2A, MEN2B, FMTC and 'sporadic' MTC families.

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MEN 2A, 2B and Familial MTC are relatively rare, dominant cancer syndromes characterised by an inherited predisposition to medullary thyroid carcinoma often associated with c-cell hyperplasia The 2A and 2B families also have an additional risk of phaeochromocytomas and/or parathyroid tumours, while the 2B families suffer from further complications including mucosal ganglioneuromas, and a marfanoid habitus The recent demonstrations that these three syndromes are due to allelic mutations in the RET proto-oncogene permits the accurate and early diagnosis of gene carriers and the exclusion from further follow up of those who have failed to inherit a causative mutation in families with characterised mutations In addition, given that an estimated 25% of all MTC cases diagnosed annually are the result of an inherited mutation, RET screening in newly diagnosed MTC cases could offer a means of targeting resources to these families while excluding those truly sporadic families from unnecessary screening and anxiety This paper reports our experience of providing such a diagnostic service since 1992 We have screened 170 individuals from 37 known families, and 54 'sporadic' patients with a variety of symptoms and no clear family history The observed mutation spectra are discussed in relation to recorded phenotype and direct mutation analysis is evaluated as part of a combined management strategy We conclude that mutation screening is a powerful and cost effective tool in the long term treatment and counselling of both familial cases and sporadic MTC patients

4.026

Pediatric brain tumours: loss of heterozygosity at 17p and p53 mutations.

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Cytogenetic analysis of primitive neuroectodermal tumours of the Central Nervous System (PNET) has demonstrated isochromosome 17q, as the most frequent chromosomal abnormality Molecular studies by RFLP's analysis has confirmed loss of genetic information on 17p The common area of loss has been restricted to 17p11 2 to 17pter This region contains the p53 gene, a role for this gene in PNET has been hypothesised However, mutations in p53 gene are very rare in this tumours This strongly suggests that another, as yet unidentified gene on 17p may be involved in these tumours We have performed a search of both loss of heterozigosity on 17p and p53 mutations (exons 5-8) by microsatellite markers and SSCP analysis respectively, on 20 childhood CNS tumours of distinct histologic types We have detected loss of heterozigosity in 5 cases all of them PNET Four of them did not imply the p53 locus while the other one presented a mutation in exon 7, not yet sequenced The area of common loss of heterozigosity in our cases includes the markers D17S926 and D17S938, and currently we are working with new markers in this interval to further delimit the region containing a new supressor gene

4.027

Search of mutations in the RB1 gene in patients with retinoblastoma

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The retinoblatoma gene (RB1) is the prototype for a class of recesive human cancer in which loss of activity of both normal alelles is associated with tumorigenesis This is a tumor supressor gene located in the long arm of the chromosome 13 (13q14) that have been cloned and sequenced The disease, the most common ocular tumor in children, arises when a mutation is inherited through the germ line and other mutation occurs somaticaly (40% of the cases), or when two mutations occur somaticaly (60% of the cases) The first type of cases (hereditary) are substancially all bilateral, including familiar and sporadic cases, and about 50% of the offsprings of these patients are affected So we are using a protocol of diagnostic based in the detection of mutations in the RB1 gene in order to 1 -Differentiate between hereditary and non hereditary cases. 2 -Offer to the families with risk of retinoblastoma an appropriate genetic counceling, prenatal and presymptomatic diagnostic With these objetives we have studied 44 families, 4 with family history and 40 sporadic cases The results obtained up today show a majority number of deletions So we have observed deletions in 4 patients with hereditary RB and 2 patients with nonhereditary RB, detecting this type of mutation with cDNA probes and polymorphic markers In two sporadic cases we have found puntual mutations in the codons 255 and 579 that generate a stop codon, analyzing leucocytes DNA

4.028

Molecular genetic diagnosis of hereditary non-polyposis colon cancer (HNPCC)

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HNPCC is one of the most common genetic diseases and may underlie as much as 15% of all colon cancers Members of two very large families with HNPCC have received genetic counselling over a period of several years Recently the mutations responsible for HNPCC in these families have been identified Molecular genetic tests have been devised to facilitate routine diagnostic testing in these families One of these mutations is a C to T transition at codon 601 of the MSH2 gene and is detected by digestion of an MslI restriction site introduced during PCR amplification The other mutation is a T insertion at codon 519 of the MLH1 gene and can be detected by heteroduplex analysis Predictive testing has been offered to over 100 people at 50 % risk of HNPCC in these families To date forty one predictive tests have been performed with 17 individuals being identified as mutation carriers and 24 being identified as non-carriers Only one person has declined predictive testing and one person has chosen to defer testing This high level of uptake for predictive testing in HNPCC families is in contrast with the low levels of uptake observed in other late onset genetic diseases such as Huntington's disease and familial breast cancer and probably reflects the availability of effective clinical management offered to at risk family members

4.029

6p abnormalities in acute lymphoblastic leukemia - nonrandom occurrence of a t(6;14)(p21-23;q32)

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In acute lymphoblastic leukemia (ALL) abnormalities of the short arm of chromosome 6 have been reported sporadically only To assess the frequency and the type of 6p aberrations we undertook a study in 553 patients with primary diagnosis (455 adults, 98 children) and in 65 patients with relapse of ALL (45 adults, 20 children) In 9 cases at diagnosis (1 6%) and in 4 cases at relapse (8 8%) anomalies of 6p were found Loss of material of 6p due to a deletion or an unbalanced translocation was present in 4 cases Reciprocal translocations were observed in 9 patients affecting various chromosomes apart from 6p Most remarkably, a translocation t(6,14)(p21-23,q32) known to occur nonrandomly in multiple myeloma (MM) and plasma cell leukemia (PCL) was identified in 3 cases, 2 with a pre-B- and 1 with a mature B-ALL immunophenotype, in all three cases accompanied by other chromosome changes In only 3 patients 6p rearrangements

were the sole cytogenetically detectable change, in the remaining cases mostly complex karyotypic changes were present in addition. In conclusion, because 6p anomalies were associated with additional aberrations in most cases, 6p rearrangements may rather be a late than an early event in ALL development which is also substantiated by the higher frequency of 6p anomalies in relapsed patients On the other hand, the occurrence of a t(6,14) in 3 of our patients may point to an early transforming event which due to additional aberrations may result in MM, PCL or ALL.

4.030

Variability in the breakpoints of 7q deletions in myeloid malignancies

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Deletions of the long arm of chromosome 7 are a non-random finding in acute myeloid leukemia (AML) and the myelodysplastic syndromes (MDS) In order to define the critical deleted region on 7q, we applied fluorescent in situ hybridization (FISH) to leukaemic cells from three patients with myeloid malignancies and the myeloid leukemia cell line GFD8 In each case the reported abnormalities of 7q involved deletions having a breakpoint within band q22 FISH with a whole chromosome 7 paint and a 7q telomere-specific probe demonstrated that one case was a "pure" interstitial deletion, one involved a terminal deletion of 7q, and the remaining two had an interstitial deletion of 7q and translocation of 7q terminal sequences FISH using a series of ordered cosmids from the regions 7q21 3-q22, 7q22, and 7q31 2-q31 3 showed that in each case the breakpoints were different, localised between the anonymous marker D7S811 (7q21 3-q22) and the cystic fibrosis transmembrane regulator (CFTR) gene (7q31 2-q31 3) (see Table)

Patient	Disease	Type of 7q abnormality	Breakpoint
A K	MDS - RAEB	Intersitial deletion	D7S811-D7S651
GFD8	AML - M1	Unbalanced translocation	D7S651- GNB2
S H	MDS	Unbalanced translocation	GNB2-CUTL1
E S	AML	TD	CUTL1-CFTR

GNB2= guanine nucleotide binding protein beta polypeptide 2, CUTL1= cut- like 1

These findings are in contrast to previous studies suggesting that the 7q22 breakpoint may be consistent We are continuing these studies on a larger series of patients in order to identify any critical region of gene loss

4.031

Von Hippel-Lindau (VHL) disease: germline mutation spectrum in the VHL tumor suppressor gene in families from Central Europe and correlation with phenotype

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VHL disease is an autosomal dominantly inheritable syndrome with multiple organ tumors, including hemangioblastomas of the central nervous system and retina, pheochromocytoma, renal cell carcinoma and pancreatic cysts We analyzed 84 VHL families from Central Europe for germline mutations in the VHL tumor suppressor gene to correlate genotypes with phenotypes and to provide non invasive presymptomatic diagnosis Large deletions were identified by Southern blotting with g7 cDNA and intragenic mutations by PCR/SSCP and sequencing

There was a wide spectrum of VHL mutations with phenotypic and geographic variations Thirty-three VHL mutations were identified in 65/84 families (76%), most being unique to this population All mutations in 22 families with pheochromocytoma were of the missense type Mutations in 43 families without pheochromocytoma but frequent renal cell carcinoma and pancreatic cysts consisted of 15 missense, 9 nonsense, 6 splice, and 5 frameshift mutations, 7 large deletions, and one inframe insértion Presymptomatic diagnosis is now available for the majority of Central European VHL families To produce pheochromocytoma the VHL mutant protein must be full length and retain some function In contrast, loss of function variants frequently give rise to renal cell carcinomas and pancreatic cysts.

4.032

Mutations analysis of the BRCA1 gene in familial and early onset sporadic breast cancer.

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Mutation of the BRCA1 gene conveys an increased risk of breast and ovarian cancer We have ascertained 155 individuals for BRCA1 screening who fit one of the following criteria, (i) a diagnosis of breast cancer under an age of 35 years, (ii) a family history of breast and ovarian cancer, (iii) at least one or more first degree relative with breast cancer diagnosed under age 50 years We have screened approximately 40% of the coding region in 41 patients, and four mutations have been identified by SSCP analysis and DNA sequencing Two frameshift mutations, including a novel two base pair deletion in exon 15 and two missense mutations, one a novel Met18Thr substitution in exon 2, which are both absent from 100 control chromosomes have been characterised We are evaluating the enzyme mismatch cleavage technique (EMC) as a potential alternative to SSCP analysis Using known heterozygous sequence changes, we have detected 9/11 mismatches through cleavage of a radioactively end-labelled PCR with T4 endonuclease VII, without any clean up steps Four of the detected mismatches have a very high signal to noise ratio and our results suggest that when dealing with heterozygous mutations, it is not necessary to include control DNA as a probe to form heteroduplex molecules

4.033

Cytogenetic analysis of the erythroleukaemic cell line, K562, using fluorescence in situ hybridization (FISH) and chromosome-specific paint probes.

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The hypotriploid, Ph^1 positive, human leukaemic cell line, K562, derived from a patient with chronic myelogenous leukaemia (Lozzio and Lozzio, 1975 Blood 45, 321), has been used extensively to evaluate the expression of erythroid and other enzyme markers As part of an investigation into the deficiency of PGM1 enzyme activity, we have used FISH to analyse the whole karyotype (modal no 65) Chromosome paints have so far shown two apparently normal chromosomes 1, and four additional chromosomes containing No 1 material der(1)t(1,11)(p32,q21), der(18)t(1,18) (p32,q23), der(21)t(1,21)(q32,p13)

and der(1)t(1,?)(p21,q12,?) The 6p+ and 7q+ markers seen in previous studies are both intra chromosomal extensions, and the two markers containing amplified C-lambda (from 22), and C-abl, (from 9), (Selden et al 1983 PNAS 80, 7289), appear to light up with paints 22 and 13 The composition of other unidentified markers is being analysed with twenty three chromosome specific paints, in rder to clarify the rearrangements for future investigations

4.034

Molecular and cytological investigation of the abnormallyexpressed phosphoglucomutase protein (PGM1) in K562 cells.

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The K562 erythroleukaemic cell line is associated with total deficiency of PGM1 enzyme activity, but the underlying molecular basis for this is not known The PGM1 protein is unusually polymorphic and it has been shown that the eight most common allelozymes, (1+, 1-, 2+, 2-, 3+, 3-, 7+, 7-), are generated by intragenic recombination between only three point mutations in the coding sequence (March et al, PNAS, 90, 10730, 1993; Takahashi & Neel, PNAS, 90, 10725, 1993) We report studies at the level of the protein, the gene and the RNA to investigate the possibility that the enzyme deficiency may have resulted from rearrangements of the PGM1 gene(s), perhaps involving non-reciprocal crossovers between the two recombinagenic regions Western blot analysis confirmed the absence of PGM1 antigen, karyotyping and fluorescence in situ hybridisation revealed the presence of three chromosomes 1, each carrying the PGM1 gene and Southern analysis using a PGM1 cDNA probe provided no evidence for structural rearrangements Very low levels of full length PGM1 mRNA transcripts were detected by RT-PCR, indicating that the cause of PGM1 deficiency in K562 is abnormal regulation

4.035

Microsatellite instability in non-HNPCC familial colorectal cancer

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The incidence of the hereditary non-polyposis colon cancer syndrome (HNPCC) has been estimated to range from 1-10% of all colorectal cancers. However, there is no distinctive phenotypic marker for HNPCC and the current diagnostic criteria may exclude many patients with an inherited predisposition to colorectal cancer Most colorectal cancers from HNPCC patients display microsatellite instability, a phenomenon ascribed to replication error the RER+ phenotype Genetic linkage studies in British HNPCC kindreds suggest that most families have mutations in *hMSHG2* and *hMLH1*. Relatively few kindreds, however, with familial colorectal cancer, will satisfy strict HNPCC criteria We have investigated two groups of such patients for evidence of mismatch repair gene mutations The RER+ phenotype was assigned if the tumours showed instability in at least 2 out of 5 microsatellite markers. In 30 kindreds where the age of onset was over 50 years for all affected members, only 2 had RER+ tumours Similarly, in 30 families where at least one affected member had an age of onset of below 50 years, only 3 had the RER+ phenotype We conclude, therefore, that most non-Amsterdam criteria familial colorectal cancer is not caused by mismatch repair gene mutations

4.036

Analysis of *hMSH2* and *hMLH1* in HNPCC kindreds.

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HNPCC may account for up to 5% of all cases of colorectal cancer Recently, four human homologues of bacterial DNA mismatch repair genes involved in the pathogenesis of HNPCC have been isolated (*MSH2, MLH1, PMS1, PMS2*) However, genetic linkage studies have suggested that mutations in *MSH2* and *MLH1* will account for the majority of English HNPCC families (Froggatt et al J Med Genet 1995,**32** 352-357] We have investigated 17 HNPCC families for whom lymphoblastoid cell lines were available for mutations in these two genes, initially by RT-PCR and the protein truncation test To date we have identified *MSH2* mutations in 4 families, and *MLH1* mutations in 2 others One *MSH2* mutation was detected in 2 families, and has subsequently been identified in a further 2 families [Froggatt et al Lancet 1995,**345** 727], with haplotype analysis suggesting independent mutations rather than a founder effect. Direct sequencing of cDNA from the remaining 11 families has since detected 2 missense mutations, one in each gene Each substitution tracks with the disease through the family concerned, and each is predicted to alter a conserved amino acid In 5 of the remaining 9 families, linkage analysis was possible and implicates *MSH2* Further cDNA sequencing is in progress

4.037

Molecular genetic determinants of phenotype in von Hippel-Lindau disease (VHL):

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VHL disease is a dominantly inherited familial cancer syndrome predisposing to retinal and central nervous system haemangioblastomas, renal cell carcinoma (RCC) and phaeochromocytoma We have previously demonstrated that allelic heterogeneity explains interfamilial differences in predisposition to phaeochromocytoma as there is a significant association between phaeochromocytoma and missense mutations. To determine if this association was caused by a "dominant-negative" mechanism, we calculated the age-related tumour risks for different classes of VHL gene mutations in 106 kindreds Large deletions and mutations predicted to cause a truncated protein were associated with a lower risk of phaeochromocytoma (6% and 9% at 30 and 50 years respectively) than missense mutations (40% and 59% respectively) and that missense mutations at codon 167 were associated with a particularly high risk of phaeochromocytoma However cumulative probabilities of RCC, and retinal and cerebellar haemangioblastomas did not differ between the two groups of mutations In addition we have identified germline missense VHL gene mutations in kindreds with familial phaeochromocytoma only These findings suggest that missense mutations predisposing to phaeochromocytoma do not act in a generalised dominant-negative manner, but that the VHL protein has multiple tissue specific effects

4.039

Ascertainment of families with a history of breast and bowel cancer in the Aberdeen Genetic Clinic

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Between January 1992 and November 1995, 1015 families have been seen at the Aberdeen Genetic Clinic with a family history of cancer drawn from a population of 500,000 Of these, a total of 86 families had a history of breast and colorectal (CRC) cancer which had presented at any age Of these, 53 families had an autosomal dominant pattern of inheritance, with five families including individuals with breast and CRC at less than 50 years of age, 28 at less than 60 years and 5 families with individuals who suffered from both breast and CRC Linkage and mutation detection studies have been performed in some of these families and tumours studied for evidence of replication error Results for one large family showed no evidence of linkage of the predisposition to cancer to BRCA1, hMLH1 and hMSH2

4.040

FISH and molecular studies applied to Retinoblastoma

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Retinoblastoma has both sporadic and hereditary forms 10% of the cases are familiar About 5-10% of the patients have a prezigotic predisposition to tumor formation associated with chromosome detectable alterations, 80-90% of them correspond to deletions involving 13q14 band Fluorescence in situ hibridization (FISH) has been used to screen deletions and structural abnormalities involving 13q14 band Furthermore, in familiar cases we tried to follow segregation of mutant allele using polymorphic molecular markers detected by Polymerase Chain Reaction (PCR).FISH was performed in 17 unrelated subjects, two of them had unilateral tumors and no familiar antecedents, 11 were bilaterally affected, two of them had positive familiar history, 4 subjects were studied as controls The Oncor biotinylated cosmid probe was used to test samples of peripheral blood by FISH, according to manufacturer's instructions Lynkage analysis by PCR was possible in four families DNA was obtained from peripheral blood We have studied polymorphic sites for Bam HI, Xba I and Tth 111I We have no definitive FISH results Most of them were obtained before optimizyng FISH In spite of this, it seems that two cases stand out against the rest, probably corresponding to deletion mosaicism Our results indicate that it is necessary to expand control sample to stablish the normality pattern One constitutional mutation resulting from de novo balanced translocation, t(5,13) in a patient with bilateral Retinoblastoma was also detected. Two patients were informative for lynkage analysis, using Bam HI and Xba I polymorphisms. Actually we are trying more informative markers Acknowledgments: Dr A Seres-Santamaria, Prenatal Genetics (Barcelona) This work was partially financed by "Marató TV3" on Cancer Research

4.041

Germ-line attenuated-APC mutations are a rare cause of predisposition to colorectal adenomas

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Spirio et al (Cell (1993) 75, 951-957) have described an attenuated form of familial adenomatous polyposis (FAP) termed AAPC, where affected individuals have been found to have mutations in exons 3 and 4 of the APC gene. AAPC expression within a family appears to be extremely variable and can overlap clinically with FAP, giving rise to between zero and a few hundred colorectal adenomas The phenotypic range associated with AAPC mutations is undefined and it is as yet unclear how many cases of sporadic colorectal adenomas might have AAPC. In order to address this we have identified 44 individuals having a phenotype compatible with a diagnosis of AAPC This group comprises 23 males and 21 females (median age 55y, range 19 - 78y) with colorectal adenomas (median = 34, range 1 - 300) Seventeen cases have had colorectal cancer; 6 cases have an extra-colonic feature of FAP either desmoid tumours (4 cases, including 2 without colonic adenomas) or sebaceous cysts (2 cases) A positive family history (FH) of colorectal adenomas and/or carcinomas and/or extra-colonic features of FAP has been obtained in 27 cases, in 8 cases there is no such FH, in the other 9 cases a FH is not ascertainable Genomic DNA has been obtained from these 44 individuals, either from blood or histologically-normal tissue Mutation screening of exons 1 - 4 of the APC gene is being carried out by chemical cleavage of mismatch Thus far 24 individuals have had all 4 exons tested, revealing two that have potential mutations in exon 3. One of these has been shown to cause a truncated protein (by PTT test on RNA from a lymphoblastoid cell-line) Thus it appears that only a minority of such individuals have AAPC-type germ-line mutations

4.042

The region of 3p22-p21.3 is non randomly eliminated from mouse-human microcell hybrids during tumor growth in SCID mice.

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Chromosome aberrations and allelic losses at 3p21 are frequently observed during the development of many types of solid tumors Several homozygous deletions have been found within the 3p21 band in lung carcinoma cell lines Our previous findings have indicated non random loss of human chr 3 regions from mouse-human microcell hybrids (MCH) following progressive growth in SCID mice. We have tentatively suggested that preferential elimination might be correlated with tumor suppressor activity (Imreh et al , 1994) We have performed additional experiments with the four, previously tested MCH lines, containing complete or deleted human chr 3, one new line MCH 910 6, containing chr 3 in intact form and, as controls, microcell hybrids that carried other human chromosomes than no 3 They included two lines containing chr 13 (MCH203 4 and MCH 240 3), one line containing chr 17 (MCH313 4) and one line containing chr 8 (MCH904 11) We could confirm the non random elimination of 3p segments during tumor growth in SCID mice Using 44 human chr 3 specific PCR markers to examine 29 tumors, we have defined the 3p22-p21 3 region, bordered by D3S643 and D3S1260 loci as the minimum common eliminated part We have identified the small fragment at 3p21 3-p21.2 (containing D3S643, MST1, D3F15S2, UBE1L and GNAI2 loci) between D3S32 and D3S663 / D3S1578 markers that was maintained in 2 tumors of MCH906 8 as a separate fragment This fragment partly overlaps the heterozygous and some homozygous deletions reported in the literature Taken together our results indicate that putative tumor suppressor gene could reside telomeric to the D3S643 locus within a region between the D3S643 and D3S1260 markers

4.043

Increased Risk of Pancreatic Cancer in Italian Melanoma-prone Families with p16 (GLy93Trp) Mutation.

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Goldstein and Whelan studies indicate that p16 mutations are required for pancreatic cancer development in 10 9p21 linked melanoma-prone kindreds We have found evidence relating an increased risk of pancreatic adenocarcinoma to the presence of a p16 mutation (p16 M) in bloodline members of Italian-melanoma-prone families. So far, within the same small geographic area of Italy (possibly due to "founder effect"), we have detected the same Gly93Trp mutation in 7 apparently unrelated families, and in none of 50 control individuals 19 melanoma and 3 displastic naevus cases, were diagnosed at ages ranging 21 to 70 years (median 37) in the kindreds harboring the mutation In addition, 15 cases of cancer at other sites have been found in such kindres, between them 3 pancreatic cancers The latter arose in three different families at age 48, 51 and 60 By contrast, 18 melanoma and 4 displastic naevus cases (median age 42,5) and 9 cancers at toher sites, including no pancreatic cancers, were detected in 7 melanoma-prone p16Wild-type families Our data are consistent with association of pancreatic cancer risk with impairment of p16 function, and suggest a possible specific role of the CDKN2 in rumorigenesis

4.044

Cytogenetic and molecular involvement of 6q in the pathogenesis of Surface Epithelial Ovarian Tumors.

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Recent cytogenetic and molecular studies have shown that alterations of chromosome 6 are common in surface epithelial ovarian tumors (SEOTs) Different authors described loss of heterozigosity (LOH) on long arm of cromosome 6 and suggested that the region 6q26-27 may harbor tumor suppressor genes Chromosome 6 abnormalities were analyzed in a series of 33 SEOTs including 26 serous (2 benign, 4 borderline and 20 malignant), 3 mucinous (1 benign, 1 borderline and 1 malignant),1 clear cell, 1 endometrioid and 2 undifferentiated tumors Conventional histologic features (grade, mitotic index, necrosis, mucus content) were evaluated, proliferative status (Ki 67) and CA-125 expression were immunohistochemically investigated Cytogenetic analysis was performed on direct preparations, using QFQ banding technique Fluorescence in situ hybrydization (FISH) was employed using as probes chromosome 6 library and YACs which map to 6q26-27 Abnormalities of chromosome 6 namely 6q deletion and chromosome 6 loss were found in 96% of SEOTs The 6q deletion was the most frequent chromosome abnormality in this series (88% of cases) and was detected in benign, borderline, low and high grade malignant SEOTs, including endometrioid, mucinous and tumors with simple near diploid chromosome complement These findings suggest that abnormalities of terminal region of chromosome 6 represent an early event in the ovarian tumorigenesis and do not seem to be restricted to serous papillary SEOTs

4.045

Identification of familial cancers in a cohort of gynecological malignancies.

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Some individuals are genetically prone to develop particular type of cancers they probably account for fewer than 5% of all diagnosed neoplasms Patients with inherited cancers may benefit from screening programs and early diagnosis, moreover, genetic investigation of these families may provide opportunities for the identification of involved genes and better knwoledge on their effects From october to march 1995, 50 patients, surgically treated for gynecological cancers (ovary and endometrium), underwent a psycologically assisted genetic counselling during their clinical follow-up In this cohort of patients we identified 4 different types of pedigrees Families without history of cancer (33 out of 50 cases, 66 %) Families with neoplasms aggregation (13 out of 50 cases, 26 %) Environmental-related neoplasms (lung, bladder and stomach tumors) were frequently reported in this group, but interesting associations were also observed, endometrial-endometrial cancers (4 families), ovarian-endometrial cancers (2 families) Suspected familial cancers (2 out of 50 cases, 4%) one HNPCC and one ovarian carcinoma family Verified familial cancers (2 out of 50 cases, 4%) one ovarian and one breast-ovarian carcinoma family These preliminary data seem to indicate that a careful genetic counselling is of paramount importance for the identification of familial cancer syndromes also in patients harboring gynecological malignancies, being more accurate than routine anamnestical investigations useful to identify patients at risk among individuals from such kindreds useful for planning surveillance and management programs for patients at risk

4.046

The Oxford Cancer Genetic Clinic; experience of the first five years.

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There is increasing awareness, fuelled by exaggeratedly alarming publicity in the media, of Mendelian determination of a minor proportion of cases of common forms of cancer (mainly of the breast, the ovary and the colorectum) The anxieties which result represent a major problem in genetical counselling and in management, pointing to a need for the integration of clinical genetics within multidisciplinary approaches to cancer treatment and, in the realm of public health, to cancer prevention The main functions in medical care of the clinic lie in providing reassurance through realistic risk assessments and through initiating periodical cancer screening programmes when appropriate Experience with 1099 individuals from 841 families referred between 1990 and 1994 is described The collaborative research functions of the clinic involve the recognition and definition of familial patterns of cancer occurrence associated with mutant alleles determining susceptibility, as pointers towards investigation of the chromosomal location and of the mode of action of these alleles As a result of advances in understanding the molecular biology of the role of single mutant alleles in the causation of cancer, important developments may be expected in connection with the definition of individual risk profiles, allowing the application of preventive measures and of advances in early detection to be directed towards specific groups

4.047

Mutational analysis of p53 and CDKN2p16 genes in bladder tumors by PCR-SSCP multiplexed analysis; preliminary study

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Mutations in the p53 gene are commonly found in bladder cancer, and they have been associated with an aggressive clinical course An increasing and sometimes conflicting data have been accumulated regarding the frequency of homozygous deletions and the importance of CDKN2p16 in primary tumors We analyzed 100 DNA samples from primary bladder tumors (50% high degree II-III) in comparation with 100 DNA peripheral blood samples drawn from the same patients Amplifications of exons 5 to 9 of the p53 gene and the 3 exons of the CDKN2p16 gene were done by PCR-SSCP multiplexed analysis One single reaction was performed to screen each gene Mutations were identified by cyclic sequencing The number of CDKN2p16 mutations found in bladder tumors (28%) was quite similar to other author's reports However we have found an important lower number of mutations on p53 gene (11%) P53 mutations were frequently found in high degree tumors (72%) Inversaly, CDKN2p16 mutations were primerily found in low degree tumors (61%) Our results suggest that mutations of the CDKN2p16 and p53 genes may be involved in early and late events of tumorigenesis, respectively

4.048

Predictive molecular genetic diagnosis in families with herediatry non-polyposis colorectal cancer (HNPCC)

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Hereditary non-polyposis colorectal cancer (Lynch-Syndrome, HNPCC) is an autosomal dominant predisposition for colorectal cancer and other associated tumours The disease is caused by germline mutations in at least four genes that are involved in DNA mismatch repair Germline mutations in the hMSH2 aor hMLH1 genes are responsible for up to 90% of HNPCC cases Tumours of HNPCC

patients exhibit a pronounced genetic instability that can be detected by analysis of microsatellite markers in normal and tumour tissue Within a cancer prevention programme we ascertained 56 patients with a family history suggestive of HNPCC and 86 patients with a colorectal carcinoma at the age below 50 years Where available, tumour tissue was examined for microsatellite instability So far screening for germline mutations in the hMSH2 and hMLH1 genese by heteroduplex and SSC analysis has been completed in 29 families 3 inactivating mutations in the hMSH2 gene (518delT at codon 173, 1683delA at codon 561 and TGT->TTT at codon 697) and 5 mutations in the hMLH1 gene (ATG->AAG at codon 1, 73delA at codon 25, CCA->CTA at codon 28, CAA->TAA at codon 62 and CGgt->TGgt at codon 226) have been identified In addition, we observed serval polymorphisms and rare silent variants Mutation analysis in HNPCC patients may add new information on possible genotype-phenotype relationships However, predictive testing in persons at risk may have deep psychosocial implications Therefore, predictive diagnosis should be performed only on request of adult persons and after careful genetic counselling

4.049

Molecular mapping of the gonadoblastoma locus on the Y chromosome (GBY)

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The incidence of gonadoblastoma is high in female patients with Y-chromosomal material Gonadal tumors are found in 25-30% of patients with XY gonadal dysgenesis and in 15-20% of 45,X/46,XY individuals The existence of a gene predisposing dysgenetic gonads to the development of gonadoblastoma in females with Y-chromosomal material has been suggested and named the gonadoblastoma locus on the Y chromosome (GBY) The aim of this study was to localize GBY more precisely We studied two female patients with a partial Y chromosome and gonadoblastoma. Both patients were tested for the presence or absence of 31 Y-chromosomal sequence-tagged sites (STSs) covering the Y-specific region of the short and long arms of the Y chromosome A single region of overlap extending from probe pDP97 in interval 4B (which contains the centromere) to marker sY182 in interval 5E of the proximal long arm was delineated This region is contained in a YAC contig that spans approximately 4 Mb of DNA Y-linked testis-specific protein (TSPY) genes have been suggested as candidates for GBY The role of these genes as candidates for GBY is discussed based on results in the two patients studied

4.050

Molecular genetic studies in a Li-Fraumeni syndrome family with a novel p53 germline mutation.

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Families with Li-Fraumeni syndrome (LFS) show familial clustering of different cancers, mainly sarcomas, often with early onset Germline mutations in the p53 tumour supporessor gene have been identified in a high proportion of the LFS families studied We have searched for p53 germline mutations in a collection of families with high incidence of cancer Using a non-radioactive SSCP analysis, direct sequencing and confirmation by restriction digestion, we have identified a novel p53 germline mutation in one family conforming to the criteria for LFS We describe in detail and discuss the constitutional mutation, possible mechanisms of formation of two paediatric tumours an dpossibilities of carriership diagnosis in other members of the family

4.051

Loss of heterozygosity and genome instability in a Spanish series of sporadic colorectal adenocarcinomas.

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Tumour supressor genes, located in chromosomes 5, 17 and 18, play important roles in hereditary colorectal cancer (CRC) Recently, mismatch repair genes located in chromosomes 2, 3 and 7 have been implicated in a type (HNPCC) of hereditary CRC in such a way that their mutation produces wholesale errors during DNA replication yielding in genome instability The aim of the present work is to assess the importance of these genes in a Spanish series of sporadic CRCs We have examined five loci containing dinucleotide repeats sequences representing chromosomes 2, 5, 17 and 18, to analyse the genome instability and the loss of heterozygosity (LOH) in 96 individuals affected of primary sporadic CRC DNA was obtained from fresh tumours, using DNA from normal mucosa and peripheral blood as controls PCR products were electrophoresed in polyacrylamide gels and Ag-stained We have stated genome instability in 38 5% of cases and LOH on chromosome regions 2p (2%), 5q (9 37), 17p (47 9%) and 18q (17 7%) Genome instability is associated with tumours located in the right colon of young patients LOH on 17p seems to be involved in the origin of some tumours whereas LOH on 5q and 18q seem to be markers of worse prognosis

4.052

New mutations in hMSH2 and hMLH1 genes in Russian and Moldavian populations.

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Hereditary Nonpolyposis Colon Cancer (HNPCC) is one of the most common types of hereditary cancer predisposition syndromes that is inherited in autosomal dominant fashion and is believed to be account for 1-6% of all colon cancer cases Mutations in hMSH2 and hMLH1 genes are responsible for a disease in a majority of HNPCC families The identification of causative mutations in these families is essential to confirm diagnosis and to determine the carrier status of unaffected relatives at risk DNA samples of HNPCC patients selected from the Moscow and the Kishenev (Moldova) cancer registers were subjected to sequencing of exons and exon/intron boundaries of hMSH2 and hMLH1 genes The mutations (five in hMSH2 and three in hMLH1) in eight out of thirteen patients were identified and were expected to be causative Interestingly, one of these mutations was described already elsewhere and was found in different population Since HNPCC still remains a rare diagnosis even among the patients of centers for proctology on the one hand and methods for the mutation detection are quite expensive on the other hand we conclude that creation of regional or national cancer registers is necessary step while designing screening programs for some cancer predisposition syndromes

4.053

A comparative genomic hybridization -study on diffuse centroblastic lymphoma and BCL2 amplification without t(14;18) translocation

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We used comparative genomic hybridization (CGH) to detect DNA copy number changes in diffuse centroblastic lymphoma for 19 primary and 9 recurrent tumors in order to assess gains, losses and high-level amplifications Twenty-five of the 28 cases showed copy number changes On average, the recurrent tumors had more changes than the primary tumors Chromosomal regions at 1q, 3, 7, 8, 11, 12, 18, and X were most frequently gained and the most common losses involved 1p, 6q, 8p, and X High-level amplifications were observed at 10p12-14, 17p11 2, 18q21-23, and Xq22-28 Translocation t(14,18) and a BCL2 rearrangement were not detected by cytogenetic analysis and PCR in cases with a gain or high-level amplification at 18q by CGH Southern blot analysis using a probe for the BCL2 major breakpoint region (MBR) revealed amplification of the MBR of the BCL2 gene Overexpression of BCL2 protein was detected using the Western blot technique which suggests that in addition to t(14,18) the amplification of the BCL2 gene is another mechanism for BCL2 overexpression in diffuse centroblastic lymphoma

4.054

Familial desmoid disease without colonic polyposis, may be due to a tumour suppressor gene on chromosome 5q.

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We have investigated a three generation family with a dominantly inherited predisposition to desmoid disease There is no evidence of polyposis of the colon in affected members Desmoid tumours present in childhood, are widespread and multifocal and in most family memebers pursue a benign course In two members of the third generation however (one male and one female) there is a more severe phenotyope with rapid and aggressive tumour growth resistant to all hormonal, chemo- and radiotherapeutic measures attempted Carcinoma of unknown primary site in a 64 year old affected male and carcinoma of the ampulla of Vater in a second affected male at 54 years may be significant Cytogenetic analysis of the intraabdominal desmoid tumour from the proband showed a deletion of chromosome 5q21-23 (the site to which the APC gene maps) in all cells examined This abnormality was not present in skin or blood Linkage analysis using polymorphic markers flanking the APC gene suggests the likelihood of a gene in this region Heteroduplex analysis of exons 1 - 15l of the APC gene has not detected any alteration in the coding sequence The absence of polyps in the colons of at least three affected individuals age 26, 30 and 54 makes it unlikely that the condition is due to a mutation in the APC gene A mutation in a nearby tumour suppressor gene may be responsible for this rare condition

4.055

APC intragenic haplotypes in familial adenomatous polyposis

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Genetic epidemiological studies are useful for the knowledge of the association of markers and genes involved in diseases In the present work we studied the frequency of 4 APC intragenic RFLP markers often used in risk evaluation in one population of 10 FAP patients belonging to 10 unrelated Portuguese FAP families not sharing the same mutation and in a population of 55 unrelated healthy Portuguese volunteers We compared the frequency obtained to normal and to affected populations and to results already reported to other populations We observed allelic frequencies for the Portuguese population which agree with the published one The intragenic polymorphisms shows strong gametic disequilibrium suggesting little recombination between them We could observe haplotype frequencies significantly different between patients and controls Haplotypes in FAP patients χ^2= 13 97, df=5, p≤0 02, informative FAP associated haplotypes in patients χ^2=14 82, df=5, p≤0 02 The gametic disequilibrium may be due to a common founder for a proportion of apparently unrelated probands

4.056

PCR detection of residual disease in leukemia patients

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Fusion genese resulting from nonrandom chromosome translocations are specific molecular markers of leukemias The use of reverse transcription - polymerase chain reaction (RT-PCR) allows sensitive detection of leukemic cells for diagnostic and moitoring studies Here, we have focused on the clinical relevance of RT-PCR evaluation of residual disease following treatment in chronic myeloid leukemia (CML) and in acute promyelocytic leukemia (APL) The t(9,22) found in about 95% of CML patients forms a novel chimeric BCR-ABL gene Twenty CML patients were monitored for the level of BCR-ABL mRNA by quantitative PCR (Cross et al,1993) during interferon (IFN) treatment or after bone marrow transplantation Statistical analysis showed a good correlation between the proportion of t(9,22) positive cells in bone marrow and the levels of BCR-ABL mRNA Further studies on sequential samples are being performed in order to evaluate the predictive significance of different BCR-ABL mRNA levels in CML patients of IFN The characteristic translocation in APL is the t(15,17) which transposes the PML and RARA genes RT-PCR can distinguish two predominant PML-RARA isoforms which may be more clinically informative that the cytogenetical detection of t(15,17) alone Our study in 8 patients showed that the current therapeutic regimes could effectively induce sustained molecular remission Serial negative PCR tests were correlated with long-term clinical remission In summary, these studies demonstrate the usefulness of RT-PCR for monitoring therapeutic response in CML and APL patients

4.057

Loss Of Heterozygosity studies in Primary Breast Cancers

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Tumour suppressor genes can be identified by loss of heterozygosity (LOH) studies We have developed a sensitive and robust PCR-based assay to measure loss of heterozygosity in archival paraffin-wax embedded breast cancer specimens Sixty-three pairs of low grade primary breast cancers were screened for loss of heterozygosity on 11 chromosomal arms, using commercially available microsatellite DNA primers High LOH (over 39%) was detected in informative

tumours with selected markers at 4 chromosomal loci 3p (47%), 11q (39%), 13q (50%) and 16q (41%) This suggests the presence of tumour suppressor genes at these loci, the loss of which allows cancer to develop. Tumour suppressor genes which may have been affected are the ataxia telangiectasia gene (ATM) at 11q and the retinoblastoma (Rb) gene at 13q More detailed analysis of chromosome 11q identified 2 distinct regions of deletion The first spans approximately 3cM and lies distal to D11S901 The second region spans approximately 5cM and lies distal to D11S940 Whilst no known tumour suppressor has been identified in the former location, the latter segment coincides with the ataxia telangiectasia gene (ATM) This is of significance since female carriers of a mutated ATM gene are reported to have a 3 9-fold increased relative risk of breast cancer compared to the general population Both regions are subject to further investigation

4.058

Feasibility of a genetic and epidemiological study of renal cell carcinoma in Bas-Rhin county (France)

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Renal cell carcinoma (RCC) is twice to 3 times more frequent in Bas-Rhin (Alsace region) than in the rest of France and its incidence is constantly increasing in this county, suggesting involvement of specific risk factors To detect such risk factors, we initiated a systematic study of clinical, familial, environmental, and molecular data for living patients (probands) treated for a RCC since 1992 in the department of urology of the 'Hôpital Civil' in Strasbourg We present results of the feasibility study Between January 1992 and October 1995, 184 patients were treated in the department They were representative of the local cancer registry data according to age at diagnosis, histopathalogy, or town of residence Familial and environmental questionnaires were given or sent to 135 probands Sixty-one percent of contacted people participated Biological samples were obtained for 91 probands either at diagnosis (tumor and blood) or during a visit (blood) No proband clearly refused to participate In the definitive study, the participation rate should be improved and we should have one of the largest RCC cases samples ever studied This study should allow to better understand the high incidence of RCC in Alsace and the molecular mechanisms of its carcinogenesis

4.059

Comparative genomic hybridization (CGH) is an excellent tool for determination of chromosome abnormalities in hyperdiploid acute lymphoblastic leukemia (ALL)

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Cytogenetic analysis of hyperdiploid ALL of childhood is commonly hampered by the poor in vitro growth, the incufficient metaphase and chromosome quality and/or by the high proportion of normal metaphases It is also not always clear whether the few analysable metaphases are representative of the leukemic clone We have therefore studied 17 hyperdiploid ALL with the new CGH technique which is an excellent tool for evaluation of quantitative karyotype abnormalities in tissues which are not accessible to conventional cytogenetic analysis Because CGH only detects those clonal changes that are present in a substantial proportion of tumor cells, we have also determined the size of the clone by measuring the DNA-content with flow-cytometry The results of our studies indicate that the identical abnormal clone is detected by all three methods However, as a result of the often poor quality of the cytogenetic preparations, similar sized and shaped chromosomes were in several instances not assigned correctly Careful revision of the karyotypes and/or FISH analysis showed that in all instances the CGH results were correct On the other hand, owing to the homogeneous nature of the clonal abnormalities, even a single hyperdiploid metaphase is likely to be representative of the genetic changes in the leukemic cell population Based on the results obtained in our investigation, we suggest that CGH should eventually be included into the routine diagnostic program of childhood ALL

4.060

Genetic screening for brca1 mutations in breast cancer patients from low risk families

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A gene in which mutations predispose to familial early-onset breast and ovarian cancer was recently elucidated and termed BRCA1 Mutations in this gene are supposed to be responsible for app 45% of inherited breast cancer, more than 80% of inherited ovarian cancer and for a small proportion of sporadic ovarian tumors So far more than 60 different BRCA1 mutations have been identified (Szabo and King, Hum Mol Genet 4R 1811-1817) Mutation screening in the BRCA1 gene of 40 breast cancer patients using SSCP- ASO- and sequence-analysis was performed Nineteen patients were from families with two cases (low risk, group a) and thirteen index patients were from families with three cases (low risk, group b) Seven index patients were from families with at least four cases of isolated breast cancer (high risk, group c) In addition, we analysed five patients from multitumor families (group d) As an internal control we were able to detect most of the more common described mutations and most of the known polymorphisms by SSCP analysis One mutation each was found so far in each group except for group d No further mutations could be identified by sequence analysis in ten index patients from group a, five from group b and two from the high risk families Our data indicate a significant lower frequency of BRCA1 mutations for both low risk (a and b) and high risk families (c) than determined for other populations Whether cases from low risk families are mutated at the BRCA2 locus or whether they have to be considered as sporadic cases remains to be evaluated

4.061

Involvement of the NF2 gene in predisposition to schwannomas

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Schwannomas and meningiomas are the commonest benign CNS tumours Their causation is almost entirely determined by inactivation of the NF2 gene on chromosome 22 Schwannomas in particular follow Knudson's two hit hypothesis for retinoblastoma better than any other tumour individuals with inherited mutations in the NF2 gene developing tumours at an average of 20 years and sporadic tumours occurring at an average of 50 years The lack of involvement of other genes in the tumorigenic process would also make all these tumours an ideal target for gene therapy While 5% of individuals with certain schwannomas (eighth cranial nerve) have unequivocal NF2 (bilateral vestibular schwannomas and other tumours), we have shown that a further 5% have a history suggestive of the disease with a unilateral eighth nerve tumour and other NF2 related tumours or a family history of vestibular schwannomas We are currently investigating the molecular basis of this in 10 of these individuals We have also collected 6 multi-generational families with multiple schwannomas sparing the cranium These families show evidence of linkage to the NF2 gene and mutation analysis and loss of heterozygosity studies are underway in the individuals and their tumours We believe that the great majority of cases of multiple schwannomas (2 or more) and around 10% of all cases have inherited mutations in the NF2 gene

10% of cases presenting with a schwannoma are likely to harbour an inherited mutation in the NF2 gene on chromosome 22

4.062

Numerical changes and structural rearrangements of chromosome 11 in leukaemia patients revealed by fluorescent in situ hybridisation (FISH).

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Numerical changes, translocations, deletions and other rearrangements involving chromosome 11 are considered as non - random changes in patients with acute leukaemia, both lymphoblastic and myeloid They seem to be a bad prognostic sign for the patients and are associated with an extremely poor clinical outcome We report 12 patients with different hematologic malignancies. myelodysplastic syndrome - five, chronic myelomonocytic leukaemia - one, acute lymphoblastic leukaemia - three, lymphoma - one, acute myeloid leukaemia - one, thrombocytemia - myeloproliferative syndrome - one Classical cytogenetic examination of 24 h cultivated bone marrow cells revealed in 4 cases trisomy of chromosome 11 , in 2 cases isochromosome 11 - i(11q) and in 6 cases translocations involving chromosome 11 The FISH with whole chromosome painting probes was used to confirm the findings of G - banding analysis The application of FISH followed extensive cytogenetic analysis Slides were examined repeatedly with several commercially available probes (AGS, BRL, Cambio) and rearrangement of 11q23 was discovered in translocations The results of our study confirmed the importance of 11q23 region in leukaemogenesis and stressed the contribution of FISH with different types of probes to its study This study was supported by grant IGA MZ ČR No 2309-3

4.063

Translocations involving chromosome 5q found in patients with myelodysplastic syndrome (MDS).

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Acquired interstitial loss of different parts of the long arm of chromosome 5 is seen in a wide range of myeloid disorders; specially in MDS and acute myeloid leukaemia The deletion is thought to occur in myeloid precursor cell as primary chromosomal defect On contrary the translocations of 5q are rather rare events as quoted by Mitelman (1990) and they were found in sporadic cases of large series of patients examined During the last two years we examined all patients with MDS and monosomy 5 or deletion of 5q by fluorescence in situ hybridization (FISH) with whole chromosome painting probes (WCP) manufactured by CAMBIO and AGS Among 15 patients with these chromosomal changes we have discovered in bone marrow cells of seven of them translocations involving different parts of 5q The partner chromosomes were different as well as the segment of the 5q involved in the translocation In three cases t(5,7) was ascertained, in one t(3,5) was found The most interesting were findings in three patients with del(5)(q12,q33) who had small segment at the distal part of 5q unstained by WCP suggesting non-reciprocal translocation with other autosome Detailed karyotype will be presented in the poster In all studied cases 5q translocation was one of the multiple acquired karyotypic changes and therefore we considered it as secondary event We believe that the involvement of 5q in all rearrangements is non-random and has close connection with progression of malignancy This study was supported by grant IGA MZ CR 2309 - 3

4.064

Detection of residual leukemic cells after bone marrow transplantation by double-colour FISH.

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Classical cytogenetic examination is limited in evaluation of the success of bone marrow transplantation (BMT) because in the early post-transplant stages mitoses are rare in hypocellular bone marrow Recently fluorescence in situ hybridization (FISH) has become one of the preferable methods for monitoring engraftment of donor's cells FISH allows rapid identifications of the genotype of mitotic as well as interphase cells In our study we used double-colour FISH with two types of DNA probes to distinguish donor's and recipient's cells after BMT α-satellite probes pY3 4 and DXZ1 (ONCOR, VYSIS) in patients treated by sex-mismatched BMT for several types of haematological malignancies cosmid BCR/ABL translocation probe (ONCOR) in Ph-positive CML patients Up-to date 22 patients after BMT were examined by interphase FISH (17 with X/Y probes and 5 with BCR/ABL probe), 9 of them repeatedly in different intervals after transplantation Fluorescent signals were scored in 200 nuclei per examination Both types of probes were used to detect BCR/ABL rearrangement together with X and Y chromosomes in four patients with CML Statistical analysis of results of classical and interphase cytogenetics studies proved high sensitivity of FISH Experimental data will be presented and contribution of interphase FISH for cytogenetic examination after BMT will be discussed in poster This study was supported by grant 2309-3 of Ministery of Health, Czech Republic

4.065

Simultaneous study of the immunophenotype and the chromosome aberrations in leukemic cells (FICTION): discrimination of distinct cytogenetically abnormal subpopulations and follow up of the minimal residual disease.

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Combined immunophenotype analysis and DNA in situ hybridization assigns cytogenetically determined aneuploidy tumor cells to a certain cell lineage The method, referred to as « Fluorescence immunophenotyping and Interphase Cytogenetics as a Tool for Investigation of Neoplasms (FICTION) », was applied on a case of acute myeloid leukemia (AML) classified as M2 At diagnosis, the immunophenotyping of the leukemic blasts from the bone marrow was performed The expression of CD34, HLA-DR, CD33 and CD15 markers was found, the lymphoid marker CD19 was partially expressed The karyotype was 46, XX [4 cells] / 45, X, -X, t(8,21)(q22,q22), del(9)(q22) [13 cells] Fluorescence in situ hybridization (FISH) study on interphase nuclei with the centromeric probe for chromosome X concluded that 80% of the cells were monosomic FICTION showed that 70% of HLA-DR+, 91,4% of CD33+, 95,5% of CD15+, 96% of CD33+CD34+ and 99% of CD34+ cells belonged to the pathologic clone During follow up, immunophenotype, chromosome, FISH and FICTION analyses were performed for each sample (table)

Table opposite

Sample	Karyotype	FISH % XO cells	FICTION % XO cells in subpops				
			HLA-DR+	CD15+	CD34+	CD33+	CD33+CD34+
13-12-94 (diagnosis)	46, XX [3] / 45, X, -X, t(8,21)(q22, q22), del(9)(q22) [13]	80	70	95	99	91 4	95 8
17-01-95 (post induction)	46, XX [27]	28 2	37	45	48 7	19 6	14 7
16-02-95 (post consolidation)	46, XX [31]	22 9	37	24	18 7	12 5	15 1
02-03-95 (before transplantation)	46, XX [31]	26	22	24	39 9	12 7	17 1

The data clearly show 1) a normal karyotype while XO cells were consistently detected by FISH during the follow up 2) a progressive decrease of the percentage of XO cells in the bone marrow except for the last sample where a slight increase occured 3) a continuous decrease of the monosomic cell number in all the subpopulations studied, except for the CD34+ cells where a 2 1 fold increase was observed just before transplantation Based on these observations, it appears that FICTION is the most sensitive technic and probably constitutes the best approach for the detection and recognition of the pathologic cells in the monitoring of minimal residual disease This project is supported by the «Fonds National de la Recherche Scientifique» Grant FNRS-Télévie, crédit n° 7 4545 94

4.066

Localization of the gene for Cowden s disease to chromosome 10q.

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Cowden disease, or multiple hamartoma syndrome, is a familial cancer syndrome with an autosomal dominant mode of inheritance Its clinical features include a wide array of abnormalities but the main characteristics are hamartomas of the skin, breast, thyroid, oral mucosa and intestinal epithelium The most important central nervous system manifestations are megalencephaly, epilepsy and dysplastic gangliocytomas of the cerebellum (L Hermitte-Duclos disease) Early diagnosis is important since female patients with Cowden disease are at high risk of developing breast cancer In addition, a high incidence of benign and malignant tumours of the thyroid gland have been observed in affected individuals By performing linkage analysis in 13 families, the gene for Cowden disease has been localized to the long arm of chromosome 10 The maximum lod score is 10 76 Haplotype analysis enabled us to identify a region of about 12 cM in which the Cowden gene cosegregates

4.067

Comparison of the cytogenetic effects induced by two different chemotherapeutic schemes in lymphocytes from patients with breast cancer.

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Differential rates of Sister Chromatid Exchanges (SCE) were observed in two groups of patients with breast cancer treated with two different schemes The one consisted of 10 CMF-treated patients and the other from 11 FEC-treated patients (CMF Cyclophosphamide, Methotrexate, 5-Fluoruacil, FEC 5-fluoruacil, Epirubicin, Cyclophosphamide) FEC was proved more efficient in inducing in vivo SCEs compared with CMF In a combined in vivo and in vitro study lymphocytes taken from 4 breast cancer patients who had been given either FEC(1 patient) or CMF (3 patients) by injection 1 5 h before and then treated with 9-Nitrocamptothecin (9-NC) in vitro were found to have synergistically increased exchange rates and cell division delays The frequency of SCEs in the patient's own lymphocytes with and without exposure to 9-NC was determined before the cytostatic therapy and was used as a control for later comparison in each individual case However in 1 futher CMF-treated and in 5 FEC-treated patients tested in this combined in vivo and in vitro study additive effects were identifyied These observations have implications for interpreting the patient's DNA repair processes involved, for monitoring the drug combinations that synergistically damage DNA in vivo and in vitro and for identifying interindividual variations in the response to the treatment (Mourelatos et al , Mutat Res 143 (1985) 225, Teratogen Carcinogen Mutagen 6 (1986) 485, Cancer Res 48 (1988) 1129; Eliopoulos et al , Mut Res 342 (1995)141)

4.068

No evidence for overexpression of the p53 protein and mutations in exons 4-9 of the p53 gene in a large family with adenomatous polyposis

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Familial adenomatous polyposis coli (FAP) is an autosomal dominant disease characterized by an early onset of numerous adenomatous polyps of the colon and a high risk of colon carcinoma Offsprings of affected individuals are at 50% risk of inheritance of the condition In the present study a big family with evidence of polyposis and colon cancer as screened for the mutations of the p53 gene and protein overexpression Nine individuals were tested Five of 9 had already developed clinical features of FAP, four of 9 revealed normal findings on colonic examination at an early age We examined p53 protein expression from 5 individuals with immunohistochemical techniques using monoclonal antibody PAb1801 Polymerase chain reaction products of exons 4-9 of the p53 were examined from 9 individuals by single-strand conformational polymorphism analysis We could find no evidence overexpression and mutations of the p53 in any lession including adenoma and carcinoma in the FAP family

4.069

Mucinous carcinoma of the colon in a 16 yr old Turkish boy with Bloom's syndrome: cytogenetic, histopathologic, p53 gene and protein expression studies

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Bloom's syndrome (BS) is a rare chromosomal brekage disease All over the world approximately more than 165 cases have been reported As far as we know this is the first case of BS from Turkey A 16-year-old Turkish, boy, born to from consanginous couple, who was firstly admitted to the Hacettepe University Children's Hospital with complaints of severe growth retardation, photosensitivity, cryptorchidism and hypospadias The patient was diagnosed as BS with clinical , cytogenetical findings at 7 years of age and registered in the Bloom's Syndrome Registry as 116 th patient He was examined regularly and suffered from intraabdominal masses localization at the transvers colon at 16 years old The chromosome analyses on the bone morrow aspirate showed that 47, XY , t (1,2) (q22, q22),mar and deletions of chromosome 1, 2, 9, 10, 11 SCE was increased (24 7 while the lymphocytes from the control subject 5-7) The presented case was screened for the presence of overexpression and mutations of the p53 gene We examined p53 expression from parafin-embedded tissue with immunohistochemical techniques using monoclonal antibody PAb1801 DNA was isolated from peripheral blood lymphocytes and polymerase chain reaction produts of exons 4, 5, 6, 7, 8-9 of the p53 gene were examined by single-strand conformational polymorphism analysis We could find no evidence overexpression and mutations of the p53 gene in BS Based on this results, we concluded that mucinous carcinoma of the colon may occur at an early age contrary to the comman knowledge that it occur at arround 35 years of age and p53 gene may not play role in BS patient

MOLECULAR GENETICS

5.001
Clinical features of Angelman Syndrome in Adulthood.

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We studied the clinical features of 28 adult patients with Angelman Syndrome (AS) (12 females, 16 males, aged 20 to 53 years, mean age 32 yrs) All were identified in eight institutions for mentally disabled people Twenty-three showed a maternal chromosome 15q11-13 deletion, in five the diagnosis was based on a combination of typical clinical features All patients were severely mentally retarded Comparing the data in the literature on frequency of clinical symptoms of AS in childhood with those in our study on adults with AS, we found coarsening of facial features (100%), thoracic scoliosis (71%) and being wheelchair-bound (39%) more frequently in adults Paroxysms of laughter were still observed in adulthood (79%), but less frequently than in childhood The majority (82%) had epileptic seizures, most of them despite anti-epileptic drugs Three patients without a chromosomal deletion never had an epileptic seizure Two other patients became seizure-free in adulthood In contrast to other studies, we found that epileptic seizures do persist in adult AS patients These findings may improve the recognition of adult AS patients in institutions for the mentally retarded

5.002
Identification of mutations in the HPRT gene in Polish patients

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Hypoxanthine-guanine phosphoribosyltransferase (HPRT, EC 2 4 2 8) is a purine-salvage enzyme. Its complete deficiency causes Lesch-Nyhan syndrome (LNS) The purpose of our study was to identify HPRT gene mutations and to determine carrier status in at-risk females Six patients with LNS and 26 members of their families were studied Genomic DNA was isolated from leucocytes and total cytoplasmic RNA from lymphocytes and/or fibroblasts DNA was digested with PstI and restriction fragments were hybridized with full-length HPRT-cDNA RNA was reverse transcribed to cDNA, which was then amplified as 3-4 overlapping HPRT-cDNA fragments Alteration in fragment size and structure was identified by agarose or acrylamide gel electrophoresis and by SSCP analysis The shifted cDNA fragments were sequenced by the dideoxy-chain termination method. Our studies did not reveal any large deletions or alterations of the normal PstI restriction pattern in the HPRT locus of any of the examined patients cDNA sequence analysis showed different point mutations in three of the index cases All identified variants were missense mutations Two of them were novel (C®T Pro176Ser and G®A Gly190Glu) and one (C®A Phe74Leu) had been reported previously. The identified mutations were used as family specific markers of the disease in carrier detection

5.003
Molecular diagnosis of Prader-Willi and Angelman syndromes by detection of DNA methylation pattern and polymorphic markers in region 15q11q13

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We studied 46 patients suspected of having either Prader-Willi syndrome (PWS) or Angelman syndrome (AS) by analysis of the methylation pattern at locus D15S63 and polymorphism of markers from the 15q11-q13 region Polymorphic analysis included CA repeat and RFLP markers An abnormal methylation pattern at locus D15S63 was observed in 20/31 PWS patients and 2/14 AS patients Among PWS patients with the abnormal methylation pattern, 70% had large deletions, 25% maternal disomy, 5% unidentified mutations Eleven of thirty-one patients had a normal methylation pattern, on follow-up examination, only one of them fulfilled the clinical diagnostic criteria for PWS An abnormal methylation pattern was detected in one AS patient with a large deletion (D15S10-D15S97) and in another with no deletion or disomy In addition, two patients with a small deletion encompassing the AS specific region demonstrated a normal methylation pattern In the remaining AS patients, including four AS familial cases, both normal methylation pattern and no deletion or disomy were identified Our results confirm that analysis of DNA methylation at locus D15S63 can be used as the initial procedure for rapid diagnosis of PWS For AS diagnosis, analysis of DNA methylation at locus D15S63 seems to play a minor role

5.004
Aneuploidy as indicator of aggressiveness of endometrial cancer(Flow cytometric study).

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Flow cytometry was used to determine the DNA ploidy of fresh tissue specimens from patients with endometrial carcinoma (n=89),atypical (n=7) and simple (n=15) hyperplasia and normal endometrium (n=28) Cell suspension was stained ethidium bromide and mytramycin and analyzed with MD-116 flow cytometer (S Vavilov Optic institute, St-Petersburg) using mercury lamp Coefficient of variation was at 5,5 or less (mean, 4,0%) Not one in a sample of normal and hyperplastic (including atypical hyperplasia) endometrium was not aneuploid Aneuploid patterns constituted 23,6% of the adenocarcinoma DNA aneuploidy rate increase with decreasing adenocarcinoma grade 6,7% of highly differentiated carcinomas, 23,1% of moderately differentiated carcinomas and 36,4% of poorly differentiated carcinomas were aneuploid DNA aneuploidy rate correlates with increasing of clinical and morphological indicators of tumor progression (depth of myometrial invasion, lymph node and distant metastases) DNA index enhancing result in increasing of tumor aggressiveness Presence of tetraploid or close lines transformed cell appears particularly unfavourable

5.005
Polymorphysms in the red/green opsin genes in the Chinese Li population revealed by SSCP and direct sequencing.

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The red/green photopigments are the light absorbing molecules that mediate red/green vision The two highly homologous genes are arranged in a head-to tail tandem array with a single red gene upstream of one or more copy of green genes and they are located at the telomeric region of the X-chromosome Previous studies revealed interesting polymorphisms in the red/green genes related to perception of light at different wavelenghts The Li population is an ancient Chinese ethnic population living in Hainan Island We have analysed polymorphisms in the 3Õ-segment of exon III of the red/green opsin genes and determined the allelic frequencies in 27 Chinese Li males with normal colour vision Furthermore, we analyzed haplotypes in this region and found six haplotypes respectively in the red and green genes Our preliminary data indicate significant differences in the frequencies of single polymorphisms and haplotypes in the red opsin gene among populations, with different frequency of 180S-> V in the red opsin gene between Li population and Caucasians reflecting polymorphism of color vision

5.006
Retinoblastoma gene (RB1) mutation analysis.

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RB1 is the tumour suppressor gene responsible for retinoblastoma (Rb), an intraocular childhood tumour which has both hereditary and non-hereditary forms Loss of function mutations in both copies of RB1 results in tumour formation Hereditary disposition is when the loss of the first copy occurs as a germline event Up to 45% of Rb cases are potentially hereditary and most of these are due to de novo mutations Identification of RB1 mutations can therefore be very important for genetic counselling and predictive testing We have recently started a mutation analysis programme in 150 hereditary and non-hereditary Rb cases analysing DNA from tumour (fresh and paraffin embedded sections) and constitutional cells of Rb patients using single strand conformational polymorphism (SSCP) analysis followed by PCR sequencing, we have so far analyzed 10 (out of 27) exons We have identified 22 (17 germline/5 somatic) different mutations in 25 patients 40% of these mutations are small deletions/insertions, the rest being point mutations most of which were C®T changes in CpG dinucleotides As more mutations are identified correlations between genotype and phenotype can be investigated along with possible mutation hot spots in the gene and mutational mechanisms involved

5.008
Endothelin-B receptor mutations in patients with isolated Hirschsprung disease from a non-inbred population

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Hirschsprung disease (HSCR), or aganglionic megacolon, is the most common cause of congenital intestinal obstruction Two different loci have been found to be tightly linked to HSCR on chromosomes 10 and 13, respectively Recently, mutations in the RET protooncogene on chromosome 10q11 2 were identified in several HSCR patients In addition, a missense mutation in the endothelin-B receptor (EDNRB) gene on chromosome 13q22 was found in an inbred Mennonite kindred affected by HSCR and associated abnormalities, demonstrating the involvement of EDNRB in HSCR pathogenesis To test whether mutations in the EDNRB could account for Hirschsprung in patients from non-inbred populations we analyzed DNA samples from HSCR patients of Italian origin We have identified two novel EDNRB mutations a missense mutation in a sporadic case, S305N, which leads to a change of a serine to an asparagine, disrupting a putative phosphorylation site, and a single nucleotide deletion in a familial case, N378I, resulting in a truncated protein Both mutations were found in one of the healthy parents, and neither of these mutations were found in any of the normal individuals tested These data confirm the involvement of EDNRB in HSCR pathogenesis and demonstrate that EDNRB mutations could contribute to HSCR disease in non-inbred populations The finding of these mutations in non-affected patients' family is consistent with the polygenic inheritance of the disease

5.010
Framework-dependent expression of two missense mutations (pro^{134}→thr and ala^{244}→val) in the coagulation factor VII gene from a Maltese kindred.

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Coagulation factor VII (CFVII) assays with a panel of tissue thromboplastins from brains of four species and CFVII antigen assays revealed phenotypic heterogeneity in a Maltese kindred with CFVII deficiency Exons 2 to 8 of the parental CFVII alleles and their respective intron-exon boundaries were amplified by PCR and sequenced Two wild type gene frameworks (framework 1 = 7,880 C, 10,975 G, and, framework 2 = 7,880 T, 10,975 A) and two new point mutations were identified The mutations were CFVII Malta I, 8,906 C→A, associated with framework 1, and, CFVII Malta II, 10,648 C→T, associated with framework 2 The four alleles resulted in a complex pattern of genotypic combinations which accounted for the phenotypic heterogeneity observed among family members The data indicated that variability in the severity of CFVII deficiency resulted in part from the allele specific mutation as well as from the CFVII framework on which it occurred

5.011
Intronic polymorphism in the PMP-22 gene provides evidence for a deletion in 17p11.2-12 in a family with hereditary neuropathy with liability to pressure palsies

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In the majority of cases, Charcot-Marie-Tooth disease type 1A (CMT1A) and hereditary neuropathy with liability to pressure palsies (HNPP) are associated with a 1 5 Mb duplication and deletion in 17p11 2-12, respectively, comprising the peripheral myelin protein 22 (PMP-22) gene In a few patients with CMT1A, HNPP and Déjerine-Sottas syndrome (DSS), point mutations in the PMP-22 gene have been found Point mutations in the P_0 genes by single strand conformation polymorphism analysis followed by direct polymerase chain reaction sequencing of genomic DNA in patients with the clinical diagnosis of a peripheral neuropathy A new polymorphism in intron 3 of the PMP-22 gene close to the exon-intron boundary was observed The overall frequency of the novel allele was calculted to be 4 7%, not differing significantly between patients and controls Segregation analysis of the polymorphic PMP-22 intron 3 allele in a family clinically diagnosed as suffering from HNPP was used as an independed approach to demonstrate a deletion in 17p11 2. In addition, results of the searching for other mutations in the genes associated with hereditary neuropathies will be reported

5.012
Detection of the intron 22 inversion mutation in Turkish hemophilia A patients using a non-radioactive method

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Hemophilia A is an X-linked bleeding disorder caused by a total or partial deficiency in the factor VIII coagulation activity The mutations in the factor VIII gene leading to the disease are heterogeneous About 50 percent of the severe cases are the result of a large DNA-inversion that split the factor VIII gene into two parts separated by 200-300 kb This inversion is caused by intrachromosomal homologous recombinations between a 9 5 kb region in intron 22 of the factor VIII gene (Int22h-1) and one of at least 2 other copies of this 9 5 kb region located 200-300 kb upstream from the factor VIII gene We report the use of a non-radioactive sourthern blot method to detect this inversion in the Turkish hemophilia A patients the F8A probe (1 1 kb EcoR 1/Sac1 digest of p482 6) was labeled with digozigenin-dUTP and detected by a chemiluminescent method using CDP-star as a substrate for th ephosphatase-conjugated antibody The inversion was found in 22 percent of the 76 hemophilia A patients with moderate and severe clinical symptoms Thirteen patients had distal, three had proximal type of the inversion and one patient had a previously undescribed pattern Since the labeled probe is stable for at least one year and can be used many times, this non-radioactive method to detect int 22 inversion is promising to be a convenient way to diagnose carriers and perform prenatal diagnosis in routine laboratory conditions

5.013
A model for estimating the origins and history of b-thalassemia mutations in the world

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Beta-thalassemia is an autosomal recessive disorder characterized by microcytic and hemolytic anemia which is the result of a reduced synthesis of the b-globin chains of hemoglobin b-Thalassemia probably accompanied humanity since the transition from hunting and gathering to agriculture. At present, over 100 million persons are carriers of the disease, primarily in regions of the world where there has been selection by malaria During the past 15 years more than 180 determinants have been identified which cause an absence or a decrease in the synthesis of the b-chains of hemoglobin Despite of this large diversity, a much more specific collection of alleles account for the inactivation of the b-globin genes in each population and ethnic group Because of the recent accumulation of data published about each population, we now have a fairly good knowledge of the spectrum of b-thalassemia mutations in different areas of the world Using these data, the present study aims at the evaluation of a mathematical model to be used in determining the origin of the most common b-thalassemia mutations in terms of time and place

5.014
Gonadal mosaicism for a submicroscopic deletion of chromosome region 22q11

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DiGeorge syndrome is characterised by a variety of phenotypic abnormalities including heart defects, absent thymus and facial dysmorphism Deletions of chromosome 22q11 have been shown to be associated with DiGeorge syndrome in a proportion of cases. The majority of cases arise sporadically but 8% are familial We describe a family in which the index case, one of male twins, was born with Tetralogy of Fallot and was subsequently shown by molecular cytogenetic techniques to have a deletion of 22q11 His twin was shown to have a normal karyotype and molecular cytogenetic analysis of both parents peripheral blood revealed normal chromosomes 22, with no evidence of a deletion In a subsequent pregnancy, however, the foetus was shown by ultrasound to have an interrupted aortic arch and VSD Postnatally she was found to have a deletion of 22q11 We have used molecular techniques to determine that the parental origin of the deletion in these two siblings is maternal The same maternal chromosome segment encompassing the DiGeorge critical region has been inherited in its deleted form by both affected children and in its non-deleted form by the non-affected brother respectively Molecular cytogenetic analysis failed to demonstrate mosaicism in a maternal skin biopsy This is the first molecular demonstration of gonadal mosaicism in a family with a 22q11 deletion

5.015
Towards cloning the gene for X-linked cleft palate and ankyloglossia

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Facial clefting disorders are amongst the commonest dysmorphologies seen in man Isolated cleft palate (CP) most commonly occurs as a result of multifactorial inheritance However, several families have been documented that inherit CP in a purely X-linked Mendelian fashion (CPX) The first positional data for CPX resulted from a genetic linkage analysis using a large Icelandic pedigree, where CPX was linked to DXYS1X in the proximal half of Xq Further linkage analysis refined the interval to a region of approximately 3 Mb between DXS95 and DXYS1X This interval was subsequently cloned to form a YAC contig Breakpoints for an inversion and a translocation in two patients with cleft palate and cleft lip respectively (46,Xinv (X) (p11,q21)Y,46,Xt(X,9) (q21 3,q33), were mapped within this interval We are now attempting to isolate gene sequences from this interval using the techniques of exon trapping, cDNA selection and direct screening of cDNA libraries Candidate cDNA sequences will be tested for disruption in the chromosomal rearrangements and for mutations in the Icelandic pedigree

5.016
Title: Detection of mutations in the gene encoding 21-hydroxylase by solid-phase minisequencing.

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Steroid 21-hydroxylase deficiency is the leading cause of congenital adrenal hyperplasia (CAH) At present, the detection of the mutations causing the disease is made by usage of time-consuming procedures such as allele specific PCR and restriction enzyme analysis We have developed a new method, using solid-phase minisequencing, to detect the pathological mutations in Danish patients with classical CAH due to 21-hydroxylase deficiency The solid-phase minisequencing method allows accurate and sensitive quantitation of two sequences which differ from each other by one nucleotide and are present as a mixture in a sample Enzymatically amplified DNA, 5'-biotinylated in one strand, is bound to a solid phase (streptavidin-coated microtiter plates) and denaturated A detection primer, constructed to end immediately before the mutation, is annealed to the immobilized single-stranded template and elongated with a single 3H-labeled deoxynucleoside, depending on the sequence of the DNA investigated Several of the most common mutations are located relatively close to one another, so that the same PCR product can be used for the majority of the analysis The solid-phase minisequencing method is fast and reliable, and can be used with success for detection of the most frequent point mutations involved in 21-hydroxylase deficiency

5.018
Characterisation of cystic fibrosis mutations in the United Arab Emirates.

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We designed a study aimed at identifying the genetic mutations responsible for cystic fibrosis (CF) in the population of the UAE, using a strategy that was based on the technique of denaturing gradient gel electrophoresis combined with asymmetric amplification DNA sequencing In ten unrelated, true UAE national families of Bedouin descent, all fifteen CF patients were homozygous for mutation S549R (G→T) This mutation probably results from a founder effect in an ancestral nomadic Bedouin tribe from this area of the Gulf Another sample of eight CF patients consisted of subjects from families that were either Pakistani Baluch or of Baluch origin (Baluchistan is the western province of Pakistan), six of these eight CF patients were ΔF508 homozygotes This data indicates that ΔF508 was introduced to the UAE by the Baluch and also that Baluchistan (the most Eastern part of the Fertile Crescent) could have been the site of origin of the ancestral ΔF508 mutation Although the number of CF families that we could survey was small (16 UAE national families including a total of 21 patients), the unique distribution of CF mutations in the UAE population (two mutations, S549R and ΔF508, characterise 90% of CF patients) will allow efficient organising and delivering of CF carrier screening programmes on the country's limited population size

5.019
Psychosocial aspects of predictive testing in HNPCC

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Two fully characterized mutations account for numerous known Finnish HNPCC families This provides an opportunity to offer predictive genetic testing to hundreds of high risk individuals Here we report aspects of a population-scale predictive testing programme in which we initially targeted 250 high risk individuals An initial psychological assessment questionnaire was sent to those 78% who consented to be counselled Two individual counselling sessions were set up During the first session the counselee is informed about the disease, its mode of inheritance, the testing procedure and follow-up scheme If, after an interval of 2 weeks, the counselee decides to be tested a blood sample is drawn, the test is done and disclosure of the result occurs during a second counselling session Approximately one month and one year after the disclosure the counselee is asked to complete the questionnaire again The project is ongoing since early 1995 Some unexpected dilemmas have arisen For instance, the protocol calls for individual counselling while siblings wish to be counselled together The plan does not provide for counselling of testing of children, however, some parents wish to have their children tested Experience from the initial stages of the project will be reported

5.020
Analysis of trinucleotide repeat expansion in patients with spinal and bulbar muscular atrophy.

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Spinal and bulbar muscular atrophy (SBMA) is a rare neuromuscular disease caused by trinucleotide expansion in the androgene receptor gene (AR) Such feartures as anticipation and instability of large trinucleotide block, differencies in paternal and maternal transmission, correlation between severity and number of trinucleotide repeats are typical for diseases caused by trinucleotide expansion However, they are less investigated for SBMA Twenty five persons from 3 families with cases of SBMA were investigated Clinical and biochemical investigations revealed 3 affected males in 3 families Pathological expansion of trinucleotide repeats was found in all these patients, and additionally detected in one person with some symptoms insufficient for reliable diagnosis, and two boys without any symptoms We have detected one case of increasing and one case of decreasing of number of trinucleotide repeats during female transmission One female carrier has mild symptoms of SBMA Analysis of lionization reveals prevalence of inactivation of normal chromosome Three patients with unclear diagnosis with bulbar symptoms were analyzed for possible trinucleotyde expansion in AR gene In all cases diagnosis of SBMA was rejected PCR amplification could be effectively used for exact diagnosis of SBMA, carrier detection, and potentially for prenatal diagnosis of this disease

5.021
Analysis of deletion of SMN gene in different forms of recessive and dominant spinal muscular atrophy.

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Spinal muscular atrophy is a set of hereditary diseases characterized by degeneration of anterior horn cells of the spinal cord, leading to progressive paralysis with muscular atrophy Three most common recessive forms (SMA I, SMA II and SMA III) was localized in 5q13 However, it was considered that other loci or noninherited cases are responsible for 5%, 20%, and 60% cases of SMA I, SMA II and SMA III The most frequent mutation specific for all 3 forms is deletion of DNA fragment including genes SMN and NAIP, probably resulting from recombinations between this region and highly homologous DNA region containing pseudogenes We have investigated the deletion of SMN gene by SSCP analysis of 8th exon of this gene and corresponding pseudogene Deletion (or gene conversion) was detected in all 34 chromosomes in patients with SMA I, 28 of 28 chromosomes (SMA II), and in 32 of 38 chromosomes (SMA III) Deletion was not detected in chromosomes of patients with dominant Kugelberg-Welander disease (158600), Finkel late-adult type SMA (182980), distal spinal muscular atrophy (182960) and atypical dominant SMA have been described in Russia It could be suggested that other genes, or other mutations are responsible for these diseases

5.022
Characterization of mutations in the 21-hydroxylase gene in patients with classical adrenal hyperplasia in Russia.

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37 patients with classical forms of adrenal hyperplasia were analyzed for 2 possible mutations in promoter region, splice mutation in intron 2, deletion of 8 nucleotides in exon 3, nonsence mutation in 8th exon Quantitative analysis and investigation of heterozygous relatives were carried out to distinguish recombinations and gene conversions All chromosomes were haplotyped for HLA A, B, and DQA1 genes 26% of affected chromosomes carry splice mutation in intron 2 This mutation is weakly associated with B35 allele, and has no association with HLA DQA1 and HLA A genes Another common mutation (10% of affected chromosomes) is CYP21A/CYP21B chimeric gene linked with A3-B47-DQA1 0201 or 0601 These two mutations are also common in West Europe and North America 20% of affected chromosomes carry chimeric CYP21A/CYP21B gene and CYP21A pseudogene and linked with B14-DQA1 0101 or 0102 This mutation was not described in Western counties, and probably originates in East Absence of association with HLA A gene could be explained by more ancient age of introduction of this mutation in Russian population 23% of affected chromosomes carry other mutations that could be detected by our approach Altogether more than 80% of mutations were identified in patients with classical adrenal hyperplasia

5.023
The multiplex systems for DNA-diagnosis of X-linked Alport syndrome.

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Alport syndrome (AS) is an inherited renal disease associated with ocular and hearing abnormality resulted in early death from end stage of chronic renal insufficiency There are at least 3 different genes responsible for X-linked and autosomal forms of AS Large fraction of patients with AS have X-linked form associated with mutations in COL4A5. We have developed three multiplex systems for DNA diagnosis of X-linked AS One system includes three polymorphic markers linked with COL4A5 (2B6, 2B20 and DXS178) for determination type of inheritance and indirect diagnosis Another system includes some exons from 5' and 3' ends of COL4A5 and promoter region for detection of deletions in COL4A5 Third system is based on SSCP detection of point mutations in 8 exons of COL4A5 We have studied 35 Russian families with presumable clinical diagnosis of AS Two large deletions and two point mutations in unrelated affected persons have been described Heterogeneity complicates indirect DNA diagnosis in families with undetermined mutations Linkage analysis in large families could increase accuracy of genetic risk estimation to reasonable level

5.024

Deletion pattern of Moscow's patients with Duchenne/Bekker muscular dystrophy reveals some population specificity.

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DNA samples from 244 Duchenne/Bekker muscular dystrophy patients in Moscow were analyzed for deletions in the dystrophin gene by means of multiplex amplification for promoter region and 19 different exons 98 deletions were detected (40%). This value is less than in West European populations Comparizon of deletion breakpoints spectra and deletion spectra reveals differencies between corresponding data from other populations Some differencies could 'be found between deletion breakpoints and deletion spectra in different European populations Thus population specificity of deletion patterns seems probable The difference in deletion spectrum can reflect polymorphism in intragenic structure responsible for higher or lower probability of deletion, or the difference in mutagenic factors Investigation of deletion breakpoint distribution within middle part of intron 7 has not revealed obvious hot point However, 5 deletion breakpoints were detected within less than 1 kb region of intron 45 in vicinity to str 45 It could be possible that several hot spots responsible for considerable part of deletions Some of these points could reveal population-specific polymorphism Aknowledgments This is a part of collaborative work with Laboratory of Prenatal Diagnosis, Institute of Obstetrics and Gynaecology, St Petersburg, and Department of Molecular and Medical Genetics, Guy's Hospital, London

5.025

Molecular genetics of some hereditary diseases in Belarus

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Molecular basis of the most frequent hereditary diseases (PKU, CF, CAH, Fra-X) are being studied in Belarus. Examination of 418 PKU chromosomes showed that R408W covered 68%, R158Q - 1 67%, R261Q - 1.4%, IVS12nt1- 0 96% R408W mutation in all the cases was associated with haplotype 2 (VNTR 3) A combination of mutation and VNTR analyses made 90% of our PKU families informative for prenatal diagnosis 11 prenatal diagnoses were performed in PKU families in the first trimester Delta F-508 heterozygote carrier frequency in Belarus newborn population (2598 chromosomes studied) was 1 72 This mutation covered 65% of 102 CF-chromosomes studied N1303K mutation was revealed twice, G551D and R553X mutations were not detected 10 first trimester prenatal diagnoses were performed in CF-families A limited population newborn screening for CF followed by mutation analysis of primary positive samples is now in progress as a pilot study Number of CGG-repeats and haplotypes were studied in 48 X-chromosomes Number of CGG-repeats varied from 9 to 34 (29 the most frequent) in 42 normal X-chromosomes A premutation with 156 CGG-repeats was revealed in one mother and a full mutation with more than 2,000 bases was determined in one patient 8bp-deletion homozygotes in exon 3 of 21-OHase gene was not revealed in 10 CAH-patients

5.026

Genetic heterogeneity of the a-globin gene Major Regulatory Element (a-MRE)

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The Human a-globin gene cluster is located on the tip of the short arm of chromosome 16. The functional genes are organized as 5'-z2-a2-a1-3' The globin genes are coordinately regulated in order of their expression during development The major positive regulatory activity for expression of the a-globin gene cluster is restricted to a 350 bp Major Regulatory Element (a-MRE) located 40 kb upstream of the z2-globin mRNA Cap-site A number of putative binding sites for nuclear factors GATA-1 and AP-1/NFE-2 have been identified within this 350 bp fragment which show high conservation in man and mouse and in old world apes To study the importance of the conserved sequences and the possible effect on a-globin gene expression we developed a one step method to identify aMRE polymorphisms within and between different ethnic populations Denaturing Gradient Gel Electrophoresis identified different patterns due to the combination of six different aMRE haplotypes (designated A, B, C, D, E and F) in a North-European, a South-European, an Indian, an Indonesian, a Chinese and two African populations Direct sequencing showed that these six haplotypes were characterized by six polymorphic sites, five in between putative binding sites for nuclear factors and one within a NFE-2 binding site The frequencies of the aMRE haplotypes were established in the different populations, haplotypes A and B were the most frequent in all populations tested The E and F were found in a Pygmy population only The D haplotype showed alteration of the NFE-2 consensus sequence and was exclusively found in the African population with a 15% frequency Comparison of hematological parameters between carriers of the D and A allele in combination with a+ thalassemia, suggested no difference in a-globin gene expression Furthermore no aMRE point mutations were found in a panel of non-deletional a-thalassemia carriers which could explain the reduced a-globin gene expression

5.027

HbC [β6(A3)Glu->Lys] in combination with HbH disease in a Surinam patient

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A middle-aged man of Surinam origin was referred to our laboratory for hemoglobin analysis He was suffering from an intermediate microcytic hypochromic haemolytic anaemia and was apparently a heterozygous carrier of haemoglobin C The Hb electrophoresis showed only the HbA and HbC bands but the amount of the mutant haemoglobin, whose identity was confirmed at the DNA level, was clearly less than the expected 30-40% usually observed in HbC heterozygotes We therefore considered the possibility of an interfering a-thalassaemia In vitro globin chains synthesis revealed a b/a imbalance compatible with the coexistence of two non-functional a genes Yet the combination of HbC with heterozygous a° or homozygous a+ thalassemia could not account for the degree of haemolysis. After having excluded enzyme-deficiencies and erythrocyte membrane defects, we performed DNA analysis on the a clusters Restriction analysis with EcoRI and BglII enzymes revealed the presence of two distinct deletion defects Southeast Asia aa/--18 8 (SEA) and Rightward aa/a-3.7 (RW) We discuss the physiopathology of this unusual case of HbH disease

5.028

Molecular structure of the Menkes disease gene

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Menkes disease (MNK, ATP7A) is an X-linked recessive disorder of copper metabolism Affected individuals suffer an overall deficiency of copper from both reduced intestinal absorption and defective intracellular distribution The disease is characterised by progressive neurological degeneration, defective keratinisation of hair, and skin and bone abnormalities The disease progresses rapidly and results in death in early childhood We have constructed a detailed restriction map of the Menkes gene which spans a genomic region of approximately 140kb and contains 23 exons The coding exons range in size from 77bp to 726bp Exon 23 is over 4kb long and is predominantly non-coding Exon 1 is separated from exon 2 by a large intron of 60kb Within this intron an alternatively spliced non-coding exon was found RT-PCR analysis has demonstrated differential usage of this exon in various tissues which may suggest a role in the regulation of MNK We have commenced analysis of mutations in our patients with Menkes disease Southern blot hybridisations with cDNA clones spanning the Menkes gene have identified a

deletion in one of 14 patients This rather substantial deletion results in the loss of all coding sequence 5' to exon 16 Other mutations undetected by Southern analysis are currently being screened using RT-PCR and enzyme mismatch cleavage

5.029

Linkage of autosomal dominant split hand/split foot to markers located in 10q23-q25

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The split hand/split foot anomaly (SHSF) is a developmental defect of the central digital rays which may occur sporadically, usually affecting a single limb, or in familial forms with an autosomal dominant pattern of inheritance Familial cases mostly show variable penetrance and expressivity, possibly explained on the basis of genetic heterogeneity, genomic imprinting, epistasis or germinal mosaicism SHSF may present not only as an isolated defect, but also in association with other skeletal and non skeletal defects (syndromal ectrodactyly), such as the ectrodactyly-ectodermal dysplasia-cleft palate syndrome (EEC) or the ectrodactyly-cleft palate syndrome (ECP) Regarding the genetic causes of SHSF, there have been several reports in the literature of SHSF patients who carry specific chromosomal rearrangements involving the 7q22 1, 6q21 and 2q24-q31 regions On this basis, it has been postulated that these regions harbour genes whose disruption leads to the SHSF phenotype The 7q22 1 region has been designated as the SHSF1 locus and an additional X-linked locus at Xq26 has been designated as SHSF2 Segregation analysis in families with autosomal dominant SHSF or EEC using DNA markers from the three autosomal candidate regions, failed to detect linkage supporting evidence for the genetic heterogeneity of SHSF Recently, a potential SHSF locus has been identified with the mapping of the Dac locus in the dactylaplastic mouse within a region synthenic to 10q23-q25 in humans and the observation of linkage to D10S583 in a family with SHSF These findings prompted genotyping of SHSF families with DNA markers from the 10q23-q25 region Our findings suggest the existence of two groups 1 cosegregating with 10q23-q25 markers and 1 discordant These results confirm genetic linkage to 10q25 and suggest genetic heterogeneity of SHSF

5.030

Study of the survival motor neuron gene in spinal muscular atrophy patients

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A candidate gene for spinal muscular atrophy (SMA), termed survival motor neuron gene (SMN) has recently been isolated from the 5q13 region and found deleted in most patients with childhood-onset SMA A highly homologous copy of this gene has also been isolated and located in a centromeric position We have studied 138 Italian patients (SMA type I - IV) for deletions and other mutations in the SMN gene by SSCP and, in part, by sequence analysis SMN exon 7 was found homozygously deleted in 96% of patients Two patients showed a homozygous deletion of exons 7 and 8 of the centromeric copy but had intact telomeric exons One SMA type I patient, who was not deleted for exons 7 and 8, had a 5-basepair deletion in exon 3 resulting in a frameshift and premature stop codon We have also studied one patient with an unusual early onset distal SMA restricted to hands and feet and found a homozygous deletion of SMN exon 7 suggesting that this distal variant is allelic with proximal SMA Thus, our results provide further evidence that the SMN gene is responsible for the SMA phenotype

5.031

A high resolution physical map of the distal part of chromosome 21

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The distal part of the chromosomal region 21q22 3 is gene rich and includes loci responsible for progressive myoclonus epilepsy, holoprosencephaly, autoimmune polyglandular disease type I and susceptibility for one form of bipolar affective disorder. The isolation of genes from this region would be facilitated by the availability of a detailed physical and contig map However, so far no such maps are available because this region is poorly represented and unstable in YAC clones To overcome this problem we have aligned nonchimaeric YACs and used these as framework and starting points for the development of cosmid contigs We have isolated 130 cosmids from the Lawrence Livermore National Laboratory Library that map in 21q22 3 and constructed sets of overlapping cosmids Restriction maps of the contigs have been determined These YAC and cosmid contigs cover more than 2 Mb of the 2 6 Mb region extending from the PFKL locus to the telomere We are currently filling in the gaps with P1 and BAC and additional cosmid clones The ordered cloned genomic DNA fragments provide a valuable resource for the construction of a transcription map of distal 21q Several cDNAs have already been isolated and mapped back to the cosmid clones

5.032

Further genetic and physical mapping of X-linked retinoschisis

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X-linked recessive retinoschisis (RS, MIM 312700)is the commonest cause of juvenile macular degeneration and is characterised by cystic changes at the macula causing poor vision in affected males Vitreous haemorrhage and retinal detachment are common complications and may lead to blindness The defect is thought to be in the Muller cell RS has been mapped to Xp22.1-Xp22 2 We have performed linkage studies in 14 families with RS, including 49 affected males and 42 obligate female carriers Linkage was confirmed to the microsatellite markers DXS207, DXS1053, DXS43, DXS999, DXS443 and DXS365 and multipoint linkage analysis revealed the following locus order Xpter - (DXS207, DXS1053, DXS43) - 1cM - RS - 1cM - DXS999 - DXS443 - DXS365 - Xpcen, with individual recombinants mapping RS distal to DXS999 and proximal to DXS43 YACs from this region are being fully characterised and we are developing a comprehensive restriction map around the RS locus PAC and cosmid libraries are being screened with YACs and STSs with the aim of constructing a contig across the interval This collection of smaller clones, together with the physical map of the region will provide a unique mapping resource and will assist in the cloning of genes from this region

5.033

Mutation analysis in Emery-Dreifuss muscular dystrophy

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Emery-Dreifuss muscular dystrophy (EMD) manifests in childhood with progressive weakness and wasting of the limb muscles and contractures of the elbows, ankles and spine By adult life the mobility of most patients is severely restricted Involvement of the heart is a consistent feature associated with arrhythmias and a

substantial risk of sudden death Most families show X-linked recessive inheritance The gene causing X-linked EMD has recently been identified and comprises 6 small exons coding for a novel serine-rich protein of 254 amino acids called emerin We have amplified the gene from genomic DNA and used the purified product as a template for sequencing In our initial studies we have screened exons 1 and 2 in 60 unrelated EMD patients and identified mutations in 12 cases Mutations were only found in families showing unequivocal X-linked recessive inheritance (n = 25) and not in sporadic cases (n = 20), families with two or more affected brothers (n = 7) or cases in which the family history and/or diagnosis were uncertain (n = 8) These data confirm that emerin mutations are responsible for X-linked EMD but mutations in the emerin gene are unlikely to be the major cause of sporadic cases

5.035
Mutation detection in Tuberous Sclerosis : restriction endonuclease fingerprinting (REF) and protein truncation test (PTT)

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The TSC2 gene on chromosome 16p13 3, responsible for about 50% of tuberous sclerosis, has recently been cloned The gene has 41 small exons covering 50 kb encoding a 5 5kb cDNA Large germline deletions of TSC2 occur in only 5% of cases We analysed TSC2 mRNA in lymphoblastoid cells from 21 cases of TSC, generating TSC2 cDNA in six overlapping RT-PCR fragments For restriction endonuclease fingerprinting (REF), each fragment was digested by restriction enzymes, end-labelled, and analysed on a non-denaturing polyacrylamide gel Interpretation of these results was difficult due to high background in several fragments We analysed 67% of the TSC2 gene by REF, and have to date found several REF alterations, but no confirmed mutations As TSC2 mutations are likely to be inactivating, we analysed TSC2 mRNA for mutations by the protein truncation test (PTT) The same 6 RT-PCR fragments were generated, using a primer for each which allowed transcription/translation of the PCR product in a reticulocyte lysate The protein product was ^{35}S labelled, and analysed by SDS/PAGE 65% of the TSC2 cDNA has been analysed by PTT in 19 cases, and 4 PTT shifts have been detected to date These PCR products are being sequenced PTT may offer more convenient detection of inactivating TSC2 mutations than SSCP based techniques

5.036
Rapid fragile X syndrome screening using a non-radioactive Southern blot test

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Purpose--To develop a rapid and safe non-radioactive test using the Southern blot capable of detecting fragile X mutations *Methods*--Non-radioactive DIG DNA labeling and detection for Southern blot was performed After labeling the Xho/PstI probe with digoxigenin-dUTP, hybridization followed by reaction with anti-digoxigenin alkaline phosphatase conjugate was done Colormetric detection with NBT and X-phosphate produces a brown color at sites of hybridization directly on the membrane *Results*--A rapid non-radioactive screening protocol for the fragile X mutation was developed Analysis of 94 referred cases showed as followed affected patients were 4 5% and carriers were 5 2% The complete procedure of digesting the DNA and hybridization to detection of the first visible signal can be accomplished within four days *Conclusions*--This rapid nonradioactive blotting protocol allows faster results with safer handling

5.037
Screening of point mutations by heteroduplex analysis in Turkish DMD/BMD patients

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In this study, 168 unrelated Turkish D/BMD patients were screened with the Multiplex I and Multiplex II gene amplification systems and deletions were detected in 52 % of cases The remaining 48 % of cases are most likely the result of point mutations and small deletions and insertions The identification of point mutations in the dystrophin gene represents a challenge because of its large size and complexity Their detection will help to reveal the molecular mechanisms underlying the disease and enable one to understand the biological function of the protein For this purpose we have developed and established a novel screening technique based on the differential mobility of homoduplexes and heteroduplexes on mildly denaturing polyacrylamide gels We have screened our nondeletion DMD population for point mutations using this conformation-sensitive gel electrophoresis system using multiple sets of exons We were able to detect heteroduplexes in nine out of 45 patients, four of them were in the third exon and five in exon 17 Direct sequencing of amplified products of the third exon in two cases revealed a T-insertion in the second intron at position (-9)-(-17) relative to the first codon of the third exon Therefore, the method developed here can be considered an easy, rapid, sensitive, cost and labour-effective method to detect small mutations

5.038
Molecular analysis of childhood onset spinal muscular atrophy in Turkish families

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Childhood onset Spinal Muscular Atrophy (SMA) is an autosomal recessive, neurodegeneratve genetic disorder which have been classified as Type I, II and III Recently, two duplicated candidate genes, SMN and NAIP, have been found to be associated with the pathogenicity of this disease In most SMA patients exons of the telomeric copy of SMN gene are found to be either deleted or disrupted The centromeric and the telomeric copies of the SMN gene differ by only two base substitutions in the coding regions, one being in exon 7 and the other in exon 8 which allow the two copies to be discriminated by SSCP analysis (Lefebvre et al , 1995). These sequence variations can also be distinguished by restriction enzyme analysis Exon 8 of the centromeric copy contains a recognition site for Dde I whereas a mismatch primer was designed to create a recognition site for Dra I on exon 7 of the centromeric copy (Steege et al , 1995) We have analyzed 50 unrelated Turkish SMA chromosomes for deletions in exon 8 and exon 7 of the telomeric copy by Dde I and Dra I digestion The results were further tested by a non-radioactive SSCP technique using digoxigenin The direct sequencing of DNA samples from SMA patients with no deletion of exon 7 and 8 but displaying sequence variation on a SSCP gel is in progress

5.039
Identification of the CFTR gene mutations in Turkey

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164 CF chromosomes from 82 Turkish CF families were screened for the presence or absence of 15 CF mutations Heteroduplex formation analysis revealed a frequency of 15% for DF508 and 1677delTA was found to be present in 5% of CF cases, all originating from the Black Sea region of Turkey The frequency of G542X, N1303K, and W1282X mutations were calculated as 2 2%, 1 5% and 0 7%

respectively R560T, R553X, G551D, S549X, V520F, C524X, R337X and R334W were found to be absent in the Turkish population This study has indicated a high molecular heterogeneity of the disease in Turkey Exons 4, 10, 11, 19 and 20 were screened by DGGE and the samples displaying an altered migration pattern were sequenced The screening of Exon 10 revealed the presence of a homozygous 1525-1 G->A splice site mutation in one patient and a frequency of 49% for 1540 A-G polymorphism in CF cases A nucleotide variation T1220I in Exon 19 was identified in one patient The association of mutations and the three intragenic microsatellite markers, IVS8CA, IVS17bTA and IVS17bCA has been investigated in Turkish CF families

5.040

Structure and chromosomal localization of the human Dr1 gene and exclusion as Stagardt's disease causal gene.

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Purpose In order to identify the Stargardt's disease gene, Dr1 was isolated from a YACs contig covering the chromosomal region of interest Methods Using a new long size PCR amplification procedure, we have identified the complete gene structure One of these long size PCR fragments of Dr1 was used for FISH experiments Results. Dr1 is about 15 kb in length and is composed of three exons and two introns The gene has been mapped to chromosome 1p22 1 by fluorescence in situ hybridization. In addition this gene has been studied in 35 patients with Stargardt's disease or Fundus Flavimaculatus (FFM) using SSCP analysis, and in 13/35 using direct sequencing Conclusions Taken together the results of this study support the exclusion of Dr1 as the causal disease gene of either Stargardt's disease or FFM

5.042

Mutations in extracellular domain of FGFR-3 produce unpaired cysteine residues in thanatophoric dysplasia type I.

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Thanatophoric dysplasia is a relatively common lethal chondrodysplasia with short limbs, macrocephaly and narrow thorax Two distinct subtypes based on the shape of femurs were recognized In the most common type, TDI, femurs are straight and associated with cloverleaf skull Mutations in the FGFR-3 gene were shown to produce radiologically related disorders including achondroplasia, hypochondroplasia and thanatophoric dysplasia Analyses of FGFR-3 sequence in 27 TDI patients reveal that five patients had a single base substitution at position 2416 and 2418 which disrupted the codon stop resulting in an extra 141 amino-acids in the amino terminal region Mutations R248C and S249C which occur in the extracellular domain of the receptor were identified in 9 and 2 patients respectively Two additional mutations were detected in 9 patients which converted glycine 370 and tyrosine 373 into cysteine residues These results indicate that TDI, is both clinically and genetically homogenous as more than 95 % of TDI patients are accounted for by FGFR-3 mutations Since mutations in the extracellular domain created only new unpaired cysteine residues we suggest atth constitutive activation of the receptor due to sulfide bonding might be responsible for the severity of the disease

5.043

Linkage studies in Finnish Lysinuric protein intolerance (LPI) families

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Lysinuric protein intolerance (MIM 222700) is a recessively inherited amino acid disorder characterized by defective efflux of cationic amino acids at the basolateral membrane of intestinal and renal tubular epithelium LPI is enriched in the genetically isolated Finnish population The random mapping approach was adopted to localize the LPI gene to a specific chromosomal region. Our study material consists of 16 Finnish LPI families including 23 affected individuals and 63 healthy family members Linkage analyses have been performed under the assumption of an autosomal recessive mode of inheritance and an estimated gene frequency of 1 100 in the Finnish population and equal recombination rates for males and females. After testing over 220 polymorphic DNA markers scattered at about 20 cM distances throughout the genome a promising chromosomal region has been found, which still needs to be confirmed

5.045

Limitation of the region for the long QT syndrome locus (LQT1) on chromosome 11p15.5

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The Romano-Ward syndrome, a form of long QT syndrome (LQT) is an autosomal dominantly inherited disorder Major clinical findings are prolonged QT intervals in the elec-trocardiogram and/or syncopes due to ventricular tachyarrhythmias, frequently leading to sudden death Predictive genetic analysis of (asymptomatic) individuals at risk for the disease is of great importance since clinical diagnosis is often difficult The disorder is genetically heterogeneous since linkage with markers in regions 3p21-p24, 4q25-q27, 7q35-q36 and 11p15 5 has been found in several families Mutations in a cardiac sodium channel gene (SCN5A) and a potassium channel gene (HERG) in the 3p and 7q regions, respectively, have recently been characterized in LQT patients In a large pedigree with 11 affected idividuals we found strong linkage of the disease locus to markers on chromosome 11p15 5 To date, for Q=0 maximum lod scores for markers tyrosine hydroxylase, D11S860, D11S988, HBB were estimated between 4 2 and 4 9 Based on three recombinants found in this family, we could conclude that the disease locus should be placed proximally of the mucine-2 genloe cus and distally to the D11S1331 locus These results confirm the exclusion of the previously suggested involvement of the HRAS gene and significantly delimitate the region of the LQT1 locus

5.047

Identification of a region involved in the regulation of the human UBE1L gene

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The UBE1L gene is located in 3p21, a region frequently heterozygously deleted in small cell lung cancer (SCLC) Despite the presence of one copy of the gene, there is a virtual absence of expression in SCLC-derived cell lines Since no mutations could be detected in the structural part of the gene, we wanted to identify its promoter region For this purpose, subcloning of a 1 28 kb fragment from the region immediately upstream of the initiation start site of the gene into a luciferase reporter vector in both orientations was followed by generation of a series of 8 deletion mutants with the use of exonucleaseIII or PCR using appropriate primers and sucloning into the same luciferase vector, sequencing of the deletion mutants,

transfection to CHO cells and luciferase assay to determine the promoter activity of the different constructs As a positive control, a reporter vector containing an SV40 promoter was used, whereas a promoterless vector was used as a negative control The construct containing the UBE1L promoter region in the 5'->3' orientation was shown to exhibit a clear promoter activity, at a level of about 60% to 70% of the SV40 promoter activity, indeed suggesting presence of promoter elements in the 1 28 kb fragment from the 5'-flanking region of the UBE1L gene Transfection experiments using the deleted clones and subsequent measurement of promoter activity have shown that when the region from -152 to +138 is deleted, no promoter activity is observed, suggesting that this region, which coincides with the region of a DNaseI hypersensitive site, contains sequences involved in the regulation of the UBE1L gene To identify more precisely the regulatory elements of the gene, gel retardation assays as well as footprinting analysis are currently being applied

5.048

Mutation screening of RET in HSCR patients.

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Hirschsprung disease (HSCR) is a congenital disorder characterised by the absence oinf tramural ganglion cells along variable lengths of the hindgut, resulting in intestinal obstruction The incidence is 1 in 5,000 births Here we report mutation analysis of the RET gene in 40 unselected HSCR patients Of these 40, 9 were known to be familial Preliminary SSCP, DGGE and sequence data for RET revealed five missense mutations (exon 3, Cys157®Tyr, exon 6, Asn 359®Lys, exon 10, Cys609®Tyr, exon 10, Cys620®Arg, exon 19, Leu 1061®Pro) and two mutations leading to a truncated protein (exon 16, Glu921 Stop, deletion Asn1059) Of these 7 mutations 4 were found in familial cases (44%), 3 in sporadic cases (10%) Two of the mutations are also known to occur in MEN 2A The patient with the Cys609®Tyr mutation did not show any MEN 2A symptoms (at the age of 30) No MEN 2A was found in any relative of this patient A family history of MEN 2A was known for the patient with the Cys620®Arg mutation Modifying factors presumably determine whether such RET mutations lead to HSCR, to MEN 2A or to both RET apparently accounts for a substantial part of the HSCR cases in particular for the familial cases An explanation of the major part of HSCR, however, still awaits identification of other genes involved in the development of colonic aganglionosis

5.049

Mutations in three genes are found associated with the development of Hirschsprung disease: RET, EDNRB and EDN3.

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Hirschsprung disease (HSCR) or colonic aganglionosis is a congenital disorder characterised by an absence of intramural ganglia along variable lengths of the colon resulting in intestinal obstruction The incidence of HSCR is 1 per 5000 live births Mutations in two genes, RET and EDNRB, have been shown to be associated with HSCR in humans We report further evidence for the involvement of EDNRB, an endothelin receptor gene, in HSCR by reporting a novel mutation detected in one of 40 unselected HSCR patients(Met374®Ile) Furthermore, we describe a mutation of the human gene EDN3, coding for the receptor ligand, endothelin 3, homozygously present in a patient with a combined Waardenburg type 2 (WS2) and HSCR phenotype. The mutation, Cys159®Phe, in exon 3 encoding the ET-3-like domain of endothelin 3, presumably affects the proteolytic processing of the preproendothelin to the active peptide endothelin 3 The patient's parents were first cousins A previous child in this family had been diagnosed with a similar combination of HSCR, depigmentation and deafness The family history suggests that the described EDN3 mutation may lead to variable expression of symptoms of WS2 in a heterozygous state whereas a homozygous state resulted in combined WS2-HSCR phenotype

5.050

New connexin32 mutations in Charcot-Marie-Tooth disease.

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We selected 26 unrelated families with a phenotype corresponding to Charcot-Marie-Tooth (CMT) disease and showing 1) no duplication on 17p11 2-12, 2) medianNCV values below or around 38 m/s, and 3) no evidence for male to male transmission Some of these patients, mostly males, are sporadic or apparently sporadic A patient from each family was screened, by SSCP analysis, for the presence of mutations in the gene for connexin32 located on chromosome X Subsequent sequencing of DNA from patients with aberrant electrophoresis profiles revealed 10 mis-sense and 2 non-sense mutations Eight of them have not yet been reported Three mutations concern amino acid 22 two of them are new and one has been described earlier and is a non-sense mutation resulting in the absence of connexin32 protein in males. Amino acid 22 seems prone to mutagenesis since 4 other cases have been reported bringing the total number of different mutations at this position to 6 No new mutations were found in transmembrane region 4 and in the C-terminal region, confirming earlier observations Mutations in connexin32 are found in 1) apparently sporadic cases, 2) electrophysiologically so- called " intermediate " families and 3) in families where women show rather CMT of type 2

5.051

A gene for FG syndrome maps in the proximal Xq region.

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FG syndrome (MIM 305450) is an X-linked recessive disorder characterized by mental retardation, facial dysmorphia, macrocephaly, congenital hypotonia and anal abnormalities Provisional localization in the Xq21 31-q22 region obtained by linkage analysis in the two generation family originally described by Keller in 1976 was reported by Zhu et al (Cytogenet Cell Genet, 1991, 58, 2091) A collaborative linkage analysis using STR polymorphic markers was performed in ten unrelated families A significant Lod Score was obtained with seven families The nearest flanking loci demonstrating recombination with the disease gene defined a 2 cM region in Xq proximal region For the three other families, localization in this region was excluded by multipoint linkage analysis These results support genetic heterogeneity of FG syndrome

5.052

Mutations in the liver a-subunit of phosphorylase kinase (PHKA2) are responsible for X-linked liver glycogenosis type I and II.

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X-linked liver glycogenosis is a benign glycogen storage disease characterized by growth retardation and hepatomegaly Two types of XLG have been described Patients with the classical type (XLGI) show a deficiency of phosphorylase kinase (PHK) in liver, erythrocytes and leucocytes In contrast, patients with the variant type of XLG (XLGII) show normal activity of PHK We have previously identified

mutations, including three stop codons and a deletion of 34 amino acids, in the gene encoding the liver a-subunit of PHK (PHKA2) from XLGI patients Recently, we also identified four different mutations in the PHKA2 gene from XLGII patients These mutations include a missense mutation leading to the substitution of arginine 186 by cysteine (R186C), a deletion of 3 nucleotides leading to deletion of threonine 251 (DT251), an insertion of 6 nucleotides resulting in the insertion of a threonine and an arginine residue between arginine 1111 and glutamic acid 1112 (R1111insTR), and a missense mutation leading to the replacement of threonine 1114 by isoleucine (T1114I) Whereas XLGI mutations probably affect the stability of the PHKA2 protein, XLGII mutations might have a regulatory effect on the PHK activity, which might explain the normal in vitro PHK activity observed in XLGII patients

5.053

Physical mapping of the Meckel syndrome locus at 17q21-q24

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Autosomal recessive Meckel syndrome (MKS) is a lethal malformation syndrome with neural tube defect (occipital meningo-encephalocele in most cases), very large kidneys with multicystic dysplasia, cystic and fibrotic changes of the liver, polydactyly, and ambiguous genitals in males The phenotype is suggestive of inductive interaction disturbances during early embryonic development This syndrome is not encountered only in Finland (birth prevalence of 1 9000) but also in other isolated populations with even higher reported birth prevalences We initially mapped the Meckel locus at 17q21-q24 in about 13 cM region that we have now been able to restrict to about 7 cM No evidence for locus heterogeneity was observed in our family material in which strict diagnostic criteria were applied Opposite to many recessive diseases enriched in the Finnish population, in MKS we have not observed any linkage disequilibrium with the markers on the critical chromosomal region, possibly suggesting several ancestor mutations in this population We have excluded some potential known candidate genes on 17q21-q24 including the homeo box B gene cluster as well as the low affinity nerve growth factor receptor Now we proceed to analyze other candidate genes on the critical chromosomal region and to construct a physical contig over the MKS region

5.054

Mutation Analysis in a European Batten disease Resource

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Batten disease occurs world-wide, with an increased prevalence in Northern Europe The batten disease gene, CLN3, is most commonly disrupted by a 1 02 Kb deletion (Cell, Vol 82, 949-957) We have tested for the presence of the 1 02 Kb deletion in 124 unrelated Batten families originating from 12 European countries Using primers F2 and G3 which flank the deletion, PCR amplification of genomic DNA demonstrates the presence of the '56' haplotype A small number of chromosomes with closely related haplotypes (66, 36, 46, 57, 55) also carry this deletion Thirty-eight families are heterozygous for the 1 02 Kb deletion Thus, chromosomes with other haplotypes clearly possess new mutations, independent of the '56' chromosome deletion To screen for all possible mutations, PCR primers have been designed to each of the gene's fifteen exons and PCR amplification of genomic DNA has been carried out on the 32 families heterozygous for the 1 02 Kb deletion Twelve exons are presently being screened using two parallel approaches of SSCP and automated sequence analysis Currently two SSCP changes have been observed, one in exon 5, the other in exon 15

5.055

Screening of G6PD Mediterranean mutation causing to severe glucose-6-phosphate dehydrogenase deficiency in Turkish population.

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common clinically significant inherited metabolic disorders It causes acute hemolytic anemia induced by specific drugs and food, and neonatal jaundice The objective of this study is to screen G6PD Mediterranean mutation in the severely G6PD deficient cases, in order to define the molecular basis of severe G6PD deficiency Turkey Sample population consisted of 160 people with severe G6PD deficiency confirmed by fluorescence spot test screening DNA from blood samples were isolated according to either phenol-chloroform extraction or Nucleon DNA extraction kit methodology PCR+MboII digestion+ mini horizontal agarose gel electrophoresis techniques were used in screening mediterranean mutation 91 and 92 numbered primers designed by Luzzatto,L(1994) were preferred in the amplification of exon 6-7 fragment covering mediterranean mutation External control DNA samples with med and nonmed mutations were employed as a standard All obtained results were also confirmed by Haematology Dept Hammersmith Hospital in London 126 out of 160 (%78 8) were found as G6PD Med and the remaining 34 (%21 2) were nonmed mutation It is experimentally approved that G6PD mediterranean variant and the mutation is predominant mainly in Aegean, Mediterranean, Eastern and Southern Anatolia regions of Turkey The ratio of % 21 2 ,which covers either G6PD nonmed or new mutations, implies the importance of international collaboration and joint studies to characterise new mutations, and also emphasizes the heterogeneity of G6PD deficiency in Turkey

5.056

Priority list of mutations causing to b-thalassemia in Aegean region of Turkey.

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Beta-thalassemia is not only the most common and preventable inherited disease but also its therapy is the most expensive The objective of this study was to determine mutations causing to beta-thalassemia in this region to make prenatal diagnosis and rational planning of therapy possible as well as informing couples before their marriage 20 different mutations were screened by using ARMS-PCR technique 1530 chromosomes covering 255 unrelated cases with beta-thalassemia and their families were studied Their hematological findings and family histories were recorded Every family was informed about the results and the nature of disease Prenatal diagnosis in the chorion villus samples was set up The results of mutation screening indicates that IVS-I-110 mutation with %44 2 is the most predominant The mutations analysed cover % 94 6 of studied patients It was seen that there is enormous genetic heterogeneity in beta-thalassemia mutations due to migration to the region from every part of Turkey and from Bulgaria, Yugoslavia, Macedonia, Albania and Aegean islands DGGE, SSCP, hydrolink gel electrophoresis and sequening methodologies were started to be used to analyse remaining mutations (% 6 4)

5.057

Title: A YAC contig and restriction map spanning the 1 Mb retinoschisis region.

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With a particular interest in X-linked juvenile retinoschisis (RS, MIM312700), we have covered the 6 Mb interval between the markers DXS16-DXS1229 with YAC clones The RS gene has previously been mapped between the markers DXS43 and DXS443 on Xp22 1-p22 2 To make a detailed genetic and physical map of the RS region, we have now isolated a new microsatellite marker from this interval and analyzed our 31 RS families also with a number of additional markers (DXS418, DXS257, DXS999, DXS7161, and DXS443) With the help of linkage data, we were able to localize the RS gene between the markers DXS418 and DXS443 We have mapped new YACs into this 1 Mb interval and isolated new STSs, as well as constructed a restriction map of the region A total of seven CpG islands were identified Cosmids are being isolated and localized into this region to be used in the ongoing gene search

5.058

A genetic predisposition to Down's syndrome? Continuing the controversy

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Genetic predisposition to nondisjunction in Down's syndrome is controversial We studied genotypes for a chromosome 21 region in parents of affected children We analysed 9 unequivocally typed RFLPs (D21S16/XbaI, D21S13/TaqI, D21S46/PvuII, D21S1/S11/EcoRI; D21S8/HindIII, D21S111/SacI, D21S3/TaqI, D21S53/SStI, D21S42/TaqI) in 127 parents of trisomic children and 54 adult controls (Northern Europeans) Parental/meiotic origin of nondisjunction were determined previously Genotype distributions differed significantly from expectation for samples from a population in Hardy-Weinberg equilibrium in disjoining parents at three loci (D21S8 p <0 05, D21S53 p<0 05, D21S42 p<0 025) and nondisjoining parents at one locus (D21S3 p< 0025) Group comparisons (contingency chi-squared) nondisjoining mothers <32 years versus controls D21S13 p<0 025, nondisjoining mothers (first meiotic division) <32 years versus controls D21S13 p<0 025, disjoining parents versus controls D21S8 p<0 025, all parents versus controls D21S8 p<0 01 Disjoining parents show unusual chromosome 21 genotype distributions at several loci All affected individuals were liveborn one explanation for these findings is a survival effect Nondisjoining parents are not significantly different in genotype distribution from controls However, nondisjoining mothers, divided by age and meiotic division, show a significantly different genotype distribution from controls for D21S13 Our results do not identify any genetic predisposition to Down's syndrome, but they continue to fuel the controversy

5.059

Single step for mutation detection

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Most of mutations causing genetic disease in man are due to intragenic deletions and duplications, or point mutations The possibility of detecting single base substitutions in PCR amplicons by double strand conformation analysis (DSCA) has been recently reported This method is based on the principle that free double-stranded DNA in aqueous solution has an intrinsic curvature, which is dependent on its nucleotide sequence A single nucleotide substitution induces a modification of the curvature profile of the double helix, the consequent variation in the friction encountered by the DNA in a porous gel should produce an electrophoretic mobility shift in nondenaturing polyacrylamide gels We show that the detectability of the mobility shift depends on the size of the amplicon and on the concentration of the gel DSCA is able to detect up to 45 % of single nucleotide substitutions in a 200-600 bp fragment The extreme simplicity of the method, associated with the high reproducibility of the results, represents a great advantage in mutation screening Moreover, since DSCA can be performed on multiplex quantitative PCR products, the same electrophoretic run may allow the contemporaneous detection of intragenic-deletions, duplications and of point mutations

5.060

21 LDL-Receptor mutations identified using DGGE and fluorescent sequencing in Familial Hypercholesterolaemia patients in Northern Ireland.

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Familial hypercholesterolaemia (FH) is an autosomal dominant disorder affecting around 1 in 500 individuals in most populations It is characterised increased serum total cholesterol and a significantly increased risk of coronary artery disease Over 200 mutations have been identified in the LDLR gene worldwide and these are spread across the entire length of the gene DGGE screening and fluorescent cycle sequencing have characterised the LDLR gene mutation in 40 families with FH Several different mutations (21) were identified largely in exons 3,4,6 and 10, nine of which were novel (211dG, 932dA, K369X, C68Y, C152X, C163Y, D461N, D461H and W462R) The most frequent were 932dA in exon 6 (n=6) and D461N in exon 10 (n=6) The total serum cholesterol in probands from families with class I receptor defects (null allele) was 11 2 mmol/l (SD 1 75, n=14) compared with families with class 2(transport) or class 5(recycling) receptor defects at 9 9 mmol/l (SD1 64, n=15, p=0 047). We conclude that a single diagnostic test for FH is difficult to achieve, however restricted screening by DGGE of exons 3,4,6 and 10 would detect 90% of the defects identified in this study In addition we have shown that mutations producing a null allele are generally associated with higher presenting total cholesterol levels than missense mutations

5.062

A retroposon-like sequence in intron 11 of the dystrophin gene associated with X-linked dilated cardiomyopathy.

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We studied a family with X-linked dilated cardiomyopathy (XLDCM), in which analysis of dystrophin expression in heart and skeletal muscle suggested a dystrophinopathy No deletion of the dystrophin gene was found Analysis of dystrophin transcription both in heart and skeletal muscle showed a higher molecular weight fragment, in addition to the normal size transcript Contrary to what found in the skeletal muscle, the normal sized fragment was very scarce in heart Cloning and sequencing of both transcripts showed that the high molecular weight fragment contained a 158 bp insert between the exons 11 and 12 of the dystrophin cDNA, the other transcript representing the normal dystrophin cDNA Sequence analysis of the 158 bp insert (that was not in-frame) revealed an high homology (>70%) to various known Alu and L1 repetitive elements and pseudogene sequences Genomic analysis in this family confirmed the presence of the inserted sequence, at 2,8 Kb from the 5' of intron 11, segregating with the disease In conclusion, we found a retroposon-like element which affects the splicing of dystrophin resulting in XLDCM Insertion of L1-like elements (but not of retroposones) have been previously reported in two families with a dystrophinopathy, and represents an extremely rare mechanism of disease in the human

5.064
Clinical evaluation and locus assignment in Bardet-Biedl syndrome:

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Bardet-Biedl syndrome (BBS) is a rare autosomal recessive condition characterised by rod/cone dystrophy, polydactyly, obesity, learning difficulties, hypogenitalism and renal dysfunction. Mapping studies indicate heterogeneity with four loci already determined. *Purpose* To determine the distribution of diagnostic features among affected patients in the U K. To ascertain the proportion of multiplex families linked to known loci. *Method s* Cardinal and minor features were surveyed in 105 patients with BBS living in the U K. DNA was collected from 20 multiplex families. Using PCR, incorporating fluorescently labelled microsatellite markers flanking known loci, we genotyped each family member with the aid of an ABI fragment analyser. Two-point and multipoint linkage analyses were performed using LINKAGE. *Results* Clinical findings: Retinitis pigmentosa 91%, polydactyly 78%, obesity 77%, learning disabilities 60%, hypogonadism 40%, kidney disease 30%, cardiovascular disease 8%, diabetes mellitus 6%, speech disorders 4%, hypothyroidism 3%, diabetes insipidus 2%. Molecular findings: Approximately 50% of our families showed linkage to chromosome 11q, 25% to 16q, 20% to 15q and 5% to 3p. *Discussion* The proportion of families showing linkage to 11q is similar to the findings of Leppert et al and probably reflects its importance as the main locus. Very few families are linked to chromosome 3.

5.065
Telomerase activity in Renal Cell Carcinomas

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Telomerase, an enzyme often present in malignant tumors and absent from most human somatic tissues, forms the ends of vertebrate chromosomal DNAs (i e telomeres) by adding hexameric (TTAGGG)n nucleotide repeats. Therefore the enzyme compensates the loss of genomic DNA that occurs in every cell cycle because of the end-replication problem, and provides stability to the telomeres. The expression of telomerase in tumor cells is concomitant with the attainment of immortality of these cells. Thus, the measurement of telomerase activity in clinically obtained tumor samples may provide important information useful as a diagnostic marker to detect immortal cancer cells in clinical materials and as a prognostic indicator of patient outcome. Based on a PCR assay described by Kim et al (Science 266, p. 2011, 1994) we determined the telomerase activity in more than 40 surgically removed primary renal cell carcinomas (tissue probes from the central tumor and from the peripher margins were tested), corresponding adjacent nonmalignant tissues and lymph nodes using a non-radioactive system. The relative telomerase activity levels were estimated by serial dilutions of extracts prepared from these specimens. Additionally, we analyzed Southern blots of HINF 1 digested genomic DNA from the corresponding tissues for changes in the lengths of telomeric repeats using the oligonucleotide probe (TTAGGG)3. Our results indicate that in 60% of the tested tumor tissues the length of the telomeres are different to the corresponding normal cells. Up to now we detected telomerase activity in the central tumors, in the tissues of the peripher margins and in the lymph nodes, but not in the analyzed corresponding normal tissue. The correlation between the level of telomerase activity and the altered telomere repeat fragments in the tumor specimens will be discussed in respect to the tumor progression.

5.066
The spectrum of Gsa gene mutations causing Albright hereditary osteodystrophy.

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Deactivating mutations in the Gsa gene (GNAS1) lead to Albright hereditary osteodystrophy (AHO) with resistance to hormones acting via adenylyl cyclase (PHPIa) and without hormone resistance (PPHP). Both PHPIa and PPHP, resulting from identical unique mutations, can occur within one kindred suggesting that a mutation alone is not sufficient to cause either form. Parental origin of mutations may play a role in the phenotypic expressision. To test this hypothesis, we have searched for GNAS1 mutations and their parental origin in our cohort of 89 patients of 57 families. Mutation detection involved PCR of individual exons and their splice consensus sequences which were subsequently analysed by DGGE. Exons 2-9 and 11-13 have been screened comprising 78% of the total coding sequence. A total of 13 mutations have been identified, 4 deletions, 1 insertion, 3 basepair substitutions and 5 splice site mutations. The identified GNAS1 mutations occur throughout the gene although 8/13 mutations occured in exons 4 and 5. The parental origin of 5 mutations could be established using previously described and newly characterised exonic polymorphisms. In keeping with the hypothesis, maternal origin of the mutation is exclusively associated with PHPIa and paternal origin with PPHP.

5.067
Polymorphic variations in Rhodopsin and RDS-Peripherin genes in the Spanish population

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Rhodopsin and Peripherin are proteins involved in the visual cascade and they are present in photoreceptors. Appart from the mutations causing retinal degeneration, different changes have been reported as intronic polymorphisms and silent mutations. We have estudied 40 Spanish patients affected with Retinitis Pigmentosa : 26 ADRP, 5 ARRP, 6 XLRP, 3 SRP and 50 control individuals. METHODS - SSCP electrophoresis was performed under two differents conditions - Restriction analysis was performed by digestion with RsaI, SacII, MvaI, MboII enzymes - PCR products was sequenced directly using US70170 Kit. RESULTS - RHO gene: Three polymorphisms were detected in the non-coding region A269G, C3982T, C5321A (Dryja et al, 1991)- RDS gene: Four polymorphisms were detected, C558T (Farrar et al, 1991) in the non-coding region and E304Q, K310R, G338D (Jordan 1992) in the third exon. DISCUSSION - Polymorphisms in the RHO gene are present in the same individuals suggesting a founder effect in the Spanish population - The polymorphism C558T in RDS gene is present with a heterozigosity of 0.49% , as it is in caucasian population - Polymorphisms in the third exon of RDS gene showed different allele frequencies comparing Irish population.

5.068
Twelve new and two rare mutations in Gaucher's disease Spanish patients.

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Gaucher's disease, the most common sphingolipid storage disease, is caused by mutations in the gene encoding the beta-glucocerebrosidase. The disease is particularly prevalent among Ashkenazi Jews. In Spain, as in other non-Jewish populations, there are two prevalent mutations: N370S and L444P which together account for about 70% of the mutant alleles. Fifty Spanish Gaucher's disease patients have been previously studied to detect the presence of the nine most

common mutations, including N370S and L444P This allowed the identification of 76 out of 100 Gaucher's disease alleles The purpose of present work was to analyse the remaining 24 mutant alleles The eleven exons of the glucocerebrosidase (GBA) gene were amplified by PCR and analysed by SSCP Those fragments producing aberrant SSCP patterns were consequently sequenced As a consequence of this study, twelve new mutations were identified They include seven missense substitutions, one nonesense mutation within the signal peptide, two one-base insertions, a two-base deletion, and one splice site mutation Beside these new mutations, two rare missense changes were also detected These mutations had only been described once before. To date, only ten Gaucher's disease alleles remain to be identified This study allowed the identification of at least 90% of the Spanish Gaucher's disease mutations

5.069

Is a mutant allele of the ROM1 gene associated with Retinitis Pigmentosa?

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Retinitis Pigmentosa is a hereditary condition characterised by slow outer-retinal degeneration Both linkage analyses and candidate gene approaches have identified several genes and loci associated with the disease, but explain only a few cases Cosegregation and SSCP analyses have been used in a panel of 48 Spanish ARRP families in search for mutations in several candidate genes Here we report the case of one family in which two amino acid substitutions (Pro60Thr and Thr108Met) in a single ROM1 allele have been found The variant, described just once in an ADRP family, is present in the two affected and one unaffected sibs, as well as in the mother None of the 76 wild-type chromosomes analysed beared these changes Analyses of the peripherin/RDS, rhodopsin and PDEB genes exclude them as the disease-causing genes in this family and also exclude the possibility of a RDS/ROM1 digenic pattern of transmission A digenic model of inheritance involving ROM1 and another unknown gene cannot be ruled out Recent clinical information seems to be consistent with a dominant pattern of inheritance assuming incomplete penetrance Further information would be needed to evaluate this variant as a disease-causing mutation

5.070

Autosomal dominant external ophthalmoplegia (CFEOM) - further delineation of the clinical phenotype.

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Congenital fibrosis of the external ocular muscles causing ophthalmoplegia is an uncommon disorder characterised by bilateral ptosis, external ophthalmoplegia and strabismus The condition follows an autosomal dominant pattern of inheritance and is highly penetrant It is not known if the primary defect is neurogenic or myopathic Linkage studies suggest that the gene for CFEOM maps to the centromeric region of chromosome 12, with strong evidence of genetic homogeneity. We describe a three-generation family in which affected members have the classical phenotype of bilateral, non-progressive ptosis, alternating exotropia/hypotropia and severely reduced extra ocular movements There is no evidence of additional neurological features One affected patient also has correctopia (distorted pupils), a feature which has not previously been included in the classical phenotype and suggests a defect in anterior segment development Investigations on this family therefore have therefore included a search for genetic defects known to give rise to anterior chamber disorders

5.071

Hirschsprung's Disease and unilateral renal agenesis in a child with a RET-protooncogene abnormality.

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Hirschsprung's disease is a relatively frequent congenital abnormality with an incidence of in 1 in 5,000 live births It is characterised by absence of intrinsic ganglion cells in the myenteric and submucosal plexuses in the gastro-intestinal tract. Males are affected 3-4 times as often as females and there is an increased risk of the condition in siblings and in the offspring of those with Hirschsprung's disease Several modes of inheritance have been suggested for this condition It has been considered to be a multifactorial disorder with several genes involved or due to a dominant gene with incomplete penetrance Cases with apparent autosomal recessive inheritance are also documented More recently, mutations in the RET- protooncogene on chromosome 10 have been identified in both familial and sporadic cases of Hirschsprung's disease When homozygous for such mutations, the mouse model has also been shown to have renal developmental anomalies We present a patient with an autosomal dominant family history of Hirschsprung's disease who also has unilateral renal agenesis Screening for point mutations in the RET-protooncogene has been carried out and an abnormality in exon 17 has been identified in the proband and her affected father We discuss the value of screening for mutations in this gene in Hirschsprung's patients

5.072

Use of novel microsatellite markers to map microdeletions in Williams syndrome

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Williams syndrome (WS) is a genetic disorder affecting 1/20 000 - 1/50 000 live births Cases are generally sporadic although familial cases have been reported Clinical phenotypic features include dysmorphic facies, mental retardation, growth deficiency, gregarious personality, hypercalcaemia and supravalvular aortic stenosis (SVAS) Patients with WS show allelic loss of elastin exhibiting a microdeletion at 7q11 23 WS is probably a contiguous gene syndrome where the other genes deleted have yet to be identified We have undertaken clinical and molecular studies of 85 sporadic cases of WS to further define the region deleted and to investigate the use of FISH and novel Genethon microsatellite markers to detect hemizygosity about the elastin locus FISH was performed with elastin cosmids on 71 patients with WS 96% with typical WS facial characteristics showed a deletion of one elastin allele. Detection of elastin hemizygosity by FISH is therefore useful for the initial diagnosis of WS The genetic order of Genethon markers 7pter-D7S672-D7S653-D7S489-D7S1870(ELN)-D7S2490-D7S2518-D7S669, has been established Our results show that heterozygosity for markers D7S653 and D7S2490 /D7S2518 indicated that these flanking loci were not involved in the deletion The marker D7S1870 was deleted in the majority of WS patients, D7S489, although deleted in 50% patients, was not very informative In cases where no elastin deletion is seen by FISH in patients with classic WS, mapping by microsatellites offers an alternative approach to identifying microdeletions These markers can therefore be used to help in the diagnosis of suspected cases of WS as well as in investigating the underlying aetiology of SVAS

5.073
Screening Klippel-Feil patients for mutations in the PAX-1 gene

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PAX genes are a family of highly conserved developmental genes Nine paired-box (Pax) genes have been identified in the murine gene family The mouse Pax-1 gene encodes a sequence specific DNA binding protein with transcriptional activating properties Expression studies of Pax-1 during mouse embryogenesis suggests a role in the development of the vertebral column, and the autosomal recessive mutation 'undulated' (un) exhibits vertebral segmentation anomalies in the mouse No human undulated equivalent has yet been described Klippel-Feil syndrome is characterised by a failure of segmentation of the cervical spine and is associated with a range of other developmental abnormalities We have investigated this syndrome as a candidate for the human homologue of the ' un' mouse 52 patients with Klippel-Feil were screened for mutations in the paired -box domain of the PAX-1 gene (HuP48) using a combination of SSCP and heteroduplex analysis A rare polymorphism was identified within the paired-box of the gene in 2 unrelated patients A base change C→G in the region upstream from the paired-box was also detected in another Klippel-Feil patient This change was not found in 100 control samples subsequently tested Further screening of the entire gene is required to determine whether mutations in PAX-1 cause Klippel-Feil syndrome and characterisation of the complete gene structure is underway

5.074
Transcription factor genes and Waardenburg syndrome

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Auditory-pigmentary syndromes such as Waardenburg syndrome (WS) are caused by 1) failure of melanocytes to differentiate in the neural crest, 2) failure of melanocytes to migrate from the embryonic neural crest to their final destinations, or 3) failure to survive in their final locations Many different human auditory-pigmentary syndromes have been described A first step in classification may be to distinguish those which affect only melanocytes (such as Type 2 WS) from those having wider effects (such as Type 1 WS) Type 1 WS is caused by haploinsufficiency for the PAX3 transcription factor Mutations include partial and total gene deletions, and splice site, nonsense and missense mutations The effects of aminoacid substitutions can be understood in the light of the recently determined 3-dimensional structure of a related protein, paired, bound to its DNA target is probably the cause of the dominant inheritance Type 2 WS is heterogeneous About 20% of families have a mutation in the MITF transcription factor gene located at 3p14 The MITF protein dimerises using helix-loop-helix and leucine zipper domains, and binds DNA via a basic domain Several mutations from families with dominant inheritance seem likely to prevent dimerisation of the MITF protein No mutations in PAX3 or MITF have been found in patients with a variety of other auditory-pigmentary syndromes, and no mutations were found in EDN3 or EDNRB in our series

5.075
Assessment of macrophage ferric reductase activity as a marker for genetic haemochromatosis

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Ferric reductase catalyses reduction of $Fe3+$ to $Fe2+$, an essential prerequisite for high-level uptake of ferric iron in both prokaryotic and eukaryotic cells Duodenal ferric reductase activity is increased in genetic haemochromatosis (HFE) compared with controls, and parallels increased 59Fe uptake rates in haemochromatosis (1) The aim of this study was to compare the ferric reductase activity (methodology as in reference 1) of haemochromatotic and control monocytes, and differentiated macrophages Controls were over 40 years of age with normal serum iron indices and no family history of liver disease or haemochromatosis, to avoid inclusion of heterozygotes A 12-fold increase in ferric reductase activity accompanied the differentiation of monocytes to macrophages ($p < 0.05$) There was no significant difference between haemochromatotic and control activities in either lymphocytes, monocytes or macrophages

Cell type		Ferric reductase activity (DA_{562}/h/10^9 cells)		n	p
		Mean	S D		
cell line	K562	1 0	0 13	3	
lymphocytes	HFE	0 66	0 47	3	>0 05
	control	0 60	0 38	3	
monocytes	HFE	0 66	0 26	3	>0 05
	control	0 56	0 11	3	
macrophages	HFE	7 7	2 0	5	>0 05
	control	7 1	3 7	5	

These results preclude use of resting macrophage ferric reductase activity as a marker of haemochromatosis in a functional complementation approach to cloning the gene Lack of a disease-related change may indicate that the haemochromatosis gene is either not expressed in resting macrophages or that expression requires a signal not present in vitro Further studies of ferric reductase activity with different iron sources and under iron loading may resolve this issue Reference 1 Raja et al, Gut, in the press

5.076
Physical mapping of haemochromatosis chromosomes in the region telomeric to D6S105

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Genetic haemochromatosis (HFE) was originally mapped to within 1cM of the HLA-A locus on chromosome 6p21 3, however recent studies have indicated a more telomeric position Previous pulsed field gel electrophoresis (PFGE) analysis of the HLA class I region did not detect any structural rearrangements in haemochromatosis (1), although a haemochromatosis family with a 6p21 1-6p23 inversion has been identified by karyotype analysis (2) We are using PFGE to to construct a physical map telomeric to the marker D6S105, and search for DNA rearrangements associated with haemochromatosis, which could define the position of the gene Yeast artificial chromosomes were hybridized to the RLDB chromosome 6 cosmid library, and positive cosmids were subcloned to obtain single copy probes DNA from 12 HFE patients and 5 controls has been digested with 5 rare-cutting enzymes and hybridized with 7 single copy probes Unlike previous studies in the HLA class I region, rare-cutting enzyme polymorphism was not detected with probes used in this telomeric region To date no disease-related differences have been detected The PFGE data are being used to construct a genomic physical map across the HFE candidate region in lymphocyte DNA, and to map and isolate CpG island-associated genes References 1 Lord et al (1990) Human Genet 85 531-536 2 Lacey et al (1994) Gastroenterol 106 A293

5.077
Identification of three novel mutations in hereditary Protein S deficiency

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Protein S (PS) deficiency is an inherited autossomal dominant disorder that accounts for less than 10% of the families with congenital thrombophilia PS is a vitamin K-dependent glycoprotein that acts as a non-enzimatic cofactor of activated

Protein C in the inactivation of activated cofactors V and VIII In the present study, we report the application of single-stranded conformation polymorphism (SSCP) analysis to the screening of 15 functionally important PS active gene (PSα) regions (4 347 kb) in 6 unrelated families patients with type I and IIa PS deficiencies PSα gene was selectively amplified using the primers previously described by Reistma et al (J Clin Invest, 1994, 93 486) A missence mutation, G to T transversion at codon 598 (Cys → Phe) was identified in a family with PS deficiency The loss of the wild-type cysteine at this position disrupts one of the dissulphide bridges of the Steroid Hormone Binding Globulin Domain, what could explain the low level of free PS antigen (14%) Two different alterations leading to premature termination of the protein translation were identified a G to A transition at codon 465 (Trp → Stop) and a 1bp T insertion in a triplet of T, starting at codon 265 This insertion, observed in three apparently unrelated families, is linked to the G allele of the Pro 626 polymorphism, suggesting a founder effect for the origin of this insertion In one subject, the insertion cosegregates with the Factor V Leiden mutation Family analysis lead us to confirm, in each case, a perfect cosegregation of the mutations with the PS deficiency Therefore, it is more than likely that these mutations represent the disease producing mutations We conclude that the SSCP analysis, in association with clinical and laboratorial findings, represent valuable tools to the understanding of the molecular pathology of the PS deficiency This research was partially supported by research grant SAU/1588/92 from Junta Nacional de Investigação Científica e Tecnológica (JNICT) T C Bustorff was supported by a research scholarship from JNICT

5.078
Genetic analysis of the molecular pathology of oculocerebrorenal syndrome of Lowe.

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The oculocerebrorenal syndrome of Lowe (OCRL, McKusick no 309000) is an X-linked disorder characterized by progressive tubular dysfunction, congenital cataract, and mental retardation The OCRL gene was localized at Xq25-q26 The 5 8 kb mRNA has an open reading frame of 2910 bp and encodes a polypeptide of 968 amino acids Recently, the predicted OCRL protein was identified as phosphatidylinositol 4,5-bisphosphate 5-phosphatase The disruption of protein trafficking in the Golgi apparatus caused by the deficiency of this 5-phosphatase has been suggested to be the primary defect in OCRL The structure of the OCRL gene is unknown at present and therefore, mutation detection requires the analysis of mRNA In eight patients affected by OCRL, we have used two approaches to characterize the molecular alterations underlying the disease On one hand, we searched for mutations in the OCRL mRNA following RT-PCR, SSCP analysis, and direct sequencing On the other hand, exon-intron boundaries, corresponding to about 40% of the coding sequence, were determined which enabled us to PCR-amplify single exons and detect mutations by this way We identified a missense mutation (R577N) affecting a residue in the active site of the enzyme, three nonsense mutations (W574X, R577X, and Y687X), a 1-bp deletion (at nucleotide position 1975), a 1-bp insertion (at nt position 2731), and a 4-bp insertion (at nt position 1877) as well as a large intragenic deletion removing at least 500 bp from the middle portion of the OCRL mRNA All but the identified missense mutation predict a premature termination of protein translation and thereby result most likely in functional null-alleles

5.079
Proximal CACCC box mutation (-90 C→T) markedly decreases b globin promoter transcriptional activity

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The b globin gene promoter contains several elements highly conserved during evolution These sequencies are necessary to regulate the b globin gene transcription level They act in cis by binding transacting factors and are likely to cooperate with other regulatory proteins In man, one of this promoter elements, the CACCC box, is duplicated at nt -90 and -105 relative to the cap site Several point mutations in the proximal CACCC box have been described Their effects decreased transcription 2 5 to 10-fold originating b thalassaemia trait We describe here the preliminary expression studies of another, naturally occurring, point mutation, -90 C→T, found in Portuguese b thalassaemia carriers (Faustino P et al, Hum Genet 89 573, 1992) To evaluate the effect of this mutation on transcription, we have cloned PCR-amplified DNA fragments, containing the mutated or the normal b globin promoter, in a pCAT reporter plasmid with an SV40 transcription enhancer HeLa cells were transfected by electroporation with the CAT constructs In order to normalize the transfection efficiencies, a control pCH110-b galactosidase construct was included in each transfection experiment The cell lysates were prepared after 48 hours and were assayed for both CAT and b galastosidase activities The results show that the -90 C→T mutation is responsible for a 8-fold decrease of the transcription level related to the wild type These preliminary results emphasise the importance of this promoter element in the regulation of transcription possibly by binding to transacting factors

5.080
Intracellular localisation of variant factor IX proteins corresponding to CRM- phenotypes expressed in CHO cells

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Qualitative or quantitative defects of factor IX (FIX) lead to haemophilia B Activated FIX (FIXa), in association with activated FVIII, phospholipids, and Ca^{2+} ions (tenase complex), converts the factor X to its active form FIX is normally synthesised by hepatocytes, where it undergoes several co- and post-translational modifications before it is secreted into the circulation When low levels of FIX C are accompanied by a similar decrease in the FIX Ag levels, patients are characterised as CRM- (cross reactive material negative). This phenotype may be the result of a subgroup of mutations which lead to defective synthesis, intracellular processing or transport of the abnormal protein The aim of this study was to determine the intracellular localisation of FIX variant proteins leading to CRM- phenotype Two missense mutations Gly207⟶Arg (FIXLuanda) and Thr380⟶Pro (FIXBarcelos) (Hum Mut, 1993, 2 355), both localised in the active site of the protein, were studied The variant cDNAs were obtained by in vitro mutagenesis and cloned into an expression vector Chinese hamster ovary (CHO) cells were then transfected with these constructs, and clones were selected and expanded Results obtained by fluorescent immunocytochemistry using an antibody towards FIX lead us to conclude that these variant proteins are most probably retained in the endoplasmic reticulum This observation was confirmed after treating the cells expressing the normal FIX with Brefeldin A Further studies are underway making use of organelle specific antibodies, and also by electron microscopy

5.081

CFTR gene expression and function in a nasal polypfrom a patient with the 3272-26A→G/ΔF508 genotype

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Nasal polyposis is said to occur in up to 48% of Cystic Fibrosis (CF) patients We have used in this study nasal polyps from a CF patient with the unusual mutation 3272-26A→G, being ΔF508 the second mutation Controls were nasal polyps from non-CF patients Although other authors have shown that this mutation affects splicing, resulting in a transcript with 25 extra nucleotide residues in exon 17b, we have not observed by RT-PCR analyses such abnormally spliced transcript in detectable amounts Epithelial cell primary cultures have been established from this polyp and immunocytochemical studies were carried out in these cells using the MATG 1031 antibody (Transgène) which recognizes an extracellular loop of the CFTR protein By using this antibody without cell permeabilization it was observed that some CFTR protein was present in the cellular membrane (CM) Since the ΔF508 protein does not reach the CM, the CFTR observed must be a product of the 3272-26A→G allele The electrophysiological analyses by Ussing chamber techniques showed a high sodium absorption in this CF nasal epithelium which is in agreement with the literature Acknowledgements This study was partially funded by a JNICT research grant The authors thank to Transgène for supplying the MATG1031 antibody

5.082

Factor VIICoimbra: a novel splice site mutation in the human factor VII gene.

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Factor VII is a vitamin-K dependent serine protease found in human plasma which on binding tissue factor initiates the blood coagulation process Its deficiency leads to hypocoagulation As part of a survey aimed at elucidating the molecular basis of factor VII deficiency we have identified a novel splice site mutation The propositus presented low antigenic and functional factor VII values (FVII Ag 9%, FVII C 7%) and haemorrhagic symptoms His parents presented intermediate values (mother FVII Ag 85%, FVII C 60%, father FVII Ag 62%, FVII C 35%) DNA from the propositus and his parents was extracted from peripheral white blood cells) Functionally important regions of factor VII gene (putative promoter, exons and intron-exon junctions) were amplified by PCR. Mutation screening was done by non-isotopic single-strand conformation analysis (SSCP) The fragment correspondent to exons 3 and 4 displayed abnormal electrophoretic mobility This fragment was further characterized by direct sequencing and a single point mutation was identified at nucleotide number 5886 The propositus was homozygous for a G to A transition at position -1 of IVS3 donor splice site This mutation was found in heterozigosity in both consaguineous parents Potential splice site usage was computed in an attempt to predict the most likely molecular consequences of this mutation Skipping of exon 3 seems to be preferred to cryptical splice site usage We are now testing this hypothesis by studying the splicing pattern of illegitimate transcripts in lymphocytes Acknowledgement HR is a recipient of a JNICT PhD grant

5.083

Integrated overlap, restriction and transcriptional map of the 21cen-21q21 region in PACs and cosmids

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Chromosome 21 map, though one of the most advanced among whole human chromosome maps, is still insufficiently complete to enable detailed transcriptional mapping and large scale sequencing The physical clone map is at the stage of near complete YAC contig and low resolution cosmid pocket maps encompassing the whole chromosome, as well as high resolution cosmid and P1-like vector contigs in limited regions in 21q22, encompassing in total ~15% of the chromosome We constructed two large PAC/cosmid contigs of 1 5 and 1 2 megabases respectively, plus a number of smaller contigs encompassing 85% of the 21cen-21q11-21q21 region So far, 49 PACs and >50 cosmids define 2 large and 9 smaller contigs We hope to close most of the gaps by the conference and present a near complete PAC and cosmid contig of this 4 megabase region A full Not I, Sal I and Xho I restriction map of the minimal tiling path clones is constructed in parallel So far, 12 potentially transcribed sequences derived by cDNA selection and/or exon trapping have been positioned onto restriction fragments The expression of these fragments in various tissues of normal, Down syndrome and Down syndrome with leukemia subjects is being analysed This map will convert further 10% of the chromosome into fully overlapping bacterial clone map status It will also enable positional derivation of new transcribed sequences as well as new polymorphic probes, that will help in elucidation of the alleged role the genes in this region may play in leukemogenesis in trisomy 21, and some forms of Alzheimer's disease

5.084

Mutational spectrum of the Neurofibromatosis type 1 (NF1) gene

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Neurofibromatosis type 1 (NF1) is one of the most common disorders with an incidence of 1 in 3000 The mutation rate is very high with approximately 50% of all NF1 patients having no previous family history of NF1 We have screened over 320 NF1 patients and have so far identified mutations in 68 patients Six patients have molecular deletion, involving one or more exons, all of which are of maternal origin A cytogenetically identifiable deletion was observed in another patient which was paternally derived Approximately 35 alterations identified in our study represent novel changes in the gene The mutation detection techniques employed include pulsed field gel electrophoresis, FISH analysis, single strand conformation polymorphism (SSCP), heteroduplex analysis and direct sequencing This extensive search for NF1 mutations indicates that approximately 70% of mutations so far identified are predicted to generate a truncated neurofibromin protein

5.085

Molecular analysis in Charcot-Marie-Tooth disease type 1

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Recent studies have identified three peripheral myelin genes peripheral myelin protein 22 (PMP22), myelin protein zero (P0), and connexin 32 (Cx32) genes, and their mutational mechanisms that underlie several inherited diseases of the peripheral nervous system, commonly Charcot-Marie-Tooth disease type 1 (CMT1) These studies have allowed subclassification of these disorders according to the

genetic basis, and provided a wider understanding of their biological basis Using the single strand conformation polymorphism analysis and heteroduplex analysis, we have screened the three genes in 76 unrelated non-duplicated CMT1 patients and in 50 normal controls By direct sequencing we have characterized four novel mutations in the P0 gene (Y68C, I112T, P132L, V58F), and seven novel mutations in the Cx32 gene (R15W, S72S, T86A, 335delGG, 337delCC, H100Y, N205S) Functional studies will be required to determine the underlying mechanisms by which these mutations cause disease in these patients

5.086
DNA comparison in Romanov family for identification of putative remains of last Russian Tsar

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It was widely believed that the last Russian Tsar Nicholas II Romanov and his family were murdered, and their bodies completely destroyed on July 16/17, 1918 Recently a shallow grave containing human skeletal remains was discovered The details within grave and mitochondrial (mt) DNA analysis reported by the BHO Forensic Science Service research team supported the hypothesis that these grave might contain the bodies of Romanov family However, the significance of match and mismatch ("heteroplasmy") between mtDNA from putative Tsar's bones and distant descendants of Nicholas II is questionable We undertook to analyze the DNA of the closest relative of the Tsar, the son of Grand Dutchess Olga and direct nephew (K-R) of Tsar Nicholas II Comparison of the mtDNA hypervariable regions (HVR1 and HVR2) revealed near matches between mt sequences of K-R and reported sequences of femur skeleton 4 (putative Nicholas II) However, the mt16169 nucleotide, which was heteroplasmic for the femur of skeleton 4, is homoplasmic for K-R and different from two distant related maternal descendants These data might be interpreted as showing recent mutation event in Imperial family (Louise of Hesse-Cassel line) The unknown rate of spontaneous mutations and population substructure are discussed as a problems for positive DNA identifications The additional analysis of the putative Tsar's remains and more informative STR analysis may be applied to prove the authenticity of bones

5.087
A point mutation in the 5' splice site of the first intron of the dystrophin gene responsible for X-Linked dilated cardiomyopathy

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X-linked dilated cardiomyopathy (XLDC) is a familial heart disease presenting in young males as a rapidly progressive congestive heart failure, without clinical signs of skeletal myopathy *This condition has recently been linked to the dystrophin gene* in some families, and deletions encompassing the region of the first muscle exon have been detected In order to identify the defect responsible for this disease at the molecular level, and to understand the reasons for the selective heart involvement, a family with a severe form of XLDC was studied In the affected members, no deletions of the dystrophin gene were observed Analysis of the muscle promoter, first exon and intron regions revealed the presence of a single point mutation at the first exon-intron boundary, removing the universally conserved 5' splice site consensus sequence of the first intron This mutation introduces a new restriction site for Mse I, which cosegregates with the disease in this family Expression of the major dystrophin mRNA isoforms (from the muscle-, brain- and Purkinje cell-promoters) was completely abolished in the myocardium, while the brain- and Purkinje cell-(but not the muscle-) isoforms were detectable in the skeletal muscle Immunocytochemical studies with anti-dystrophin antibodies showed that the protein was reduced in quantity but normally distributed in the skeletal muscle, while it was undetectable in the cardiac muscle These findings indicate that expression of the muscle dystrophin isoform is critical for myocardial function, and suggest that selective heart involvement in dystrophin-linked dilated cardiomyopathy is related to the absence in the heart of compensatory expression of dystrophin from alternative promoters

5.088
Linkage of autosomal dominant familial dilated cardiomyopathy to chromosome 9

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Idiopathic dilated cardiomyopathy is a heart muscle disease of unknown etiology, characterized by impaired myocardial contractility and ventricular dilatation Familial transmission is often recognized (FDC), mostly with autosomal dominant inheritance In order to understand the molecular genetic basis of the disease, a large six-generation kindred with autosomal dominant FDC was studied for linkage analysis Eighty family members were investigated and the disease status was established after a clinical follow-up of 3 years According to strict diagnostic criteria, 13 members were considered to be affected, and 2 of unknown status, while members under the age of 16 were excluded from the linkage study to avoid the low penetrance of this age group The analysis was then extended to two other informative families with autosomal dominant pattern of transmission and identical clinical features (with 8 affected members, and 1 of unknown status) A genome wide search was undertaken after a large series of candidate genes were excluded Co-inheritance of the disease gene was excluded for over 95% of the genome, analyzing 251 polymorphic markers Linkage was found for chromosome 9q13-q22, with a maximum cumulative two-point lod score of 3 69 (locus d9S153, at q = 0 08) and a maximum multipoint lod score of 4 2 There was no evidence of heterogeneity According to the two-point, multipoint and haplotype analyses, the gene for FDC in these families was placed in the interval between loci D9S153 and D9S152 A gene for autosomal dominant FDC was localized on the long arm of chromosome 9 several candidate genes for causing dilated cardiomyopathy map in this region

5.089
A new locus for arrhythmogenic right ventricular dysplasia on the long arm of chromosome14

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Familial arrhythmogenic right ventricular cardiomyopathy or dysplasia (ARVD) is an idiopathic heart muscle disease with autosomal dominant pattern of transmission, characterized by fibro-fatty replacement of the right ventricular myocardium and ventricular arrhythmias Recently, linkage to the chromosome 14q23-q24 (locus D14S42) has been reported in two families In the present study, three unrelated families with ARVD were investigated According to strict diagnostic criteria, 13 out of 37 members were considered to be affected Linkage to the D14S42 locus was excluded On the other hand, linkage was found in the region 14q12-q22 in all three families (cumulative two-point lod score=3 26 for D14S252), with no recombination between the detected locus and the disease gene With multipoint linkage analysis, a maximal cumulative lod score of 4 7 was obtained in the region between loci D14S252 and D14S257 These date indicate that a novel gene causing familial

ARVD (provisionally named ARVD2) maps to the long arm of chromosome 14, thus supporting the hypothesis of genetic heterogeneity in this disease

5.090
Identical size of 17p11.2 deletion in unrelated patients with tomaculous neuropathy demonstrated by cosH1 probe.

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Hereditary neuropathy with liability to pressure palsies (HNPP), also called tomaculous neuropathy, is an autosomal dominant disorder that produce an episodic, recurrent demyelinating neuropathy HNPP is associated with a 1 5 Mb deletion of the 17p11 2 region which is duplicated in Charcot-Marie-Tooth (CMT1A) disease The deletion was identified in most clinically diagnosed HNPP patients We analyzed ten pedigrees (22 patients) in which HNPP phenotype segregates and five isolated cases The genetic study was carried out with chromosome 17 probe from the CMT1A REP element, cosH1, which is able to reveal the 17p11 2 duplication/deletion found in most CMT1A/HNPP patients In PFGE filters from normal subjects probe cosH1 recognizes two EagI fragments (270 and 220 Kb), in HNPP patients this probe identifies an extra fragment of 320 Kb The 320 Kb fragment was detected in all affected individuals both in familial and in isolated cases This finding confirms that this mutation is the most common genetic defect underlying the disease in our population PFGE study demonstrated the homogeneity of the molecular defect, being the size of extra EagI fragment identical in all our patients althought they had different clinical features

5.091
Mutation analysis in the NF1 gene in Greek families with neurofibromatosis type 1

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Neurofibromatosis type 1 (NF1) is a common autosomal dominant disorder characterised by variable expressivity, including cafe'-au-lait spots, Lisch nodules, cutaneous or subcutaneous neurofibromas or even a variety of tumours of the nervous system , The Nf1 gene, which encodes for a GTPase-activating protein, (neurofibromin), is thought to be a tumor suppressor gene Because of its large size (350 kb) and the very high mutation rate it displays, the detection of a possible large number of different unique mutations has become a very difficult task Here we report preliminary results of the mutation analysis of 65 Greek families containing 108 patients with NF1 Screening for large size rearrangements with Southern blotting and hybridization with cDNA probes has not yet revealed any mutations in the unrelated patients tested On the other hand, PCR amplification studies of exons 1-10 of the Nf1 gene detected 21 candidate DNA alterations We have found 3 candidate mutations in exon 3, 4 in exon 4, 5 in exon 5, 2 in exon 6, 2 in exon 7 and 5 candidates in exons 8-9 The investigation of the nature of these candidate alterations in the DNA sequence is under way The findings will be discussed in the light of the proportion of true mutations versus polymorphisms to be found It is worth mentioning that the region of exons 3 to 9 is one of the less studied in the NF1 gene, with no mutation reported so far, to our knowledge

5.092
An integrated Map of human Chromosome 10

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We present an integrated map of human chromosome 10 by correlation of genetic,cytogenetic and physical mapping data Our genetic linkage map was constructed using 153 loci (120 microsatellites and 33 RFLPs) It contains147 markers consisting 67 framework markers, i e 40 haplotype systems and 27 unique markers, placed in a single order (odds > 1000 1) Six markers were placed in more than one interval Genotypic data were generated at IMBB (40 markers), or selected from CEPH and CHLC databases, i e 28 and 85 markers, respectively The sex-averaged map length is 187 7 cM with a mean genetic distance of 2 8 cM Nine IMBB microsatellites were used in YAC library screening Isolated ICI YAC sets were employed in FISH experiments on metaphase chromosomes The cytogenetic data are in agreement with the loci positions determined by linkage analysis Finally, we have screened a panel of 23 cytogenetically characterised chromosome 10 fragments (collected from human-hamster somatic cell hybrids or obtained from a monochromosomal cell hybrid by the radiation-reduction method, R Marzella et al , GDB ID G00-636-617) by 50 microsatellites distributed along our framework map This work will further facilitate the integration of chromosome 10 genetic, cytogenetic and physical mapping data

5.093
Molecular studies in nondeleted patients with Prader-Willi or Angelman syndrome.

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Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are caused by loss of function of genes, located in 15q11-q13 This region is subject to genomic imprinting, possibly under control of an Imprinting Control Element The vast majority of typical PWS patients show loss of paternal alleles due to de novo deletion or maternal uniparental disomy 15 In AS, these alterations are of opposite parental origin A parent of origin specific methylation imprint has been found in all, including nondeleted nondisomic, classical PWS patients, but not all classical AS patients We present data from 18 patients with PWS or AS, who were nondeleted on FISH analysis Included are 2 AS families with two affected siblings each All patients were seen by one of us (AS) and their phenotype was considered classical for the disorder The parent of origin specific methylation imprint at different loci (D15S63 and SNRPN-1) was determined All 8 PWS patients, but only one of 10 AS patients showed an abnormal methylation pattern Using CA repeat polymorphisms, uniparental disomy could be detected in 2 PWS patients so far Further investigations are in progress in order to exclude uniparental disomy in the remaining cases and to identify putative imprinting mutations

5.095
A 1.5 Mb deletion in 17p11.2 causing tomaculous neuropathy due to a mutation besides the recombination "hot spot" in CMT1A-REP elements

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Hereditary neuropathy with liability to pressure palsies (HNPP) is an autosomal dominantly inherited disorder of the peripheral nervous system The vast majority of

patients reveal a 1 5 Mb deletion in 17p11 2 due to meiotic crossing-over between flanking repetitive sequences, so called CMT1A-REP elements EcoRI fragments (7 8 and 6 0kb) located within these elements provide proof to a recombination by dosage differences on Southern hybridization with probe pNEA102 One of 9 unrelated HNPP patients revealed a EcoRI/Southern hybridization pattern (pNEA102) typical for a CMT1A duplication with a dosage of 7 8 6 0 kb as 1 2 FISH analysis with probes pVAW409R3, PMP22, c20G8 and c122H12 indicates a 1 5 Mb sized deletion in 17p11 2. A recombination hot spot leading to a 7 8 kb EcoRI junction fragment is frequently observed in HNPP deletions (for detail see abstracts of Reiter et al , Timmerman et al) EcoRI/SacI Southern hybridization with the CMT1A-REP probe pLR7 8, however, revealed no junction fragment of 7 8 kb but dosage differences indicating a recombination outside the "hot spot"

5.096

Homologous recombination involving flanking repeats as a molecular mechanism for the Smith-Magenis del(17)(p11.2) microdeletion syndrome

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Smith-Magenis syndrome (SMS) is a contiguous gene syndrome associated with deletion of the short arm of chromosome 17 in band p11 2 The SMS deletion is a recurrent event since it occurs in multiple de novo patients and most patients appear to have a similar size deletion (estimated at 5-6Mb) To facilitate the study of the molecular mechanism involved in this deletion and the cloning of gene(s) involved in its phenotype, an overlapping Yeast Artificial Chromosome (YAC) contig of the critical region has been constructed We have identified three low copy number repeats in this region Interestingly, one of these repeats (194) corresponds to a cDNA which maps to both ends of the SMS critical region (SMS-REP) This cDNA when utilized to probe pulsed-field gels from 16 unrelated SMS patients identified a specific junction fragment of a similar size This result suggests a precise homologous recombination mechanism between the flanking SMS-REP repeat sequence as the molecular mechanism for the SMS deletion

5.097

A recombination "hot spot" mutation in 17p11.2 is frequently found in European CMT1A and HNPP patients.

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Charcot-Marie-Tooth disease (CMT) and hereditary neuropathy with liability to pressure palsies (HNPP) are the most frequently inherited disorders of the peripheral nervous system A recent European collaborative study demonstrated that 70 7% of unrelated CMT type 1 patients have a 1 5 Mb tandem duplication in 17p11 2 and that the reciprocal deletion is observed in 84 0% of unrelated HNPP patients (Nelis et al 1996, Eur. J Hum Genet , in press) It has been shown that the CMT1A duplication and HNPP deletion are products of unequal crossing-over events between misaligned flanking ~30 kb CMT1A-REP elements We analyzed 130 unrelated CMT1A duplication patients and 32 unrelated HNPP deletion patients for the presence of a mutation "hot spot" in the CMT1A-REP sequences 100 (76,9 %) CMT1A patients revealed a 3 2 kb EcoRI/SacI duplication junction fragment and 23 (71 9 %) HNPP patients showed a 7 8 kb EcoRI/EcoRI deletion junction fragment with the CMT1A-REP probe pLR7 8 The junction fragments were also found in 8/9 de novo CMT1A duplication and 1/2 de novo HNPP deletion patients These data support the hypothesis of a recombination "hot spot" within the CMT1A-REP for crossing-overs that result in the CMT1A duplication or HNPP deletion

5.098

Altered frequency of Gaucher Disease carriers among heterozygotes of Tay-Sachs Disease

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Gaucher's Disease (GD) and Tay-Sachs Disease (TSD) are autosomal recessive disorders caused by impaired activity of the enzymes glucocerebrosidase and hexosaminidase A, leading to pathological accumulation of glycolipids Frequency of heterozygotes of these diseases is highest among Ashkenazy Jews (1 29 for TSD and 1 16 for GD) Two theories attempt to explain this unusually high frequency one assumes a founder effect with subsequent genetic drift and the other postulates a selective heterozygote advantage We addressed this issue by analyzing the frequency of heterozygotes of GD most common mutation (1226A→G) among carriers of the common TSD mutation (+TATC1278-1282) as compared to the frequency of each mutation in the general Ashkenazy population The frequency of GD carriers among 188 TSD heterozygotes was 1 31 which is half the frequency found in 517 control individuals (1 15 5) (χ2,,p=0 09) Concurrently we found lower frequency of TSD heterozygotes among 121 GD carriers (1 60) than in controls (1 34) The altered frequencies might indicate that these mutations are relatively recent and have not reached an equilibrium as yet Alternatively, double heterozygotes for both GD and TSD may be associated with a selective disadvantage

5.099

Prenatal diagnosis of X linked Ichthyosis affected fetus and abnormal karyotype (XYY) in uncultured amniotic cells using Fluorescence -In-Situ-Hybridization (FISH).

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X linked Ichthyosis - a skin disorder that affects between 1 2000-1 6000 males, is caused mostly (80-90%) by deletion, in the steroid sulfatase gene located on Xp22 3 A 44 years old female, (GP), known to be a X-linked Ichthyosis carrier, came to genetic counseling, due to her advanced age, prior amniocentesis Maternal serum screening showed low levels of uE3 (0 02MOM), while the αFP and βhCG were normal FISH was performed on uncultured and cultured amniotic fluid cells with direct labeling X(alpha satellite) and Y(satellite III) probes (VYSIS) and with indirect labeled Steroid Sulfatase (STS), region (Xp22 3) and X centromer control probes (Oncor) On the uncultured amniotic cells a XYY fetus was identified four hours after amniotic procedure was carried out Using the STS probe on the uncultured amniotic cells, we identified that the fetus had a steroid sulfatase deficiency Our results were confirmed using the same technique on cultured amniotic cells The abnormal karyotyping was confirmed by the routine cytogenetic analysis Multiplex PCR amplifying fragments along the STS gene indicated a deletion of the whole gene FISH technology on uncultured amniotic cells is of emerging value as a rapid and accurate identification of unique deleted genes and aneuploidies in the fetus

5.100

Evaluation of chromosome 17p11.2 specific probes for duplication and deletion analysis in hereditary peripheral neuropathies.

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More than 70 % of patients with Charcot-Marie-Tooth disease type 1 (CMT1, HMSN I) carry a 1 5 Mb tandem duplication in chromosome 17p11 2 (designated CMT1A) Hereditary neuropathy with liability to pressure palsies (HNPP) is associated with a reciprocal deletion In 124 patients and relatives with CMT1 or HNPP we performed

MspI/Southern hybridization with probes pVAW409R3a, pEW401HE and pVAW412Hec (provided by C Van Broeckhoven, Antwerp) and/or EcoRI /Southern hybridization with probe pNEA102 (provided by J R Lupski, Houston), looking for characteristic dosage differences of informative alleles Independently we performed FISH diagnostics with probe pVAW409R1 in 92 of the patients In 57 hybridizations with pVAW409R3a we detected 9 duplications (15 8 %), 15 (32 63 %) wild type patterns and 33 (57 9 %) non-informative patients 11 of the 33 non-informative patients were diagnosed with probes pEW401HE and pVAW412Hec FISH diagnostics confirmed the diagnosis in all 28 patients tested The recently available probe pNEA102 provided a definite result in all non-informative patients In two of 37 patients there was evidence for a recombination at 17p11 2 in contrast to the results of the previously used probes and FISH diagnostics In 89 hybridizations with pNEA102 we surprisingly found two clinically and in FISH diagnostics determined HNPP cases which showed a density pattern typical for the CMT1A recombination Probe pNEA102 was always informative and may be more sensitive for recombination detection than the previously used probes However, it should only be used in connection with a second well-established diagnostic method Acknowledgement KDB holds a DFG research fellowship, HG and BR are supported by the DFG, TL holds a Herbert-Quandt fellowship

5.101
Development of an adhesion test system for pathogenic mutations within the Charcot-Marie-Tooth type 1B canditate gene P0.

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P0 (MPZ, myelin protein zero) is a protein located in the myelin sheath of the peripheral nervous system As shown earlier the P0 wt protein is able to form homodimers and is essential for the compaction of the myelin sheath of peripheral nerves Recently point mutations within the P0 gene as cause of Charcot-Marie-Tooth (CMT) disease type 1B were described However the in vivo effects of these mutations remain to be determined Analysis of sural biopsies ist more and more reduced due to the rapid methods of genetic detection for mutations in DNA extracted from whole blood cells Thus, studies of the expression of candidate genes have to be carried out more and more in cell culture or animal systems We are developping an adhesion test system based on cloned P0 cDNAs in Schneider2 (S2) insect cells P0 mutations are introduced by PCR-directed in vitro mutagenesis experiments The following mutations are used Frame shift Ins663GC (Hum Mol Gen [1994] 3, 1701-1702) Deletion of amino acid no 34 (Ser, numbering refers to the mature PO protein) and exchange of Ser 34 to Cys (TIG [1994] 10, 128-133) The first results are very promising In the aggregation assay only the wildtype case shows an aggregation behaviour The functional analysis will be an important step to understand better the genotype-phenotype correlation of the slowly progredient CMT disease Acknowledgement KDB holds a DFG research fellowship, HG and BR are supported by the DFG

5.102
Dramatically prolonged cell cycles in the yeast Saccharomyces cerevisae and growth arrest for E. coli bacteria: induced by high dose of human peripheral mylein protein 22 (PMP22)

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The human PMP22 gene was cloned by PCR screening of two cDNA-libraries fetal brain and brain stem The PCR products of app 500bp length were ligated into the high copy yeast vector pVTU102 under control of the strong constitutive ADH promototer and terminator Afterwards an inducible shuttle vector pYES2 was also used for heterologous expression of the human PMP22 cDNA Sequence analysis was carried out to confirm the integrity of the cloned PMP22 DNA Induction of expression in every first used expression system in E coli directs the exponentially growing culture immediatley into the stationary phase Using the yeast as lower eukaryotic expression system with a high copy number plasmid pVTU had similar results Freshly transformed cells reveal a strongly decreased growth rate compared to the wild type Use of an inducible pYES2 system for the whole PMP22 gene leads also to a reduced growth PMP22 transcripts could be detected by northern blot analysis and RT PCR However, Western blot analysis with three different PMP22-specific antibodies showed no reasonable amount of PMP22 protein Genetic engineering of the human PMP22 cDNA is now carried out to detect the cytotoxic domain within the cloned open reading frame Expression of the first extracytosolic domain of PMP22 had no effect on the cell cycle Hence other fragments have to be analyzed for growth arrest effects Both the negative effects on the growth of yeast and E coli and the description of PMP22 as gene inducing an apoptosis -like phenotype in NIH3T3 cells after overexpression indicates a basic function of PMP22 not exactly restricted to myelination However, the biological function of PMP22 remains to be determined Acknowledgements BR is funded by the DFG, OP holds a korean fellowship, HA holds a fellowship of the „Studienstiftung des Deutschen Volkes", TL holds a Herbert-Quandt-fellowship We wish to thank Prof Dr E Gebhart and Prof Dr RA Pfeiffer for their support

5.103
Screening the SMN and NAIP genes in SMA families

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Alterations in two candidate genes in the 5q 11 2-q13 3 region have been identified as the putative cause of childhood onset spinal muscular atrophies One of the two genes the so called survival motor neuron (SMN) gene was shown to be deleted or interrupted in the majority of patients regardless of the SMA type they belong to The other gene called the neuronal apoptosis inhibitory protein (NAIP) gene was shown to be deleted in certain patients Eighty-five patients were screened for the presence or absence of exon 5 from the NAIP gene and of exons 7 and 8 from the SMN gene Following PCR, the copy gene was separated from the functional gene in SMN exons 7 and 8 with the use of restriction enzymes Deletions were detected in 94 9% of our patients Forty-one percent of patients had deletions of NAIP and SMN exons 7 and 8, while 43 6% of patients had deletions of exons 7 and 8 and 10 3% had deletions of only exon 7 The majority of SMA I patients had deletions of NAIP, SMN 7 and 8 (63 6%) while the majority of SMA II patients had deletions of only SMN exons 7 and 8 (70 4%) Deletions of NAIP were not detected in SMA III patients SSCP was performed in the remaining 5% of patients with no detectable deletions, in order to detect possible mutations in exons 7 and 8 Mutations were not revealed in these exons Fifty sibs of affected patients were screened for possible deletions of one of these exons but no deletion was observed

5.104
A 3' acceptor splice site mutation in intron 50 leads to mild Duchenne muscular dystrophy phenotype

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Point mutations are the cause of Duchenne or Becker muscular dystrophies in almost 30% of patients with no detectable deletions Using a variety of point mutation detection techniques such as chemical cleavage of mismatch, SSCP, heteroduplex analysis and PTT a number of mutations have been identified so far During screening of our DMD/BMD patients with no detectable deletions in the dystrophin gene, using chemical cleavage of mismatch and SSCP analysis, we detected an alteration in the DNA of three patients belonging to a DMD family Affected individuals became wheelchair bound at the age of 10-12 but the disease progressed slowly until the age of 25-30 with no major respiratory complications They could move their hands, and keep their body up straight Muscle biopsy could not be performed Following sequencing a G^{-1} to A point mutation in the 3'

acceptor splice site of intron 50 was identified Following RT PCR of this region, only one RNA transcript of altered size was observed, indicating the absence of an alternative splice site Sequencing of this product revealed a deletion of 11 bases from exon 51 (positions 7518 to 7528) Predicting from the sequence analysis, a cryptic splice site is activated at position 7527-28 which results in a frameshift transcript and a premature termination codon at position 7561-63 The mutation was traced using CMM in the other affected individuals and carriers in this family Results were backed up by linkage analysis data

5.105

A new microsatellite marker at the RFP locus on chromosome 6p22 definitely locates the haemochromatosis gene at least one megabase telomeric to HLA-A.

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Genetic haemochromatosis is a common autosomal recessive disorder of iron metabolism characterized by excessive iron absorption through the duodenal mucosa and progressive iron overload in parenchymal organs This results in the midlife onset of severe clinical complications, including cirrhosis, diabetes, cardiopathy, and arthropathy The exact nature of the biochemical defect remains unknown Analysis of affected pedigrees has demonstrated linkage between the putative haemochromatosis gene and the HLA-A gene on chromosome 6 There is however no agreement as to the position of the gene locus relative to HLA-A Available recombinant data place the centromeric boundary of the candidate region at HLA-F locus and linkage disequilibrium data suggest that the gene may even lie beyond D6S105 Therefore, with respect to physically localizing the gene, investigators must still contend with a candidate region spanning at least 2000 kb Efforts at mapping the haemochromatosis gene have been hindered by the lack of informative markers precisely localized in the region The RFP gene has recently been mapped by in situ hybridization on chromosomal band 6p22 and by pulsed-field gel electrophoresis 1030 to 1180 bp telomeric to HLA-A We have screened a chromosome 6-specific cosmid library with a 387-bp probe encoding the cysteine-rich N-terminal domain of RFP and identified a cosmid containing the repeated sequence (TC)11(AC)20 This microsatellite was shown to be located within 4 kb of the 5'-end of the gene and was characterized in a sample of 91 healthy unrelated individuals by a method combining fluorescence labeling of PCR products and use of an automated DNA sequencer It is highly polymorphic (90% heterozygosity) Pedigree members of an informative recombinant family were typed for this new marker, and the proband's recombination was shown to have occurred in the maternally derived chromosome, between RFP and D6S306 This definitely moves the centromeric boundary of the candidate region at the RFP locus, i e at least 1000 kb telomeric to HLA-A

5.106

Deletion and methylation pattern analysis on patients with typicaland atypical Prader-Willi syndrome.

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Prader-Willi syndrome (PWS) is usually caused by a paternally derived del(15)(q11q13) (about 70%), or by maternal uniparental disomy (UPD) of chromosome 15, in both cases associated with loss of function of imprinted genes in the region Molecular studies were performed in a group of 16 patients described as typical (10) or atypical (6) Prader-Willi syndrome We have utilized six DNA-probes pML34 (D15S9), pTD3-21(D15S10), IR4-3 (D15S11), IR10-1 (D15S12), pTD189-1 (D15S13), pIR39 (D15S18) Methylation pattern in the region was tested by probe PW71B (D15S63) (gift of Prof B Horsthemke) and exon a of the SNRPN gene 6 patients from the typical group have visible deletions which were confirmed by dosage analysis, where at least three DNA-markers were deleted on the molecular level Patients without visible deletion have shown molecular deletion in two cases and UPD in two cases as well Chromosome anomalies in atypical group were revealed in 3 cases: del(15)(q11 2), inv(15)(p11,q13) and partial trisomy 15(p-q13) due to maternal translocation - t(1,15)(q43,q13) Deletion was confirmed by DNA-probes Patient with inversion apparently have not lost DNA from the region and had methylation pattern corresponding to PWS as well as patient with trisomy In other three patients molecular defect was not found We suggest that such unusual chromosome anomalies with incomplete phenotype of the PWS will give us more information about regulation system in the imprinted region This work was supported in part by the Russian Human Genome Project, grant No 62

5.107

Mapping the gene for familial juvenile hyperuricaemic nephropathy by linkage analysis

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Familial juvenile hyperuricaemic nephropathy (FJHN), is an autosomal dominant disorder, characterised by early onset hyperuricaemia (sometimes gout) and progressive renal disease Renal failure and possibly death, occurs at 30-40 years of age Although early diagnosis may permit effective treatment, the only diagnostic tool (fractional uric acid clearance) can be inaccurate Our aim is to map the gene for FJHN by linkage analysis, which is the first step towards cloning the gene and providing rapid, genetic testing for presymptomatic and prenatal diagnosis We typed DNA from 16 FJHN families (134 individuals) with markers from three candidate loci Autosomal dominant polycystic kidney disease type 1 (associated with an increased incidence of gout), Juvenile nephronopthesis (sometimes exhibits a similar phenotype to FJHN); Adenine phosphoribosyltransferase (APRT) deficiency, (an APRT gene mutation has been found in 8 FJHN patients) Rapid typing of microsatellite markers linked to these candidate loci was facilitated by use of a fluorescent labelling and automated detection system Calculation (using MLINK) of two point LOD scores between the FJHN locus and markers showed no significantly positive results and we conclude that FJHN is not linked to any of these candidate loci This project is now continuing as a systematic linkage study of all human autosomes

5.108

Construction of a contig in the region of 21q22.3 involved in several human diseases.

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The 21q22 3 region, particularly between D21S19-PFKL, is linked to several rare human genetic diseases progressive myoclonic epilepsy (EPM1), bipolar disease and an autoimmune disorder, APECED In order to construct à contiguous physical map, start point of the construction of a transcriptional map, we first analyzed the YACs of the chr21 YAC continuum (Chumakov et al 1992) We found that only 38% contained the expected STSs and were localized on chromosome 21 by ISH on chromosome metaphasis These YACs with those of the St Louis library, allowed to construct a contig from D21S19 to CRYA1 Only one YAC was found positive with PFKL Lack of YACs in the two libraries between CRYA1 and PFKL , was also found in the ICI library after screening These results suggested that the composition of sequences in this region might lead to instability of YACs Therefore other libraries were screened to realize the junction between CRYA1 and PFKL P1 and PAC total human libraries as well as chr21 cosmid libraries were screened with new STSs isolated in the lab YACs, P1 and PACs were also used to identify cosmids of the region Two contigs are constructed one from D21S19-CRYA1, and the other from PFKL The progress towards the completion of the contigs will be presented

5.109
The CFTR mutation 1154insTC is more common than previously reported

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Heteroduplex analysis of a series of unidentified CF mutations revealed a recurring abnormal banding pattern in CFTR exon 7 Sequence analysis revealed a TC insertion at nucleotide position 1154 This mutation has previously been characterised(1) and reported on only eight CF chromosomes worldwide (Cystic Fibrosis Genetics Consortium Report, May 1992) We have established a simple, non-isotopic method for detecting this mutation using PCR and polyacrylamide gel electrophoresis This test is suitable for high-throughput analysis Analysis of CF patients referred to the North Thames (West) Region Molecular Genetics Laboratory showed that the 1154insTC mutation occurred on 10 out of 950 CF chromosomes (ie at approximately 1%) This frequency is similar to that reported for other CF mutations (eg 621+1G→T) which are routinely tested for in many diagnostic laboratories in the UK and elsewhere Haplotype analysis is in progress to establish whether the 1154insTC mutations seen in our population are due to a common "founder" effect or whether they represent recurrent mutational events (the TC insertion occurs adjacent to a pair of consecutive TC dinucleotides and could arise from "replication slippage") This data will be presented Reference (1)Iannuzzi, M C et al (1991) Am J Hum Genet 48, 227-231

5.111
An autosomal dominant triphalangeal thumb, polysyndactyly with variable expression in a large Indian family maps to chromosome 7q36.

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Hereditary limb deformities involving digit malformations are genetically heterogenous and occur as isolated anomalies or as part of particular syndromes We have ascertained several families in the Gujarat region of India with pre- and postaxial polydactylies and selected a large family (UR002) with autosomal dominant triphalangeal thumb (TPT) and polysyndactyly (PSD) for linkage analysis The pedigree consists of 112 affected individuals (58 males and 54 females) Over 90 individuals are severely affected with unilateral to bilateral TPT, preaxial duplication, triplication or quadruplication of thumb or syndactyly of multiple thumbs, hypoplastic additional fingers, articulated TPT with additional finger or duplication of TPT Less severely affected subjects showed small additional or rudimentary fingers Previous linkage analysis on apparently similar phenotypes have identified a locus on chromosome 7q36 (Heutnik et al, Tsukurov et al, Nat Genet 6 282 and 287, 94) To map the gene for the TPT-PSD in family UR002 we have performed linkage analysis in DNAS from 47 affected and 7 normal individuals Marker D7S550 of 7q36, revealed a maximum Lod score of 11 31 at θ=0 00 Additional markers in the same region (D7S559 and EN2) also showed no recombinations These data indicate that the gene responsible for the hand anomaly in this family is the same as described in pedigrees with similar phenotypes or this locus includes a cluster of genes each of which is involved in various stages of hand /foot development Further analysis of recombinants among all linked families using new polymorphisms will narrow down the critical region and facilitate positional cloning of the elucive gene

5.112
Mapping of the CAF1p60 and HCS genes in the Down syndrome critical region suggests their potential involvement in the trisomy 21 phenotype.

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To identify genes in the Down syndrome critical region (DSCR) of chromosome 21, we isolated exons from cosmids of the LL21NC02-Q library that map in the DSCR Trapped sequence JLD14b01 (110 nucleotides) showed identity to bp 440-543 of human Chromatin assembly factor I-p60 subunit gene (CAF1-p60) (gb U20280) and amino acid residues 127-160 of its predicted polypeptide Two more trapped sequences of 121 and 76 nucleotides, respectively JLL23G01 and JL74AE09, showed identity to bp 1968-2084 and bp 1739-1812 of human Holocarboxylase synthase gene (HCS) (gb D23672) and amino acid residues 561-598 and 486-506 of its predicted polypeptide Using somatic cell hybrids, YACs and cosmids we mapped both CAF1-p60 and HCS genes to 21q in the DSCR between markers D21S17 to ETS2 Overexpression of the CAF1p60 gene may result in subunit imbalance of the multimeric protein leading to defective assembly of histones with newly replicated DNA Overexpression of the HCS gene, which is involved in fatty acid synthesis, gluconeogenesis and amino-acid catabolism, may result in a disequilibrium of a metabolic pathway CAF1-p60 and HCS are therefore candidate genes for contributing to the pathophysiology of particular phenotypes of Down syndrome Cellular and transgenic animal models for overexpression of these genes have not yet been reported, and will be necessary to assess their potential role in Down syndrome

5.113
Genomic organisation of the Human Survival Motor Neuron gene (SMN).

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Spinal muscular atrophy (SMA) is a common fatal autosomal recessive disorder characterized by degeneration of lower motor neurons, leading to progressive paralysis with muscular atrophy Recently, our group described the inverted duplication of a 500 kb element in normal chromosomes We identified a SMA determining gene called SMN (Survival Motor Neuron) in the telomeric element of the duplication and an highly homologous gene, cBCD541 in the centromeric element The SMN gene is either lacking or interrupted in 98,6% of SMA patients and those retaining this gene carried intragenic mutations (1,4%) Here, we characterized the genomic DNA clones that span the entire SMN and cBCD541 genes The SMN as well as cBCD541 genes are 20 kb in length and consist of 9 exons separated by 8 introns Exon-intron boundaries as well as the nucleotide sequence of the 5' end of the non-coding region was determined These data will allow the characterisation of SMN gene defects in SMA patients retaining this gene and will be useful for studies of the tissue-specific regulation of the gene expression

5.115
Large deletions involving SMN, NAIP genes and markers C212-C272 are specific to Werdnig-Hoffmann disease.

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Spinal muscular atrophy (SMA) is characterized by degeneration of anterior horn cells of the spinal cord and represents the second most common fatal autosomal recessive disorder after cystic fibrosis The childhood SMA are divided into acute (Werdnig-Hoffmann disease, type I), intermediate (type II) and juvenile forms

(Kugelberg-Welander disease, type III) on the bases of age of onset, milestones of development and life span All three forms map to chromosome 5q13 3 in a highly unstable region characterized by the presence of low copy repeats Inherited and de novo deletions of the 5q13 region were observed in patients Recently, two genes have been identified within the 5q13 candidate region One of these, the survival motor neurone gene (SMN), is a SMA determining gene as it is absent in 98,6% of patients Yet, no genotype-phenotype correlations were observed, as SMN is lacking in all patients, independent of the type of SMA A second gene, XS2G3 or the highly homologous neuronal apoptosis inhibitory protein gene (NAIP) was found to be more frequently deleted in type I than in the milder forms (types II and III) We have tested 106 type I-III patients for SMN (exons 7 and 8), markers C212-C272 and NAIP (exon 5) mapping upstream and downstream to SMN respectively We show here that deletions involving these markers are specific to type I SMA (43%) allowing one to predict the severity of the disease

5.116
Analyses of the Survival Motor Neuron gene products (SMN).

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Spinal muscular atrophy (SMA) is characterized by degeneration of anterior horn cells of the spinal cord and represents the second most common fatal autosomal recessive disorder after cystic fibrosis Recently, we identified a SMA determining gene termed the Survival Motor Neuron gene (SMN) as it is absent or truncated in 98,6% of patients The SMN gene was found to encode a novel protein with a predicted molecular weight of 32 kDa A highly homologous gene (cBCD541) is also present in the 5q13 region Northern blot and RT-PCR analyses showed that both genes are expressed and detect 1,7 kb transcript cBCD gene was shown to undergo alternative splicing of exon 7, resulting in a truncated transcript lacking this exon and a putative protein with a different C terminal end Polyclonal rabbit antisera were generated against synthetic peptides corresponding to the amino terminus, the carboxy terminus and an internal sequence of the predicted aminoacid sequence of the SMN gene, respectively The analyses of SMN gene products from SMA patients and controls by immunoblotting experiments should allow the detection of the putative protein isoforms, the estimation of their apparent molecular weight, their cellular and subcellular distribution and the consequences of SMN gene defects in SMA patients

5.117
Genetic analysis of unusual SMA pedigrees using SMN, NAIP genes and markers C212-C272.

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Proximal spinal muscular atrophy (SMA) is characterized by degeneration of anterior horn cells of the spinal cord, leading to progressive paralysis associated with muscular atrophy SMA represents the second most common fatal autosomal recessive disorder after cystic fibrosis (1 out 6000 newborns) The childhood SMA are divided into acute (Werdnig-Hoffmann disease, type I), intermediate (type II) and juvenile forms (Kugelberg-Welander disease, type III) on the bases of age of onset, milestones of development and life span A SMA-determining gene called Survival Motor Neurone (SMN) has been recently identified and characterized However, no genotype-phenotype correlations between the SMN gene defect and the type of SMA was observed Indeed, the gene was absent or truncated in 98,6% of the patients independent of the the type of SMA Simultaneously, another gene called Neuronal Apoptosis Inhibitory Protein (NAIP) was identified and found to be deleted more frequently in type I than in type III Here, we report on 13 unusual SMA pedigrees with either children affected with different forms of SMA in the same sibship or SMA patients in different parts of the same pedigree Similar pedigrees have been previously reported and have suggested the existence of a determining gene and a modifying gene (Becker's model) In order to test this model, genetic analysis of these families was carried out using SMN, NAIP genes and markers C212-C272 SMN exon 7 was lacking in all patients However, NAIP gene and C212-C272 markers analysis did not provide additional information to explain these unusual pedigrees suggesting that modifying gene(s) might be unlinked to the SMA locus

5.118
The Study of nt 3243 Point Mutation in Turkish MELAS Patients.

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A point mutation at nt 3243 tRNAleu gene is classically associated with MELAS (Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes) We have studied 10 patients with clinical symptoms such as recurrent strokes, ophthalmoplegia and lactic acidosis which are variably associated with MELAS We identified nt 3243 mutation in only one patient by PCR amplification and Apal enzyme digestion Thus we concluded that this common mutation reported from other populations is not a founding mutation for our MELAS patient population mtDNA sequence analyses are underway to determine point mutations specific to our MELAS population

5.121
A two-step strategy for isolation of XRCC2 a gene involved in maintenance of genetic stability

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Most genes involved in the maintenance of genetic stability and in DNA repair are of considerable medical relevance Many are discernible as autosomal recessive genetic diseases in which genetic instability and increased mutation rates can be correlated with an increased risk of neoplasia Particularly valuable tools in the identification of these genes have been hamster cell mutants, selected for their extreme sensitivity towards in vitro mutagenesis The hamster cell mutant irs1 is highly sensitive to a range of genotoxic treatments (Jones et al 1987) and the gene mutated in these cells, XRCC2, is expected to play a major role in prevention of mutation and preservation of genetic stability XRCC2 has been localised by chromosome transfer experiments to human chromosome 7q36 1 (Jones et al, 1995 Genomics 26 619-622) We have been employing a two-phase strategy for identifying the XRCC2 gene First a correcting mRMA fraction was isolated which contains the XRCC2 transcript and this is then used in the second stage as a specific probe for screening a chromosome-specific cosmid library By microinjection of a human mRNA-fraction with a size range of 3500 ± 250 nucleotides, we were able to transiently complement the sensitivity of irs1 cells to the DNA cross-linking agent, Mitomycin C, with respect to survival (Bender et al, Mut Research in press) This mRNA fraction was used to prepare a 32P-labelled cDNA probe which was then used to screen the chromosome 7 cosmid library of Nizetic et al (1994,Mamm Genome 5(12) 801-2) 24 cosmid clones gave strong signals with this probe Information held in the "Reference Library Database" allowed 7 of these to be disregarded since they do not map to the appropriate sub-chromosomal region We are now examining the remaining 17 cosmids for 1) hybridisation to either of two YAC-contigs from 7q36 1, 2) complementation of Mitomycin C sensitivity after transfection into irs1 cells, 3) loss of expression or 4) genomic rearrangement in irs1 cells

5.122
Cloning of a new human NCAM-related gene and mapping to chromosome 21q21.

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We have used human chromosome 21 (HC21)-specific cosmids to trap exons that map on this chromosome The predicted sequence of one such exon showed homology with various Neural Cell Adhesion molecules (NCAM) from different species (best homology was with xenopus NCAM P16170, aa 162-206, p=5 7e-15) This exon was mapped by somatic cell hybrids between markers D21S18 and D21S282 on 21q21 HC21 cosmids Q53H7 and Q63H7 of the LL2 NCO2 "Q" library contain this gene We then cloned an almost full length cDNA from a fetal brain library Sequence analysis revealed an NCAM molecule (termed NCAM-21) which shows significant homologies to other NCAM genes from frog, chicken, rat, mouse and cow, it is also homologous but different from an unmapped human NCAM clone THC85532 of the TIGR database and the human NCAM of chromosome 11q23 The predicted protein sequence of the cDNA encodes for 5 immunoglobulin-like, two fibronectin-like, a transmembrane and an intracellular domain Northern blot analysis revealed two additional bands consistant with the existence of two novel isoforms of NCAM-21 The cloning of these putative isoforms is under way To date there are no candidate human disorders on HC21 that can be associated with mutations in NCAM-21 Furthermore, the role of a triplicated NCAM-21 in trisomy 21 will be investigated since NCAMs are important in cell-cell interaction and normal development

5.123
Linkage analysis in Spanish families with Usher syndrome.

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The Usher syndrome (USH) is an autosomal recessive disease characterized by congenital sensorineural hearing impairment and progressive pigmentary retinopathy Classically, there are established two types, type I and type II, distinguishing by the vestibular dysfunction It is also accepted a type III, clinically similar to the type II, but characterized by a progression of clinical hearing impairment A gene for USHII has been localized to chromosome 1q Up to now, there are three different genetic loci involved in USH type I 14q, 11p and 11q Unliked families have been reported in both types of USH providing evidence for genetic heterogeneity of this disorder Recently, it has been found linkage to chromosome 3q for USH type III families We have analyzed, up today, 30 Spanish families using polymorphic markers for the more usual candidate zones The molecular study has allowed us to determine that the 75% USHI Spanish families show linkage to 11q and the proportion of USHII families linked to 1q is estimated at 85% By the other hand, the remaining USHI and USHII families that have not shown linkage to these loci have been studied with different molecular markers localized in several regions 11p, 3q, 17p, 8p, 8q, 14q

5.124
Molecular analysis in true hermaphrodites with different karyotypes and similar phenotypes

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True hermaphroditism is characterized by the development of both ovarian and testicular tissue in the same individual Mullerian and Wolffian structures are usually present and external genitalia are often ambiguous The most frequent karyotype in these patients is 46, XX or various forms of mosaicism, whereas 46, XY is very rarely found The phenotype in all these subjects is similar We studied ten true hermaphrodites, six of them had a 46, XX chromosomal complement, three had been reared as males and three as females The other four patients were mosaics three were 46, XX/46, XY and one had a 46, XX/47, XXY karyotype One of the 46, XX/46, XY mosaics was reared as a female while the other three mosaics were reared as males The sex of assigment in the ten patients depend only on labio-scrotal differentiation Molecular studies in 46, XX subjects revealed the absence of Y centromeric sequences in all cases, arguing against a hidden mosaicism One patient presented Yp sequences (ZFY+, SRY+), wich contrast with South African black 46, XX true hermaphrodites where no Y sequences are found Molecular analysis in the subjects with mosaicism revealed the presence of Y centromeric and Yp sequences confirming the presence of a Y chromosome

5.125
Biochemical and molecular analysis of X-linked ichthyosis in the Mexican population.;

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X-linked Ichthyosis (XLI) and Ichtyosis Vulgaris (IV) are the most frequent types of ichtyosis However, IV (1 300) is by far more prevalent than XLI (1 2000) in all the populations reported to date XLI is due to steroid sulfatase (STS) enzymatic deficiency and it is caused by the STS gene deletion in 90% of the patients Clinical diagnosis is difficult but the enzimatic assay or molecular analysis allow the correct classification of both types of ichthyosis The present study analyzes a sample of 30 patients referred by ichthyosis to the General Hospital of Mexico using biochemical and molecular methods 25 corresponded to XLI and only 5 to IV through STS enzymatic assay This indicates that XLI has a higher prevalence than IV in this sample of the Mexican population In 80 % of the isolated cases, it was recognized a carrier mother by enzymatic assay, this was very important for genetic counseling 17 XLI patients were analyzed using PCR for the 3' and 5' ends of the STS gene and in 16 cases there was no amplification of the product, indicating a complete deletion of the STS gene Only in one case, the PCR product was present, suggesting a possible point mutation In conclusion, we observed a higher prevalence of XLI than IV, in contrast with other reports in the literature Complete deletion of the STS gene is the most frequent abnormality in XLI

5.126
Functional expression of the hypoPP mutation (R528H) of the skeletal muscle calcium channel in HEK293 cells.

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Hypokalemic periodic paralysis (hypoPP) is an autosomal dominant disorder belonging to a group of muscle diseases involving the abnormal function of ion channels The latter includes hypokalemic and hyperkalemic periodic paralysis and non-dystrophic myotonias We localized the hypoPP locus (hypoPP1) to chomosome 1q31-32, in a region including the α1 subunit of the skeletal muscle L-

type calcium channel gene (CACNL1A3) Subsequently, 3 point mutations in the CACNL1A3 gene were found in hypoPP patients The mutations R528H, R1239H and R1239G are responsible for arginine to histidine or arginine to glycine substitution in the voltage sensor segment (S4) of the α1 subunit We have reproduced the hypoPP mutation (R528H) into the rabbit α1 subunit cDNA The wild type (α1s) and the mutated (α1m) subunits were expressed in HEK293 cells Electrophysiological studies of transfected cells show that both subunits lead to the expression of functional L-type calcium channels Further studies are currently performed in order to determine electrophysiological properties of the mutated calcium channel

5.127

Evidence for a common origin of most Friedreich ataxia chromosomes in the Spanish population.

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Friedreich ataxia (FA) is an autosomal recessive neurodegenerative disorder of unknown etiology, that was mapped in chromosome 9q in 1988 (Chamberlain et al, 1988) The FRDA candidate region is restricted now to 150 Kb (Montermini et al, 1995) Haplotype analysis is a powerful approach to estimate the spectrum of mutations accounting for a disease in a specific population We had previoulsly analysed a sample of 88 FRDA chromosomes with 10 linked markers Mct112/MS-GS2-267P/BstX1-FD1-MLS1-FR1-FR2-FR8-FR7-FR5 and obtained a spectrum of FRDA as follows Haplotypes Ia and Ib (mutation I), 50% of FRDA chromosomes, Haplotypes IIa and IIb (mutation II), 25%, Haplotype III (mutation III), 7%, rare haplotypes, maximum 18% All of the above mentioned markers are microsatellites (STRPs) but 26P (RFLP) We now present the isolation of the new marker FAD1, which is a single base substitution polymorphism (SNP) that maps between FR2 and the FRDA candidate region It is a diallelic system were the rare allele is in strong disequilibrium with FA (p= 0 0001) Inclusion of the FAD1 marker in the FRDA haplotypes allowed us to associate allele B with haplotypes I and II, and it was also found in all but three "rare" chromosomes As SNP have a very low mutation rate and are not subjected to recurrent mutations, they are considered as unique events that allow reconstruction of monophyletic chromosome groups Therefore, we could parsimoniously reconstruct a genetic tree of FRDA haplotypes in the Spanish population that supports the hypothesis that FA arose from an ancient founder effect, since a single mutation associated to FAD1 B allele should account for roughly 90% of FRDA Spanish chromosomes

5.128

A 5' splicing site mutation in the peripheral myelin protein-22 gene associated with hereditary neuropathy with liability to pressure palsies.

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Hereditary neuropathy with liability to pressure palsies (HNPP) is an autosomal dominant disorder causing acute recurrent transient palsies with sensory disturbances, often related to minor trauma to the peripheral nerve HNPP is commonly associated with a 1 5 Mb deletion in chromosome 17p11 2 involving the same genomic interval that is duplicated in Charcot-Marie-Tooth disease type 1A Several point mutations at the PMP22 gene have been reported in CMT1A patients with no duplication Up to now only two point mutations have been reported in non-deleted HNPP patients in one case, a 2 bp deletion at the exon 1 of the PMP22 gene that generated a null allele with a truncated protein (Nicholson et al , Nature Genet, 1994), and in a second case, a 1 bp deletion that generated a frameshift and a premature stop codon at Val154 (Taroni et al Am J Hum Genet, 1995, abstract 1327) We present here a new point mutation within the PMP22 gene in a Spanish family segregating typical HNPP as an autosomal dominant trait The 1 5 Mb deletion was excluded by detecting no loss of heterozigosity of markers RM11-GT, VAW409R3a, VAW412R3HEc, and EW401HE in any of the six affected members, and by dosage analysis of the CMT1A-REPflanking sequences (probe pNEA102) Linkage analysis of the extended haplotype in three generations (6 affected and 7 non-affected) suggested linkage to the HNPP locus in this family (max lod = 2 7, theta = 0 0) The four exons of the PMP22 gene were screened for mutations by SSCP analysis and abnormal mobility of exon 1 was observed in all patients but not in normal individuals Exon 1 PCR product from the proband and from a non-affected individual were sequenced and a G to T transversion at the first nucleotide of the intron 1 donor splice site was detected in the patient This mutation (78+1G->T) generated a new AluI restriction site that we used to screen the family We demonstrated that the mutation segregated with the HNPP phenotype This mutation can alter the mRNA processing by exon 1 skipping or by utilization of a cryptic splice site in the vicinity creating a null allele which agree with the proposed dosage model for CMT1A duplication/HNPP deletion These results further support the pathogenic role of the PMP22 in producing HNPP

5.129

Towards an integrated chromosome 21 map: progresses of the European chromosome 21 consortium.

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The four areas of chromosome mapping- ie genetic ,physical, transcriptional and diseases- have been investigated by the consortium The main findings are 1- genetic map after completion of a genetic map with an average interval size below 1 7cM new markers have been developped in the p arm, the centromeric region and the telomeric region in which a marker reveals significant difference between male and female rates of recombination 2-physical map with new results on the q22 3 region, on the q11 region and on the p arm the high precision maps which anchor YACs , PACs, and cosmid contigs on the genomic map and determine physical distances now span more than ½ of the chromosome 3-transcriptional map exon trapping and c DNA selection have been used on cosmids from the whole chromosome or from specific regions such as the Down syndrome chromosome region and the distal half of 21q22 3 More than 700 potential coding sequences, exons and cDNA fragments, have been identified Around 200 have been located on the physical map Sequences analysis revealed similarities with known human genes not previously mapped on chromosome 21 such as GABPA, lanosterol synthase , or not accurately mapped on the chromosome such as holocarboxylase synthase or GIRK2 Other sequences were found to be homologous to genes from other species to mouse TIAM-1 gene , to drosophila sim, white, mnb genes or to human ESTs A large amount of sequences are novel (Blast analysis) Validation of the new gene fragments is obtained by comparing the products of exon trapping and cDNA enrichment and by hybridizing them on somatic cell hybrids DNA blots Genomic sequencing has also been used to identify new genes in the IFNAR region The function of these new genes is approached by analysing their expression pattern on Northern blots and tissue sections, as performed for the human sim and a new gene DSC1 found in 21q22 1 4-mapping of the diseases has also progressed allowing to define a critical region for monosomy 21 and to determine a more precise location of the candidate region for progressive myoclonic epilepsy The integration of these mapping data together with the identification of

new genes should contribute to the understanding of the diseases associated with chromosome 21

5.130

Transcriptional mapping in the Down syndrome chromosomal region reveals new transcripts and transcripts homologous to drosophila sim and mnb

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Analysis of genotype-phenotype correlations in patients with partial trisomy 21 has defined a region, duplication of which might be responsible for the main phenotypic features of Down syndrome This region spans 2 5 Mb in 21q22 2 and might contain 50-100 genes The nature of the genetic defect, duplication of several genes, renders necessary the construction of a transcriptional map as exhaustive as possible As a preliminary step a physical map of the region D21S55-D21S65 has been constructed (1) which localizes 13 YACs and defines accurately their overlaps Eight YACs spanning the region of interest (D21S55-CBR) have been selected to screen a chromosome 21 specific cosmid library (ICRF) 400 cosmids were isolated and used , together with cosmids from the LLNL library, to construct contigs covering more than 80% of the region To isolate coding sequences from this region, we are using an approach which combines two techniques exon trapping and cDNA selection CDNAs are selected from three libraries (fetal liver, fetal brain, adult muscle) and are gridded at a frequency of 4-5 clones/kb of genomic sequence (8000 clones) Arraying allows the fast identification of positive clones corresponding to a given genomic fragment isolated from a cosmid , the evaluation of redundancy of clones from the cDNA selection method and a comparison with the results of exon trapping experiments Twenty groups of coding sequences (6 including exons and cDNAs) have already been isolated and show homologies with known genes and / or ESTs Eight give specific signals on Northern blots Two correspond to genes already assigned to chromosome 21 the holocarboxylase synthase and GIRK2, a potassium channel gene Two present strong homologies with genes involved in the development of the SNC of drosophila single-minded and minibrain 1-M C Dufresne-Zacharia et al Genomics, 1994, 19, 462-469

5.131

A 5.9 Mb YAC contig spanning the APP-SOD1 region critical for the monosomy 21 syndrome.

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Phenotypic and molecular analysis of individuals with partial monosomy 21 have been used to define a region deletion of which is associated with specific features of monosomy 21 facial anomalies , hypertonia, arthrogryposis and mental retardation (1) This region is located between APP and SOD1 34 YACs from the CEPH and ICRF YACs libraries have been selected and mapped by fluorescent in situ hybridization on normal individuals and patients with partial deletion of chromosome 21 4 YACs were found on other chromosomes than chromosome 21 and 7 were shown to be chimerics 13 YACs were used to build a contig by comparing the overlap map (obtained by PCR and slot blot analysis) and the restriction map (constructed with Not1, Mlu1and Nru1) This map was anchored on the genomic Not1 map and a gap distal to the APP region was filled with PAC clones from a total human PAC library (H Lehrach, P Ioannou) This contig spans 5 9 Mb and permits to localize 47 markers The physical mapping of these markers should help to construct a transcriptional map and to identify genes potentially involved in the pathogenesis of the monosomy 21 syndrome

5.133

Localisation of a gene for central areolar choroidal dystrophy to chromosome 17P.

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Purpose Central areolar choroidal dystrophy is a rare inherited retinal disorder which causes progressive profound loss of vision in patients during their 4th decade The condition is inherited either in an autosomal recessive or dominant manner The purpose of this study was to identify linkage for this condition in a large Northern Irish family with this disorder Methods We identified and examined a 3 generation family with this disease All patients underwent detailed clinical ophthalmic examination including stereo fundus photography, fluorescein angiography and detailed electrophysiological testing. Twenty patients were found to be affected The inheritance pattern in this family is autosomal dominant A total genome search was undertaken and 150 highly polymorphic microsatellites were typed Linkage analyses were performed using the Linkmap options of the FASTLINK program Results Linkage to known retinal candidate gene loci - peripherin-RDS, ROM-1, rhodopsin and the beta-subunit of cyclic GMP were excluded Using standard linkage methodology the CACD gene was excluded from more than 80 % of the genome Significant lod-score values were then obtained for markers located on chromosome 17p (multipoint Zmax = 4 35, between D17S938 and D17S796) Analysis of recombination suggests that the gene is located in the interval between D17S5 and D17S804 Conclusions Here we report the first mapping of a gene for CACD to the short arm of chromosome 17 Candidate genes in this area are now being analyzed Supported by grants from MRC, DHSS, N I and British Council for Prevention of Blindness

5.134

Rapid HMSN1a diagnosis using fluorescent microsatellite dosage

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Approximately 70% of HMSNIa patients have a 1 5Mb duplication at chromosome 17p11 2 We have used three microsatellite markers (RM11-GT, D17S921 and D17S261) located within the duplicated region to confirm diagnosis of HMSN1a These markers were initially labelled by direct incorporation of fluoroscein-12-dUTP within a PCR reaction followed by dosage analysis using an Applied Biosystems 373 fluorescent analyser and Genescan software More recently we have multiplexed these markers using primers end labelled with different fluorescent dyes to create a rapid single tube assay In informative HMSN1a patients with a duplication either three alleles or dosage differences between two alleles were observed for at least one locus Uninformative cases were rare We reported cases as having a duplication when at least two of the microsatellites demonstrated either three alleles or an abnormal dosage pattern Patients informative for at least two markers and showing a normal pattern of dosage were reported as not duplicated Patients falling into neither of these categories were analysed further using Southern blot RFLPs located within the duplicated region Of 112 patient samples referred with a possible diagnosis of HMSN1a, 53 (47%) showed abnormal dosage for at least two microsatellites, confirming the diagnosis of HMSN1a, 36 (33%) were informative for at least two microsatellites but showed normal dosage indicating no duplication and 23 (20%) patients were uninformative for at least two markers and consequently required Southern analysis Of these 23 patients, only 4 were found to be duplicated after Southern analysis No patients gave conflicting results This method has considerably expedited and simplified our HMSN1a analysis Molecular analysis for urgent cases can now be completed within 24hrs and average turnround time for routine testing has been reduced We are currently incorporating two other microsatellites, D17S955 and D17S839, into our assay to further reduce the number of samples requiring Southern analysis

5.135
Connexin 32 mutations in Finnish families with X-linked Charcot-Marie- Tooth disease

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Charcot-Marie-Tooth disease type 1 (CMT1) is an inherited neuropathy characterized by slow conduction velocity of peripheral nerves It is genetically heterogenous About 70% of the patients have a duplication of the peripheral myelin protein 22 (PMP22) gene (at 17p11 2), while a small portion of the patients have mutations in PMP22 and in the myelin protein zero (P0) gene, located to 1q22-q23 The X-linked form of CMT1 (CMTX) represents about 10% of all cases CMTX is associated with mutations of the connexin 32 (Cx32) gene (at Xq13 1), which encodes a gap junction protein expressed in myelinating Schwann cells We screened for mutations in the coding region of Cx32 in Finnish CMT1 families with possible X-linked inheritance The whole coding region was amplified by PCR, using tailed primers The PCR fragment was then isolated and cycle sequenced with the appropriate dye-primers, and run on an ABI 377 automated DNA sequencer The mutation search was facilitated by using the SeqEd software We found 4 families with dominant point mutations in the Cx32 gene 3 of the mutations have not been described elsewhere The mutations lead to amino acid substitutions in the first, second and fourth transmembrane domains and in the second cytoplasmic domain of Cx32

5.136
A study for the detection of known and novel mutations in the CFTR gene in the Turkish patients

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Cystic fibrosis (CF) is the most severe autosomal recessive genetic disorder in the populations studied The major mutation so far in the Turkish population is ΔF508 In our studies, screening of 67 unrelated patients has revealed that the frequency of ΔF508 mutation in the Turkish population is low (16%) compared to the European and North American populations (70%) Our studies have also revealed a 4% frequency of 1677delTA The great genetic heterogeneity of the Turkish population made necessary to use a general screening strategy to facilitate the identification of the mutations responsible for CF in Turkey since a dominant single mutation is not expected We have screened the patients for three probable mutations, R347P, 3849+10kbC and Q220X, by restriction enzyme analysis of PCR products These mutations are (common in or) specific to the populations in the neighboring geographical areas However, no chromosomes carrying the mutations were identified We extended the study to mutation analysis using denaturing gradient gel electrophoresis (DGGE) in the exons coding for the second nucleotide binding domain (NBD2), which is one of the hot spot regions The two most frequent mutation detected so far in this study are W1282X in exon 20 and N1303K in exon 21, with their frequencies being nearly 2 percent and 1 percent, respectively Samples migrating with a different pattern than normal are subjected to DNA sequence analysis

5.137
Amplification of the microsatellite RB1.20 in single spermatozoa.

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Various studies report non Mendelian tranmission of mutated RB1 alleles in retinoblastoma pedigrees In order to verify this hypothesis, we chose to analyse the sperm of males who transmitted a retinoblastoma to their offspring This report describes a methodology which allows the amplification of a polymorphism in single spermatozoa RB1 20 is a highly informative microsatellite, present in intron 20 of the RB1 gene The technique requires the analysis of thousands of spermatozoa and we therefore optimized a previously described method (Brandt et al 1992) The amplification consists of two rounds using nested primers to increase the specificity of the reaction When as little as 3 pg of DNA (one target molecule) need to be amplified, the PCR conditions must be optimized The detection of the allele present in each spermatozoa is obtained after staining with ethidium bromide and visualization by UV irradiation This technique allows reliable and rapid detection of microsatellite RB1 20 in individual spermatozoa (Brandt B et al Am J Hum Genet 1992, 51 1450-1451)

5.138
Molecular cloning of the cDNA for a human homolog of the Drosophila E(z) gene and mapping to human chromosome 21q22.2

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To identify genes that map on human chromosome 21 (HC21) and contribute to the phenotype of Down syndrome, exon trapping was applied to cosmid DNA from an HC21-specific library LL21NCO2-Q More then 560 potential exons were cloned and partially characterised One of those, hmc23b04 (gb X88270) after Blast search showed strong homology to the Drosophila enhancer of zeste (Ez) protein (gb U00180) from amino acid 664 to 694 (p=7 6e-11) We have cloned a full length cDNA for a human homolog of the Drosophila (Ez) and mapped it by somatic cell hybrids, cosmids and YACs to the proximal border of the Down syndrome Critical Region in YAC 64f11 between markers D21S65 and ERG on human chromosome 21q22 2 Sequence analysis indicates that the human gene encodes a 707 amino acids polypeptide which is similar to the Drosophila E(z) protein with 60% identity of amino acids and contains a trithorax domain and a DNA binding motif Alternatively spliced isoforms have been identified in RNAs from several tissues Northern blot revealed that this human gene is expressed in almost every tissue tested The Drosophila E(z) protein is a member of the polycomb group of genes that maintain the segment identity gene repression and is thought to control gene expression by regulating chromatin The human homolog may therefore contribute to certain phenotypes of Down syndrome by altered regulation of its target genes

5.139
Localization of a human homolog of the mouse pericentrin gene to chromosome 21qter

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Exon trapping was used to identify portions of genes from cosmid DNA of a human chromosome 21 (HC21) specific library LL21NC02-Q Among 560 potential exons identified, three hmc46b12 (gb X88027), hmc22g01 (gb X88276), and hmc25g07 (gb X88231) showed strong homology to different regions of the cDNA for the mouse pericentrin gene (gb U05823) The homologies were from nucleotides 342 to 481 (85% identity, p = 8 0e-35) , 1257 to 1368 (80% identity, p = 2 1e-22) and 6282 to 6374 (64% identity, p = 9 4e-6) of the pericentrin gene respectively, and from amino acids 17 to 61 (71% identity, p = 3 8e-13) for exon hmc45b12, and 322 to 358 (78% identity, p =2 5e-12) for hmc22g01 These results indicate that the three trapped sequences are portions of a human homolog of the mouse pericentrin gene Using PCR, Southern blot and FISH we have mapped this human gene to 21q22 3 distal to D21S25 Northern blot revealed an mRNA species of about 9 5 kb in almost every tissue but particularly in muscle Pericentrin is a conserved integral component of the filamentous matrix of the centrosome involved in the initial establishment of the organized microtubule array Anti-pericentrin antibodies disrupt mitotic and meiotic divisions in vivo and block microtubule aster formation (Doxsey et al, Cell 76 639, 1994) Mutations in the pericentrin gene may be associated with abnormal phenotypes of meiosis or mitosis in human cells

5.140

Cloning of the cDNA for a human homolog of the Drosophila white gene and mapping to chromosome 21q22.3

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To contribute to the transcription map of human chromosome 21 (HC21) and the understanding of the pathophysiology of trisomy 21, we have used exon trapping to identify portions of HC21 genes Two trapped exons from pools of HC21-specific cosmids showed homology to the drosophila white (w) gene We subsequently cloned the corresponding full length cDNA for the human homolog of the Drosophila w gene (hW) from human retina and fetal brain cDNA libraries The gene belongs to the ABC (ATP binding casette) transporter gene family and is homologous to drosophila w and to a lesser extent to drosophila brown (bw) and scarlet (st) genes which are also involved in the transport of eye pigment precursor molecules This family gene family is characterized by the presence of a well concerved ATP binding domain and six transmembrane domains A DNA polymorphism with 62% heterozygosity due to variation of a poly (T) region in the 3'UTR of the hW has been identified and used for linkage mapping in the CEPH pedigrees The hW is located at 21q22 3 between DNA markers D21S212 and D21S49 in a P1 clone that also contains marker BCEI Northern blot indicated that this gene is expressed in many human tissues including the retina The contribution of this gene to the Down syndrome phenotypes, or the human eye color, or the resulting phenotypes of null or missense mutations are presently unknown

5.141

Cloning of a cDNA for a homeodomain containing polypeptide similar to the mouse Meis1 gene and mapping to chromosome 21q22.3

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To contribute to the transcript map of human chromosome 21 (HC21) and the understanding of the pathophysiology of trisomy 21, we have used exon trapping to identify portions of genes from pools of HC21-specific cosmids of the LL21NCO2-Q library One trapped sequence, hmc37a09 (gb X88106), was identical to a human EST (gd D31072) Using the trapped sequence and the EST as probes, we have cloned the corresponding full length cDNA from a human fetal kidney cDNA library This gene encodes a polypeptide with 67% to 80% identities in different regions of the mouse Meis1 gene (gb U33629), and contains homeodomain-like sequences Using PCR and Southern blot with cosmids, YACs and somatic cell hybrids, and linkage analysis with a polymorphism with 60% heterozygosity due to a (GT)n repeat in the 3'UTR of the cDNA, we have precisely localised this new gene to chromosome 21q22 3 between markers D21S49 and PFKL on YAC350F10 that also contains CBS The gene is expressed in many human tissues tested by Northern blot Meis1 is a PBX1-related homebox gene involved in myeloid leukemia in BXH-2 mice This novel HC21 gene may play a role in the development of malignancies that are associated with abnormalities of HC21 including some forms of leukemia frequent in trisomy 21 or with developmental abnormalities Elucidation of the function by targeted disruption in mice will enhance our understanding of the role of this gene in normal human development

5.142

Cloning of the cDNA for the human homolog of the rat PEP-19 gene and mapping to chromosome 21q22.2.

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Exon trapping was used to identify fragments of chromosome 21 genes One trapped sequence, hmc18h10 (GenBank X88329), showed strong homology to a region that includes the first exon of the rat PEP-19 gene (GenBank # S65225) We subsequently cloned the corresponding full cDNA for the human homolog of the rat PEP-19 and mouse pcp-4 genes and mapped it to the Down syndrome critical region (DSCR) of chromosome 21q22 2 by somatic cell hybrids and cosmid identification The predicted polypeptide sequence of the human PEP-19 gene is 95% identical to the rat peptide sequence (2 differences out of 62 amino acids) In rodents PEP-19 is a neuron-specific polypeptide only expressed in several regions of the central nervous system, notably cerebellum, thalamus, caudate putamen, and olfactory bulb and the neural retina The 62 amino acid polypeptide, is related to the S100 proteins and may be a form of brain calcium binding protein It serves as a cell-specific marker in Purkinje cells and its expression is developmentally regulated in the cerebellun The precise function of PEP-19 is unknown but the accumulation of the protein is correlated with the development and maturation of the rat cerebellum Unbalanced expression of the PEP-19 gene may play a role in certain phenotyps of Down syndrome (DS) in humans Mutations in this gene may be associated with certain disorders that map to human chromosome 21

5.143

Construction of a transcription map in the Xp22 region around the Retinoschisis disease gene

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We have previously constructed a YAC contig in the Xp22 1-p22 2 area spanning the markers DXS414 to DXS451 The region encompasses several disease genes, including RS, HYP, CLS and KFSD Currently, we are focussing on the region around RS Retinoschisis (RS, MIM 312700) is a rare hereditary vitroretinal degeneration By analysis of recombinants we have recently refined the RS candidate region to between DXS418 and DXS999 (Van de Vosse et al 1995) A cosmid contig spanning the RS region was constructed by screening X-specific cosmid libraries (ICRF, LLNL) with specific markers and YAC-derived Alu-PCR products and by subcloning a YAC containing the region in a cosmid vector We are now constructing a transcription map of the RS candidate region The techniques to identify transcripts include direct screening of cDNA libraries and exon trapping The latter was done mainly using sCOGH2, a vector system we have recently developed which allows the simultaneous isolation of all exons present in the insert (Datson et al submitted) We will present the results of the characterisation of the products obtained so far E Van de Vosse, et al A Xp22 1-p22 2 YAC contig encompassing the disease loci for RS, KFSD, CLS, and HYP, refined localization of RS 1995 Submitted N A Datson, et al Scanning for genes in large genomic regions cosmid-based exon trapping of multiple exons in a single product 1995 Submitted

5.144

Cosmid-based exon trapping in the 4q25 region containing Rieger syndrome translocation breakpoints

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Rieger syndrome (RGS) is an autosomal dominant disorder of morphogenesis causing malformation of the anterior segment of the eye and dental abnormalities RGS has been linked to epidermal growth factor (EGF) on 4q25 We used cell lines of two RGS patients with balanced translocations in the 4q25-27 region to refine the localisation of the RGS gene A YAC contig was constructed around EGF and individual YACs were mapped by FISH on the translocation cell lines A 2 4 Mb YAC was identified which crossed both translocation breakpoints An Alu-mediated YAC fragmentation strategy was used to generate a series of shorter YACs which were used to refine the breakpoint localisation and to map markers and cosmids to specific regions A 200 kb fragmentation YAC was shown to still cross both translocation breakpoints A 850 kb fragmentation YAC was subcloned in cosmids in a vector (sCOGH1) allowing scanning of entire cosmid inserts for multiple exons in a single spliced product Cosmids mapping to the 200 kb region containing both breakpoints are currently being used in cosmid-based exon trapping to isolate the RGS gene

5.145

European directory of DNA laboratories involved in genetic disorders diagnosis.

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The rapid increase in the number of Genetic diseases open to a DNA based diagnostic test has presented a requirement for a comprehensive directory of laboratories and a listing of the services they can offer National directories of this type already exist in some countries but there is no comparable European listing However during the last twelve months national coordinators have been identified in most European countries with the aim of collecting standardised data for a Europe-wide laboratory directory The data is currently being collected and formulated by the directory centre at Loverval, Belgium To date (Nov 1995) the European Directory of DNA Laboratories (EDDNAL) provides contact names and addresses for 230 DNA laboratories and details services on 260 genetic diseases The ten diseases most commonly listed are Fragile-X syndrome, Cystic Fibrosis, BMD/DMD, Myotonic Dystrophy, Huntington Chorea, Angelman Syndrome/PW, Hemophilia A, CMT disease, Hemophilia B/NF I/PKU, OPCA I The directory will be designed to be available as an on-line facility updated each month It is expected to promote information exchange between European centres and to improve the availability of services for rare genetic conditions The first version will be available soon and will initially be distributed through European genetic centres

5.146

Human minisatellite mutation rate after the Chernobyl accident

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We are studying the radiation-induced increase of mutation rate in minisatellite loci in mice and humans It has recently been shown that acute doses of ionizing g-radiation cause a significant increase in minisatellite germline mutation rate in mice, detectable by DNA fingerprint analysis of small numbers of families at doses substantially lower than can be monitored by standard genetic techniques Germline mutation at human minisatellite loci also has been studied among children born in heavily polluted areas of the Mogilev district of Belarus after the Chernobyl accident and in a control population The frequency of mutation assayed both by DNA fingerprinting and by single locus analysis was found to be two times higher in the exposed families than in the control group Furthermore, mutation rate was correlated with the level of ^{137}Cs surface contamination, consistent with radiation-induction of germline mutation The magnitude of the mutation increase suggests that mutation induction arises indirectly by some mechanism that enhances spontaneous minisatellite mutation The potential use of minisatellite loci in monitoring radiation-induced mutations in human populations will be discussed

5.147

Correction of a severe molecular defect in Hemophilia A due to errors in DNA replication and/or RNA transcription/translation.

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Hemophilia A is a common blood coagulation disorder caused by many different mutations in the X-linked factor VIII gene These mutations result in absence or reduced amount of protein or non-functional protein To date, all mutations examined correlate well with the clinical phenotype (mild, moderate or severe) of the patient We studied a Japenese family with a severe frameshift mutation (T deletion in codon 1441 within a run of adenines, A_8TA_2 to A_{10}) in the factor VIII gene In spite of this frameshift mutation, affected males show a moderately severe hemophilia with plasma factor VIII activity around 5% Immunoblotting with anti-factor VIII monoclonal antibodies showed 3-5% of normal factor VIII in patient's plasma Since there was no mosaicism, we hypothesized that errors in DNA replication or RNA transcription partially restore the reading frame producing a small amount of functional factor VIII We studied the extent of slippage of polymerases through the A_8TA_2 or A_{10} region in normal and mutant DNA and RNA molecules The error rate for the expected number of As was 4 times higher in the mutant as compared to normal presumably because of the longer run of As In 7% of RNA molecules from the patient, normal reading frame was restored leading to functional protein This study illustrates that a severe DNA mutation can be modified by errors in enzyme fidelity to produce a milder phenotype

5.149

Construction of a cosmid contig across the TSC1 region of chromosome band 9q34

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The disease Tuberous Sclerosis has been shown to be caused by mutations in one of two genes, one of which, TSC1, has been assigned by linkage analysis to 9q34 There is a general consensus that TSC1 maps between D9S149 to D9S114, a genetic distance of 3cM, although some contradictory evidence from recombination events within this interval makes the exact position undefinable Using a cosmid fingerprinting and contig assembly technique (Nahmias et al 1995 Eur J Hum Genet 3, 65-77) we have constructed a deep cosmid contig (with 3 gaps) spanning this interval The total length of genome contained within the contigs is approximately 1 2 Mb In the course of this assembly, in situ hybridisation of cosmids from the ends of the contigs to extended chromatin has enabled us to estimate the sizes of gaps between contigs and has led to the closure of some gaps Published physical mapping data based on pulsed field gel analysis and on YAC contig assembly suggest that the region corresponds to a physical distance of from 2 0 to 2 5Mb Our estimates of the remaining gaps are smaller than those implied by these figures We have isolated candidate genes across this region by exon trapping

5.150

Improvements in the genetic and physical map of chromosome 9p24

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Genetic map: A concerted effort has been made to improve the genetic map in the distal region of 9p, particularly in the p24 sub-band Ten genethon markers have been typed in the 40 CEPH families and their data analysed using the CRI-Map program to place the markers on the genetic map of chromosome 9 Typings were performed on selected families with another six STSs in order to improve the information and resolve conflicts in the existing map The genetic map was then used to construct a meiotic breakpoint panel for 9p The results of mapping further STSs using this panel will be presented Physical map: Cosmids for genes thought to be in 9p24 have been collected using the Lawrence Livermore chromosome 9 specific cosmid library (LL09) These cosmids have been used to build contigs, mainly by vectorette walking, and the physical location of the cosmids has been confirmed by FISH In order to reliably place the telomère, a sub telomeric repeat cG1a3 4 6 (Weber, B et al Cytogenet Cell Genet vol 57 179, 1991) has been used as a probe in the LL09 library to collect cosmids containing the sub-telomeric repeat and vectorette walking with these cosmids has allowed the identification of a 9pter-specific cosmid contig This contig is being searched for the most distal polymorphic marker on chromosome 9p

5.151

Integration of physical and genetic maps of human chromosome 9 using the SIGMA program.

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The System for Integrated Genome Map Assembly (SIGMA) allows the combination of mapping data from a number of different sources into one map A SIGMA map of human chromosome 9 was started in 1994, and has been regularly updated since then, and used as a map display and publication tool at Single Chromosome Workshops he initial map was based largely on linkage data, but recent additions include YACS in several chromosomal regions and cosmid contig data This has resulted in considerable reduction in "ambiguity bars" over large regions, ie refinement of the marker locations on the map The map is now scaled in kilobases, so that where physical distances have been estimated these are now used in positioning markers on the SIGMA map Conflicts in the information about the order or distances between markers are highlighted by the software The map curator can choose the order or distance which will be displayed, but conflicting information can also be stored The amount of data available is now difficult to represent in a printed version but a subset will be shown Latest Sigma maps are available by anonymous ftp from ftp gene ucl ac uk (128 40 82 1) in directory /pub/c9workshop/1995, and from the chromosome 9 homepage at URL http //www gene ucl ac uk/chr9home html on the World Wide Web

5.152

Molecular and population studies on the lactase persistence polymorphism.

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A major interest of our group is to define the molecular basis of the genetic polymorphism which determines the persistence or non-persistence of lactase activity into adult life in human beings This polymorphism is of cultural, anthropological and population genetic interest We have identified genetic variation within the lactase gene and characterised a large number of polymorphisms, mainly by a single strand confirmation analysis (SSCA) We have shown that the phenotypic polymorphism which determines lactase expression is cis-acting to the lactase gene (Wang et al [1995] Hum Mol Genet , 4, 657-662) Analysis of the haplotypes in fifty of the CEPH (Centre d'Etude du Polymorphisme Humain) families demonstrated the occurrence of only 4 common haplotypes indicating a region of linkage disequilibrium that spans approximately 70 kb of the lactase gene (Harvey et al [1995] Eur J Hum Genet , 3, 27-41) These haplotypes (like lactase persistence/non-persistence) differ in frequency in different populations None of the haplotypes are strongly associated with lactase non-persistence, though the common haplotype maybe more frequent in lactase persistent individuals We hope within the foreseeable future to be able to identify lactase persistent and lactase non-persistent alleles at the DNA level

5.153

The human homologue of the mouse T (Brachyury) gene; gene structure, cDNA sequence and assignment to chromosome 6q27.

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We have cloned the human homologue (Hu-T) of the mouse T (Brachyury) gene The T protein is a transcription factor vital for the formation of posterior mesoderm and axial development in vertebrates Brachyury mutant mice which lack T protein die in utero with abnormal notochord, posterior somites and allantois A protein motif within the DNA binding domain of T, the T-box, is highly conserved amongst T homologues from different species and defines a new family of paralogous T-box genes We have identified human T genomic clones and derived the mRNA sequence and gene structure There is 91% amino acid identity between human and mouse T proteins overall, and complete identity across the 77 amino acids of theT-box Hu-T expression is confined to cells derived from the notochord within the human fetal intervertebral disc, thus confirming that Hu-T expression is very similar to that of T genes in other vertebrate species This is only the second human member of the T-box gene family to be described We have mapped the human T gene (Hu-T) to chromosome 6q27 thus providing focus for the identification of clinical conditions associated with T abnormalities

5.154
Does the CEPH family series represent a uniform 'northern European' population?

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In the analysis of the CEPH (Centre d'Etude du Polymorphisme Humain) families little attention has been paid to their possible genetic heterogeneity The majority of the families come from Utah This population is largely due to deliberate colonisation by the Church of the Latter Day Saints (Mormons) and originates mostly from eastern USA, UK and Scandanavia The Utah population has been shown to be representative of Northern Europe by studies of multiple polymorphic enzyme markers and blood groups (McLellan et al [1984] Am J Hum Genet , 36, 836-857) The second major group comes from France In view of the marked difference in frequency of the lactase haplotypes between these subsets of the CEPH population (Harvey et al [1995] Eur J Hum Genet , 3, 27-41), we have analysed other gene and DNA markers in the French and Utah groups These involve nucleotide substitutions in the case of the genes sucrase-isomaltase (SI) (chromosomal location 3q25-26) and ABO (9q34 1-34 2), the VNTRs within the coding region of the mucin genes MUC1 (1q21), MUC4 (3q29), and MUC2 & MUC6 (11p15 5), and a microsatellite marker at the DBH locus (9q34 3) and one anonymous VNTR marker D2S44 (2q22 3-23)

5.155
A Rare Y Duplication Identified By Fluorescence In Situ Hybridization.

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We studied a boy of 7 years old who was referred for hyperactivity and lack of capacity at school The cytogenetic studies with GTL, CBG and QFQ banding showed a long Y chromosome, seemingly with only one centromere, and with extra material at the end of the long arm The characteristic, very bright quinacrine fluorescence was not localized in the distal two-thirds of the Y long arm, but was in the center of the long arm Neither the X chromosome nor the autosomes of the proband demonstrated structural changes with QFQ,CBG or GTL banding Firstly, we thought the extra material could be from an autosome, derivated from a paternal translocation, but the karyotypes of parents were normal With molecular probe DYZ3 biotin labelled (Oncor) which defines the alphoid satellite DNA, was observed two green fluorescent spot on the two ends of the abnormal Y chromosome With the probe WCP Y (Vysis) we observed an image like a spindle, with a very bright spot on the middle but with a weaker intensity on both terminal regions This was interpreted like an unusual Y duplication This case indicates the usefulness and importance of chromosome specific probes in the identification and characterization of chromosome rearrangements

5.156
Molecular mechanisms of Beckwith-Wiedemann syndrome (BWS)

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BWS is a congenital overgrowth syndrome characterised by the association of gigantism, macroglossia and visceromegaly with a variety of developmental anomalies including anterior abdominal wall defects, hemihypertrophy, congenital heart defects and Wilms' tumour Molecular studies have suggested that the BWS gene(s) is imprinted and abnormal regulation of IGF2 and H19 has been implicated We have investigated the molecular basis for BWS in 85 sporadic cases Analysis of microsatellite markers within chromosome 11p15 5 revealed paternal uniparental disomy in 15 patients (18%) In each case there was mosaic partial isodisomy resulting from a postzygotic mitotic recombination All BWS disomic cases exhibited increased methylation at IGF2/H19, whereas only 4 non-disomic patienrs were hypermethylated (Reik et al Hum Molec Genet in press) Loss of IGF2 imprinting was found in non-disomic patients with both increased and normally methylated IGF2/H19 alleles suggesting that regulation of IGF2 imprinting is complex and that a variety of molecular mechanisms may result in loss of IGF2 imprinting in BWS

5.157
A specific superoxide dismutase mutation is on the same genetic background in sporadic and familial cases of amyotrophic lateral sclerosis.

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Amyotrophic lateral sclerosis (ALS) is a degenerative disease of motor neurones causing progressive muscular atrophy, weakness and death from respiratory failure, often within 2 to 3 years Although most cases are sporadic, some 5 to 10% are inherited as autosomal dominants with age-dependent penetrance An ALS locus has been mapped to chromosome 21q and causative mutations identified in the Cu/Zn superoxide dismutase (SOD1) gene A majority of SOD1 mutations have been found in cases with a clear family history of ALS However, we and others have also described SOD1 mutations in patients where the disease appears to be sporadic This is especially true for the missense mutation in codon 113 of the SOD1 gene, which substitutes threonine for isoleucine (ile113thr) One explanation for this finding is that this codon is a mutational hotspot with sporadic cases representing new mutations Another is that the inherited nature of the cases is disguised by the reduced penetrance of this specific mutation We have now shown that each of six unrelated cases of ile113thr mutation that we have collected in an unselected Scottish ALS population occurs on the same genetic background Association analysis of multiple flanking loci on chromosome 21q supports the conclusion of a founder effect, with the original mutational event occurring at least 10 generations ago

5.158
Heterozygotes for the common ΔF508 cystic fibrosis allele are not protected against bronchial asthma

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Although over 500 different cystic fibrosis (CF) mutations have been described, Δ F508 occurs on about 70% of CF chromosomes, and is largely responsible for the prevalence of the disease amongst those of north European descent The reason for the high frequency of the ΔF508 allele is unknown, although the probable explanation lies in some form of heterozygote advantage Recently, Schroeder et al (Nature Medicine 1 703, 1995) suggested that the advantage may stem from protection of some ΔF508 heterozygotes against asthma in childhood and early adult life Tests of their hypothesis used heterozygotes identified through an index affected child We have used a different approach, taking heterozygotes who had no affected children, and who were derived from a population screening programme Inspection of the medical records of 186 CF heterozygotes and 337 matched controls showed no decrease in the incidence of asthma in the CF heterozygote group The distribution of CF alleles among asthmatics was also normal We conclude that the 'protection from asthma' hypothesis is incorrect

5.159

Is there genetic heterogeneity in Marfan Syndrome(MFS)?: A report of two families in which MFS does not segregate with the FBN1 locus

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Marfan Syndrome (MFS) is an autosomal dominantly inherited connective tissue disorder with a high mortality rate due to cardiovascular complications resulting in aortic dilatation and dissection The diagnosis of the disease is clinical with the criteria being specifically defined by the Berlin Nosology (Beighton et al Am J Med Genet 29 558-594 (1988)) The identification of the chromosome 15 gene, FBN1 as the site of the primary mutation in MFS has permitted presymptomatic diagnosis to be offered by linkage studies A recent report has identified a large family whose clinical diagnosis of MFS fulfils that of the Berlin nosology but in which the disease segregates with a locus on chromosome 3 (Collod et al Nature Genetics 8 264-269 (1994)) Two families, who fulfil the criteria for the Berlin nosology, were analysed Using informative FBN1 markers we revealed non-segregation of the disease with the FBN1 locus and subsequent immunohistochemical analysis of fibroblasts from an affected individual from each family, using an anti-fibrillin monoclonal antibody, did not clarify the situation Further analysis revealed an apparent segregation of the disease with the chromosome 3 locus MFS has always demonstrated clinical heterogeneity Further investigation is required to allow an ascertainment of the genetic heterogeneity in MFS The analysis of other FBN1 unlinked families is required to determine the contribution of this new locus in the etiology of MFS

5.160

Prenatal diagnosis in a fragile X carrier which gave discrepant results between the molecular and cytogenetic analyses

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A CVS was taken from a fragile X carrier for sexing and determination of fragile X status Direct chromosomal preparations showed a 45X karyotype indicating that the fetus would be affected with Turner's syndrome However, PCR amplification of the CVS DNA gave 2 normal sized alleles at the androgen receptor (AR) locus and a single (non-maternally inherited) FRAXA allele in the normal size range indicating a female with a fragile X mutation PCR on DNA from cultured CVS cells gave the same molecular result but one AR allele was present at a much greater concentration than the other An early amniocentesis was then performed PCR and Southern blotting showed a female fetus with a full fragile X mutation FISH performed on the amniocytes showed that the majority of cells had a normal female karyotype These results suggested that mosaicism was present in the CVS, and that the 45X line was preferentially selected in culture This case was resolved by interdisciplinary co-operation and demonstrates that caution must be applied to all CVS analyses

5.161

Screening oligo- and azoospermic patients for Y chromosome microdeletions

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Approximately 11% of men attending infertility clinics suffer idiopathic oligo- or azoospermia In 1976, Tiepolo & Zuffardi demonstrated an association between disrupted spermatogenesis and loss of the distal portion of the Y chromosome long arm, including all of the heterochromatin Subsequent screening of chromosomally normal (46, XY) infertile men with Y-specific probes revealed 4 patients with Yq microdeletions (Ma et al , 1992, 1993) It was postulated that a locus involved in regulating spermatogenesis, the azoospermia factor, AZF, was disrupted by these deletions In order to test this hypothesis, a set of Y chromosome spanning sequence tagged sites (STSs, Foote et al , 1992) have been used to PCR screen a subset of 100 oligo and azoospermic patients for Y chromosome long arm microdeletions CEPH reference families and fertile male relations, where available, were used as controls The screen was concentrated on the regions containing the RBM (Ma et al , 1993) and DAZ (Reijo et al , 1995)genes, as these have both been proposed as candidates for AZF Initial results indicate that about 1 in 10 of the men tested carry microdeletions which may be associated with their spermatogenic disturbances, suggesting a significant genetic aetiology underlying some forms of male infertility References 1 Foote (1992) Science 258, 60-66 2 Ma, (1993) Cell 75, 1287-1295 3 Ma, (1992) Human Molecular Genetics 1, 29-33 4 Reijo, (1995) Nature Genetics 10, 383-393 5 Tiepolo, (1976) Human Genetics 34, 119-124

5.162

Recent advances in haemophilia A & B molecular studies in Russia.

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RFLP analysis of some inter- & extragenic polymorphic sites of Factor VIII (FVIII) and Factor IX (FIX) genes was carried out in Slavic populations from the European part of Russia and also in native ethnic groups of some Asian republics Allele frequencies for HindIII(intron 19) & XbaI(intron 22) polymorphic sites (PS)in FVIII gene are very similar in all populations studied, but different for intron 13 (CA)n repeat Population specific alleles (4 35 & 4 2) were identified in St14/Taq1 PS 180 of 230 HA families were found informative for DNA analysis such as carrier detection (46 cases-confirmed, 55-rejected) & prenatal diagnosis (37 cases) Inversions disrupting the FVIII gene were ascertained in 24 out of 54 cases studied (18 - Type 1 mutation & 6-Type 2) with DraI restriction being mostly informative Significant variations in TaqI (intron d) & DdeI (intron-a) polymorphisms of FIX gene are evident between Russian & Asian populations New allele (380 bp) for DdeI PS of FIX is found in Asian populations SSCP analysis of 20 HB patients revealed two novel FIX mutations 10468G-A & 17755G-A Carrier status has been ascertained in 6 HB female relatives and rejected in 10 of them Origin of some HA & HB mutations was traced with relevant polymorphic markers in several at-risk families

5.163

The effect of the pericentric inversion of chromosome 9 on the reproductive function.

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Pericentric inversion of chromosome 9 - inv 9(gh) - is one of the variant for "normal" polymorphisms of human chromosomes The medical genetic counselling for the couples, where one of the members is the carrier of 9(gh), is difficult and the problem of the evaluation of risk has not been solved so far During 1988-1994 cytogenetic survey of 211 persons couples for the families with spontaneous abortion (group 1), of 1005 parents whose children had mutliple malformations (group 2) and of 378 parents of Down syndrome children (group 3), was performed The control group comprises 18884 persons (complied data of Serra et al , Amer J Med Genet Suppl 1990, 7 162) Metaphase chromosomes were prepared by standard whole blood method and stained with CBG methods Only total inversions of C-regions were taken into account Most of the cases of inv 9(gh) were found in the group 1 - 42 (1,9%), this was significantly higher comparing to the control (0,94%) This frequency of the inv 9(gh) in the group 2 was 0,8%, in the group 3 - 0,58%, and these values did not differ significantly from the control One may suppose the inversion 9(gh) in carriers to be the reason of aneuploid conceptions which causes intrauterine elimination of aneuploid foetuses

5.164

Molecular characterisation of Angelman syndrome patients

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Angelman syndrome (AS) is a neurogenetic disorder caused by a lack of maternal genetic contribution from chromosome 15q11-13, a region subject to imprinting Most cases are associated with a de novo deletion, and a few have paternal uniparental disomy Approximately 25 - 30% of cases have no detectable deletion or disomy, and there is a high recurrence risk in these families A novel type of molecular lesion has recently been identified, which accounts for some of these patients In such "imprintor mutation" cases, although both maternal and paternal chromosome 15s are present and intact, they both carry a paternal imprint These cases can be identified using two probes which detect parent-of-origin specific DNA methylation patterns, PW71B and SNRPN We have now studied 130 AS patients, initially using several microsatellite markers, to identify any atypical deletions or recombination events which might help define the AS critical region Imprintor mutations were assessed using both the PW71B and KB17 (SNRPN) probes, to determine whether the results obtained with these two probes are consistent We found two cases in which the results differed Approximately 5% of patients have imprintor mutations, indicating that this mechanism may be more common than previously suspected

5.165

Molecular genetic study of a Welsh family with incomplete congenital stationary night-blindness confirms previous reports of linkage to the proximal short arm of the X chromosome.

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Clinical and molecular genetic studies have been performed on a single family from South Wales in which congenital nystagmus and refractuive error have segregated as a sex-linked trait with variable manifestation in a some female carriers In this family, most affected males demonstrate high myopia, but some female carriers show hypermetropia Clinical examination and electrodiagnostic studies are compatible with a diagnosis of either incomplete congenital stationary night-blindness or of Aland island eye disease Both disorders have been mapped to the proximal short arm of the X chromosome Molecular genetic studies in this family show complete linkage with DXS255 in the proximal X short arm, and a single recombination with DXS7 Further studies with an array of microsatellite markers from this region will be presented Our studies suggest that incomplete congenital stationary night-blindness and Aland island eye disease may be the same condition.

5.166

Further delineation of the critical region for Noonan syndrome on the long arm of chromosome 12.

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Noonan syndrome (NS) is characterised by typical facies, short stature and congenital heart defects Despite an estimated incidence for NS between 1 1000 and 1 5000 there are very few large families available for linkage studies We have recently mapped a gene for NS to 12q22-qter [Nature Genet 8, 357-360 (1994)] In our original study a significant Lod score for CA-repeat markers on 12q was obtained in one large 3 generation Dutch family In 18 out of the 20 smaller 2 generation families positive Lod scores in this region were obtained Recombination events in the large family placed the gene in a 14cM interval Two smaller families had recombinations in this region which would place the gene between D12S354 and D12S79 (1cM) if these families were linked to 12q We have further delineated the critical region for NS on 12q using newly isolated CA-repeat markers [Nature 337(suppl), 321-333 (1995)] Microsatellite analysis in the large family now maps the gene for NS between AFMa356wa9 and NOS1 reducing the interval to approximately 7cM In one of the two small families showing recombinations within the NS region, the interval where the gene could be is now telomeric to D12S839 and centromeric to D12S79

5.168

'Database cloning' of human Δ1-pyrroline-5-carboxylate synthetase (P5CS) cDNA: a bifunctional enzyme catalyzing the first two steps in proline biosynthesis.

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P5C synthetase is an NADPH and ATP-dependent mitochondrial membrane enzyme catalyzing the first two steps of the the proline biosynthesis pathway The P5CS enzyme has not been fully characterized in animals and the studies on proline biosynthesis have been limited In this report , as an initial step in determining the molecular basis for the tissue-specific characteristics of P5CS and the factors which influence its functional roles, we describe the cloning and sequencing of human P5CS cDNA by using 'database cloning' strategy Initially, this cDNA was identified as a human EST in a search from the GenBank EST database using the BLASTN program and short streches of conserved nucleotide sequences (35-40 bases) from the various microorganisms and ESTs from the C Elegans Genome Sequencing Project Utilizing sequence data of this human EST and an appropriate 5'-RACE strategy, we were able to clone and subsequently sequence the full lenght cDNA for human P5C synthetase The deduced amino acid sequence for human P5CS has 739 residues and shows good conservation of domains common to P5CSases from other species In contrast to bacteria and yeast where P5CS activity is the result of the two separate proteins, the corresponding human genes, as well as those of plants, have evidently fused to create a bifunctional enzyme P5CS mRNA has a 4 5 kb length and is present in a wide variety of tissues, including spleen, thymus, prostate, testis, ovary, small intestine, heart, squelettal muscle and pancreas A human genetic disease caused by a deficient P5CS has been recognized The phenotypic features for deficiency of P5CS include hypoprolinemia, hypocitrullinemia hypoornithinemia, hyperlaxity and cataract Studies investigating the molecular basis of this deficiency in P5C synthetase are in progress

5.169

A/C655G mutation in patients with CYP21 deficiency in Czech population.,

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The frequency of the most frequent mutation in intron 2 of the steroid 21-hydroxylase gene(CYP21), the C~G substitution, was investigated in 51 Czech patients with congenital adrenal hyperplasia (CAH) This mutation has the incidency of 26-37% in patients with classical form of CAH in eastern countries All our patients were having classical form of disease according to the clinical diagnosis In diagnosis of this mutation we were using PCR amplification specific for intron 2 in CYP21 gene For the detection of mutation we were using specific cleavege site in PCR product for MwoI restriction enzyme We found the frequency of A/C655G mutation in allels of our CAH patients to by 33% This is consistent with the data publicated from other countries

5.170

Linkage analysis with the new marker F22 (D6S1078) in Herediatry Hemochromatosis families

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Hereditary Hemochromatosis (HH) is a common recessive disease characterized by iron overload Linkage disequilibrium in French and Italian populations suggested a candidate region for HH of about 400 kb flanking the HLA-A gene Association studies, performed in Australian and UK populations have extended this region to D6S105, estimated to be at least 2cM telomeric to HLA Recently, we concentrated our efforts around D6S105, increasing the number of polymorphic markers by isolating some cosmids clones from this region They have been used as targets for the search for repeats After hybridization, one positive clone (F22-D6S1078) was detected and characterized PCR primers have been designed and at least four alleles (ranging from 202 to 210 bp) detected in a large sample of individuals Successively, linkage analysis was carried out using F22 and two additional microsatellites lying telomeric to D6S105 (CS5 and D6S1050) 9 and 10 different alleles have been detected at D6S1050 and CS5, respectively Normal and affected chromosomes showed similar allele frequencies for all these loci suggesting a possible absence of linkage disequilibrium If these data will be further confirmed, it will be very difficult to compare our findings with data from Northern Europe Supported by grant from Italian Ministry of Health, and Telethon E149

5.171

Identification of 5 new mutations in the rBAT gene

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Ser Genet Med, IRCCS-"CSS", S Giovanni R, IClin Pediatr (TO), [2]Div Nefrol (Roma), Italy, [3]Dept Genet Mol and Dept Bioch Fisiol (Barcelona) Spain

Cystinuria is an inherited disease of amino acid transport, transmitted as an autosomal recessive trait Three phenotypes (I, II and III) have been described During the 1994 we demonstrated that an amino acide transporter gene, rBAT, was responsible for cystinuria More recently, by linkage analysis and by performing genotype-phenotype relationships, we demonstrated that the disease is heterogeneous and that rBAT is defective in type I cystinuria Up to now 12 different mutated alleles have been described Here we describe the identification of five additional rBAT mutated alleles, detected using the RNA-SSCP technology A nonsense mutation has been detected in two chromosomes from a gipsy cystinuric patient A large deletion, involving at least 5 exons of the gene was detected in an italian patient All mutated alleles belong to type I cystinuric patients, confirming our previous findings Work supported by Italian Ministry of Health, Teleton E 083, Institut Català de la Salut, DGICYT PB93/0738

5.173

A gene for premature ovarian failure associated with eyelid malformation maps to chromosome 3q22-q23

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Female infertility have been reported in association with an autosomal dominantly inherited malformation of the eyelids blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) This association distinguishes BPES type I from BPES type II in which affected females are fertile and the transmission occurs through both sexes Recently, we and others have mapped a gene responsible for BPES type II to chromosome 3q22-q23 However, no information regarding the localization of the gene for BPES type I has been available We have studied three independent families affected with BPES type I including a total of 20 affected individuals (10 infertile women) and 6 healthy relatives Linkage data shows a maximum pairwise lod score for marker AFM 268vc9 at the D3S1316 locus (Zmax = 4 52 at Q = 0) The maximum likelihood estimate for the BPES type I gene location is in the interval defined by loci D3S1292 and D3S1316 (location score in log base 10 = 4 82) This result indicates that BPES type I is localized at the BPES type II locus The clinical variation of ovarian failure observed in BPES type I indicates that an autosomal gene at 3q22-q23 is necessary for both development and maintenance of ovarian function

5.175

Mutation screening of a CF population, analysis of patients with bronchiectasis and identification of two novel mutations.

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Cystic Fibrosis, the most common autosomal recessive disorder in Caucasians, is caused by mutations in the CFTR gene We screened a CF population and patients with bronchiectasis using the DGGE method on 11 exons We identified 13 already described mutations and two novel alterations (I539T and 3272-9A→T) I539T was found in a patient with bronchiectasis In the 3' intronic sequence of intron 17a, we detected two mutations, 3272-26A→G (already described) and 3272-9A→T We confirm that the first one creates an aberrant acceptor splice site The mutated allele encodes a troncated CFTR without the NBF2 domain For the 3272-9A→T mutation, we show that this alteration does not disrupt the splicing process but can affect its efficiency In our CF population, DGGE screening allows the characterisation of 88,6% of CF alleles For the patients with bronchiectasis and normal sweat chloride concentrations, we observed a significant increase in the carriers frequency In this population, it is difficult to differentiate a mild form of cystic fibrosis from a distinct pulmonary disease Clinical and biological data are being collected and may contribute to characterise bronchiectasis as a disorder in which the CFTR mutations are involved

5.176

Molecular analysis in Familial Hypertrophic Cardiomyopathy

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Familial Hypertrophic Cardiomyopathy (FHC) is a primary myocardial disease that is mainly familial and is transmitted with an autosomal dominant trait The prevalence of FHC in the general population has been estimated to be 1/500 The natural history of FHC is heterogeneous ranging from a benign asymptomatic course to sudden death FHC exhibits also genetic heterogeneity with regard to the number of responsible genes b-myosin heavy chain (ch 14q11), troponin T (ch 1q3), a-tropomyosin (ch 15q2) and loci 11p11 2, 7q3 About 40% of families, however, does not show linkage to any of the known loci We performed linkage analysis in ten unrelated families using MLINK 5 2 program One family showed cosegregation of the disease with polymorphic markers for the chromosome 15 locus and a G->A transition in nucleotide residue 579 was identified, which caused aspartate-to-asparagine substitution (Asp175Asn) We studied by SSCP analysis 18 unrelated individuals with sporadic HCM In these individuals, we analyzed the first 23 exons of the b cardiac MHC gene and the 9 exons of the a tropomyosin gene and we identified three nucleotide substitutions in exon 7, 11 and 16 of the b MHC gene, none of these changes, however, resulted in amino acidic changes

5.177
X-Linked Hydrocephalus (HSAS): molecular analysis and genetic counseling

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X-linked hydrocephalus is the most common form of congenital, inherited hydrocephalus affecting 1 30000 male, characterized by stenosis of aqueduct of Sylvius, mental retardation, clasped thumbs The gene for HSAS has been localized in the region Xq28 where the L1CAM gene has been mapped, it codes for a surface, highly conserved, glycoprotein and it is implicated in the migration of neuronal cells L1CAM cDNA has been cloned and at least 11 mutations have been identified The lack of hot-spots for mutations and the genetic heterogeneity suggest the use of linkage analysis for the prenatal diagnosis for X-linked hydrocephalus Here we describe a male patient presenting a typical HSAS phenotype Pedigree analysis showed the presence of two proband's mother's brothers affected At first, we looked for known mutations in the propositus Molecular analysis by PCR on DNA and RNA obtained from patient fibroblasts did not show any of the known mutations Subsequently in a second pregnancy, we performed prenatal diagnosis by linkage analysis on chorionic villi Molecular analysis of the whole family using polymorphic DNA markers DXS548 (Xq27 3), DXS15 and F8C (Xq28), allowed us to identify the haplotype segregating with the disease The fetus did not inherit the affected chromosome

5.178
Involvement of the cystic fibrosis gene in adults having disseminated bronchiectasis.

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Phenotypic expression of cystic fibrosis is heterogeneous, both in the clinical manifestations and in the severity of the disease Respiratory syndromes of CF patients are made of lung infections, chronic obstructive pulmonary disease, asthma, and disseminated bronchiectasis In order to assess a possible involvement of the CFTR gene in such pulmonary diseases in adults, we have investigated 44 patients having disseminated bronchiectasis of unknown cause Scanning of all the CFTR gene exons and their flanking regions, using a DGGE approach, has shown a high incidence of CF mutations A total of 25 CF defects were found in 18 of 44 patients (41%) Twenty of these mutations are known to be deleterious (with ÆF508 in 9 instances, 3849+10kbCÆT and D1152H in two cases). The remaining five are putative disease causing mutations. One patient was homozygote for ÆF508, 6 others were found compound heterozygotes, and 9 were heterozygotes for a deleterious mutation We have also identified 3 rare missense substitutions (L997F, T1220I, R31C), heretofore considered as neutral variations but which could be involved in these mild forms, and one rare polymorphism (3041-71G/C) These results indicate that a part of bronchiectatic lung diseases may be due to CFTR gene mutations, moreover, a number of patients may have unrecognized CF In order to strenghten this hypothesis, further analysis must be performed to find defects located in other parts of the gene Henceforth, these findings have implications in genetic counselling for the patients and their family

5.179
Imprinting mutations in Angelman syndrome are relatively frequent and mainly caused by de-novo mutations or trans-acting factors.

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Recently, we described a novel mutation type in Angelman syndrome (AS), the imprinting mutation (IM), which is characterised by biparental inheritance of 15q11-q13 but aberrant genomic imprinting as suggested by abnormal DNA-methylation patterns Findings in some families of deletions on maternal chromosomes, located approximately 1 Mb centromeric to the AS gene critical region, define an imprinting centre (IC), a cis-acting element, controlling the genomic imprinting of the entire region Nevertheless, in several IM patients mutations in cis have not been found yet Therefore, IM due to mutations in trans-acting factors, whose existence has already been described in the mouse, need to be considered We have now determined the segregation of 15q11-q13 haplotypes in 9 AS families with IMs that were ascertained among 336 AS patients (182 with definite and 154 with possible clinical diagnosis) investigated Haplotypes were constructed using genotypes from 6 different microsatellites from the entire region In one family with two affected siblings a maternal deletion at D15S128, close to the proposed IC was found, in agreement with the assumption of a cis-acting factor In three other families where maternal grandparents were available for investigation, the maternal chromosome of the patient was of grandmaternal origin Moreover, in a fifth family the patient's maternal chromosome was also found in his healthy brother Four families showed results that do not exclude any mechanism Our findings can only be explained as due to cis-acting factors if either de-novo mutations or germline mosaicism are postulated Alternatively, mutations in trans-acting factors could have caused the IM in these families. Identification of such trans-acting factors is important for the elucidation of mechanisms governing the genomic imprinting process Finally, this has important consequences for genetic counselling of recurrence risk and prenatal diagnosis

5.180
A possible locus for X-linked non-specific mental retardation identified in two subjects with androgen insensitivity syndrome and mental retardation

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We present data which suggests the existence of a mental retardation (MR) locus at Xq11 2-12 between DSX1 and DXS981, identified in two subjects with complete androgen insensitivity syndrome (CAIS) and MR. Androgen insensitivity syndrome is a disorder of male sexual differentiation caused by a defect in the androgen receptor gene (Xq11-12) Two individuals with CAIS due to a complete deletion of the androgen receptor (AR) gene have previously been reported, one of which also has MR We have identified a third individual with a complete deletion of the AR gene and who has MR All deletions are submicroscopic The deletion in the two subjects with CAIS and MR extends past the AR gene and includes several loci both proximal and distal to the AR gene, these include, DSX1161, PGKP1, DXS1160, DSX908 and DXS897. In the subject with CAIS but without MR of those markers tested only the AR gene is deleted All markers were tested by PCR This data suggests the existence of a gene involved in mental retardation close to the AR gene We are continuing to test further markers to map the deletion and hence the position of the putative MR locus, more precisely

5.181

Homozygosity mapping of a new form of HMSN: autosomal recessive Hypermyelinating Neuropathy.

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We already described a large Italian pedigree with Autosomal Recessive Hypermyelinating Neuropathy (ARHN) showing distinct clinical and histopatological features (Quattrone et al , Neurology, 1995, in press) The six patients belonging to the seventh generation are related to each other through multiple consanguineous marriages starting from an original couple of founders To assess whether the mutation segregating in our pedigree is allelic to any of the other HMSN loci, we performed a linkage study using markers from the following four candidate regions 17p11 2 (CMT1A), 1q22-q23 (CMT1B), 1p35 (CMT2A), and 8q13-q23 (CMT4A) Standard two point linkage analysis yielded significant negative lod scores A search for mutations in PMP-22 (the CMT1A gene) and MP0 (the CMT1B gene) produced negative results Taking full advantage from the particular structure of this pedigree, only five patients have been typed with 362 Généthon's markers, distributed throughout the genome In this way, we have identified only two markers on chromosomes 4 and 10 at which all five patients are homozygous for the same allele and three markers on chromosomes 12, 14, and 18 at which the five patients share nine out of 10 possible haplotypes These five candidate regions are being further screened using additional markers and including the remaining family members in order to finalize the genetic map of the ARHN locus

5.182

Frequency and parental origin of de novo RET mutations in Hirschsprung disease.

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RET mutations are distributed throughout all the exons of this proto-oncogene in Hirschsprung (HSCR) patients Among the 23 RET mutations identified in our series of 121 patients, 17 were present in sporadic cases Of these the same mutation was absent in either parent in 6 cases (de novo), while it could be identified in either parent in other 6 cases and could not be investigated in the remaining 5 cases The de novo RET mutations causing HSCR amount therefore in our patients to at least 26% Among the 6 documented de novo mutations, we could trace the parental origin in 5 cases, following 3 approaches (1) PCR amplification and restriction study when an informative RFLP in the vicinity of the mutation could be analysed on the same fragment, (2) reconstruction of phase through somatic cell hybrids retaining the mutated or the normal allele of RET together with a closely linked polymorphic allele, (3) constitutional loss of heterozygosity (LOH) The parental origin of three point mutations (Ser765->Pro, Leu40->Pro, C->T at +19 of intron 12) was maternal, while paternal origin was demonstrated for two deletions, namely a 4 bp microdeletion (CTGG)[4] starting at nucleotide 3118 and a cytogenetically visible deletion in 10q11 2-21 2

5.183

Expression of mutated glucocerebrosidase alleles in human cells.

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Gaucher disease, is a very heterogeneous disease This heterogeneity results from a large number of mutations in the gene encoding the lysosomal glucocerebrosidase Aiming at understanding the correlation between the phenotypic expression exerted by different mutations and the biochemical defect associated with them, mutations were expressed in human cells using the T7/EMC/vaccinia virus derived expression system (84GG, D140H, K157Q, E326K, D140H/E326K, N370S, D409H, R415P, L444P, recNcil and recTl) RNA stability, protein stability, recognition by monoclonal antibodies and in situ activity were measured The results demonstrated that two mutations (K157Q and D409H) caused significant RNA instability Three proteins, L444P, recNcil and recTL, did not recognize two monoclonal antibodies However, none of the recombinant proteins showed significant decrease in stability Activity of the recombinant proteins was measured in cells preloaded with the fluorescent substrate, LR-12-GC, and infected with viruses harbouring the normal or mutated alleles The results demonstrated that the N370S and the D140H carrying enzymes, under the conditions used, behaved similarly to the normal allele The L444P and the D409H carrying enzymes had 50% and 40% of normal activity, respectively All the other mutations had a severe effect on the cellular enzymatic activity Except recTl carrying enzyme, the biochemical defect could explain the phenotype of the mutations

5.184

Processing of the prosaposin gene.

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Several lysosomal hydrolases involved in the catabolism of complex sphingolipids require interactions with activator proteins for optimal hydrolytic activity Four different activators known as the saposins (A-D) are encoded by one gene designated prosaposin Saposin B activates arylsulfatase A while saposin C activate glucocerebrosidase and saposin D activates ceramidase All four saposins are processed in the lysosome from prosaposin Prosaposin itself seems to be biologically active It is the major protein secreted by sertoli cells The prosaposin gene is transcribed to three mRNAs emerging from alternative splicing of a 9 bp exon Aiming at understanding the possible role of the different spliced prosaposins in different biological processes, we chose to express two spliced cDNA forms (with or without the 9bp exon) using a T7/EMC/vaccinia virus hybrid expression system Both cDNAs expressed the corresponding prosaposin When directed into lysosomes, both were proteolytically processed to yield active saposin C as measured by hydrolysis of a fluorescent glucosyl ceramide within the tissue culture cells The results demonstrated that most of the 9bp containing prosaposin is secreted to the medium while most of the 0 bp containing prosaposin is directed into the lysosomes where it is processed to the different activators We could demonstrate prosaposin expression in the hind brain, the dorsal ganglia and the genital ridge of 12 5 days old mouse embryo, suggesting importance during development Actually, the secreted form of prosaposin has been proposed lately as a neurotrophic factor (1) (1) O'Brien JS, Carson GS et al FASEB J 9 681-685 (1995)

5.185

Characterization of a pentamer repeat in the mouse prosaposin gene.

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The degradation of sphingolipids with short hydrophilic head groups by lysosomal enzymes depends on sphingolipid activator proteins (saps) Four such activator proteins (saposins) are encoded by one gene, the prosaposin The first 88 nucleotides of the mouse prosaposin cDNA have not been found in the genome yet In an effort to find these missing nucleotides, 1 5 kilobase genomic prosaposin fragment was sequenced A pentamer (GGGCT) repeating thirteen times has been found 500bp upstream the first known exon This sequence is not polymorphic as demonstrated by amplification of DNA samples from eight different mouse strains Such a repeat exists in the human and mouse immunoglobulin heavy chain switch regions and the herpes simplex I inverted repeats (ITRs), suggesting a role in recombination events This repeat binds preferentially single-stranded DNA with 5'p, as measured by electophoretic mobility shift assays This specificity resembles the one presented by the Smbp-2 protein (1) Based on published data and our results, it is tempting to speculate that the prosaposin gene is involved in recombination

(1) Fukitay , Tatsunobu-Ryushin, M et al J Biol Chem 268, 17463-17470, (1993)

5.186
Identification of factors regulating the expression of the glucocerebrosidase gene.

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The glucocerebrosidase gene is impaired in Gaucher disease, an inborn error of sphingolipid metabolism In this disease, glucocerebrosides accumulate mainly in cells of the reticuloendothelial system due to a drastic decrease in the activity of lysosomal glucocerebrosidase The glucocerebrosidase gene is expressed differentially In order to identify the factors responsible for the differential expression of the glucocerebrosidase gene, electrophoretic mobility shift assays, DNAse footprinting analyses and CAT assays were employed The results demonstrated that OCT, AP-1 (cJun and cFos), PEA-3 and a CAAT binding protein are important in the glucocerebrosidase gene expression Point mutations in any one of the consensus sequences known to bind these proteins caused a drastic decrease in CAT activity directed by the glucocerebrosidase promoter Transfection of F9 cells lacking endogenous AP-1, with a vector carrying the CAT reporter gene coupled to the glucocerebrosidase promoter, resulted in very low CAT levels Cotransfection of this plasmid with a plasmid expressing cJun resulted in somewhat higher CAT levels Cotransfection of the expressing plasmid with plasmids expressing cJun and cFos resulted in elevated CAT activity, demonstrating the dependence of glucocerebrosidase promoter activity on AP-1 binding Binding of factors from different cells to the glucocerebrosidase promoter demonstrated that the availability of the factors in different cells determine the level of glucocerebrosidase expression

5.187
Isolation and partial characterization of several members of the human axonemal dynein multigene family.

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Dynein heavy chains (DHC), which display ATPase activity, are the mechanochemical energy transducers that are responsible for ciliary and flagellar motility (axonemal dyneins) and may also be involved in some other microtubule-based motility within the cytoplasm (cytoplasmic dyneins) All these dynein chains define two related protein families that display a striking structural conservation through evolution Recent cloning studies, performed in various species except in humans, has led to the identification of as many as a dozen genes encoding axonemal dyneins With the aim to test the involvement of these latter dyneins in ciliary and flagellar dysfunctions in humans, we designed a PCR-based strategy to isolate human axonemal DHC coding sequences from genomic DNA using different sets of axonemal-specific degenerate primers This study allowed us to generate a large number of different PCR products which were subcloned and sequenced Several clones of interest were islolated Comparisons of the nucleic acid and the predicted amino acid sequences with the sequences previously characterized in other species indicate that these DNA fragments encode axonemal DHC molecules that can be further subdivided into various groups These are the first human fragments of axonemal DHC genes reported so far (supported by grants from the GREG 53 and from CRC 931421 AP-HP)

5.188
New spectrum of growth hormone receptor mutations among patients with Laron syndrome.

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Laron syndrome is a rare autosomal recessive growth resistance condition due to mutations in the growth hormone receptor (GHR) gene This receptor is a transmembrane molecule that belongs to the cytokine receptor superfamily and gives rise to a circulating GH-binding protein (GHBP) which is undetectable in the majority of the Laron patients In this study, 18 unrelated Laron patients were screened for mutations in the GHR gene A mutation was identified in the 36 chromosomes studied Fourteen different molecular defects were characterized, most patients being born from a consanguineous union Ten of these mutations are new, they include 2 splice defects, 3 nonsense, 2 frameshift, and 3 missense mutations involving conserved residues among the cytokine receptor superfamily Since all the missense mutations, which are located in the extracellular domain of the receptor, were identified in patients with no detectable GHBP, they are expected to interfere with GH binding activity Overall, these results underscore allelic heterogeneity of this disorder and point out critical residues in GHR as well as probably in related molecules The ongoing study of the functional consequences of the missense mutations should determine the underlying mechanisms by which these amino acid substitutions cause disease in the patients

5.190
Functional analysis of the Menkes disease gene

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Menkes disease is an X-linked human genetic disorder arising from a defect in copper transport The gene, ATP7A, has been isolated by various groups using positional cloning techniques (1,2,3) Deletions in patients with Menkes disease provide evidence that ATP7A is responsible for the observed phenotype Sequence analysis shows homology to ATP7B, which has been identified as the causative gene for Wilson disease (4), another copper transport disorder Both genes have functional domains that are very similar to prokaryotic cation transporters In order to further characterise ATP7A, anti-peptide antibodies, specific to the Menkes gene, are being used to localise the protein within the cell by immunofluorescence A clone containing the entire open reading frame (MNK) has been constructed from CDNA clones and RT PCR products Due to the extensive expression pattern of ATP7A, an epitope tag (MYC) has been incorporated into the open reading frame of the gene (MNK MYCTAG) This enables the expressed protein product of MNK MYCTAG to be distinguished from that of the endogenous protein produced by the cells used in functional analysis Constructs containing both the wild type and mutated variants of the Menkes gene will be used to further investigate the functional role of this protein References 1)Chelly, J et al (1993) Nature Genetics 3 14-19 2)Mercer, J F B et al (1993) Nature Genetics 3 20-25 3)Vulpe, C et al (1993) Nature Genetics 3 7-13 4)Bull, P C et al (1993) 5 327-337

5.191
Refinement of the pycnodysostosis locus at chromosome 1q21

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Pycnodysostosis (pycno) is an autosomal recessive skeletal dysplasia of unknown etiology Recently, we established a 4 cM pycno locus on chromosome 1q21 with a large Arab family with 16 affecteds in which only D1S498 failed to recombine with

disease (Nat Genet ,10 235) To further localize the pycno locus in the D1S442-D1S498-D1S305 interval, we genotyped this family with five new microsatellite repeats mapping to this region Markers were also mapped with 83 diploid radiation hybrid cell lines using RHMAP D1S2346, previously mapped between D1S498 and D1S305, was heterozygous in the affecteds from two sibships with heterozygosity at D1S305 D1S2345, D1S2343 and D1S2347 were previously mapped at no recombinant distance from D1S498 In the affecteds with heterozygosity at D1S305 and D1S2346, there was also heterozygosity for D1S2345 and D1S2343 D1S2347 was homozygous-by-descent in all affecteds D1S2344, previously mapped at no recombination distance from D1S442, was heterozygous in the affecteds heterozygous at D1S442 and likely maps between D1S442 and D1S498 Radiation hybrid mapping revealed a linkage group at LOD=3 00 comprised of D1S498, D1S2347, D1S2345, and D1S2343 These data support the order D1S442-D1S2344-(D1S498/D1S2347)-(D1S2345/D1S2343)-D1S2346-D1S305 The pycno locus lies between D1S2344 and D1S2345/D1S2343, a 2 cM interval

5.192

Analysis of rhodopsin, rds/peripherin and rom-1 gene mutations in Spanish patients with retinitis pigmentosa and macular dystrophy

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We have screened a group of retinitis pigmentosa (RP) patients for mutations in rhodopsin, RDS/peripherin and ROM1 genes by denaturant gradient gel electrophoresis (DGGE) Screening for mutations in the rhodopsin gene in 141 RP patients has been performed We have detected 4 punctual mutations that change the corresponding aminoacid Gly-106-Arg, Arg-135-Leu, Gly-188-Arg, His-211-Arg, and a G-to-T mutation in 3'acceptor splice site of intron four in 5 out of 13 autosomal dominant RP (ADRP) families A Met-44-Thr change was characterized in one isolated case out of 86 sporadic RP patients analyzed These mutations cosegregate with ADRP in each family No rhodopsin mutations have been found in 27 autosomal recessive RP (ARRP) and 15 unclassified RP families Screening of the RDS/peripherin gene has been performed in 135 RP patients previously excluded of having a rhodopsin mutation by DGGE We have detected an Ile-32-Val mutation in two out of three affected members of a RP family A missense Arg-172-Trp mutation in the RDS/peripherin gene was found in a family with autosomal dominant macular dystrophy Screening for mutations in the ROM-1 gene has revealed an Ala-118-Gly mutation in two unrelated RP patients and a Cys-253-Tyr mutation in a third RP family

5.194

Mutations of the FGFR3 Gene in 10 Sporadic Cases of Achondroplasia in Greek Patients

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Achondroplasia is the most common type of disproportionate genetic dwarfism and has been reported in individuals from different races and ethnic groups It is inherited as an autosomal dominant trait with complete penetrance Nevertheless, 80% of cases are caused by new mutations Although diagnosis is evident on physical examination and radiological assessment, confirmation of the type of mutation of an affected individual is useful for prenatal diagnosis Recently it was described that missense mutations in the gene encoding fibroblast growth factor receptor 3 (FGFR3) account for all cases Studies of individuals from several countries have demonstrated that the G1138A transition represents 97% of the achondroplasia mutations and that the G1138C transversion accounts for about 2% We have studied 10 Greek children (7 girls and 3 boys) with achondroplasia All patients represented sporadic cases PCR amplification of a genomic DNA segment spanning the transmembrane domain of the FGFR3 gene was performed and PCR products were subsequently digested with SfcI and MspI restriction enzymes Eight of 10 patients had the G1138A transition and 2 of 10 the G1138C transversion Our results are in agreement with the findings in other ethnic groups

5.195

A splice site mutation in the cardiac myosin binding protein-C gene is associated with familial hypertrophic cardiomyopathy.

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Familial hypertrophic cardiomyopathy (FHC) is a cardiac disorder transmitted as an autosomal dominant trait FHC is genetically heterogeneous and out of the five loci which were found, three genes have been identified which code for cardiac contractile proteins Recently, the cardiac myosin binding protein C (MyBP-C) gene was mapped to chromosome 11p11 2, hence making this gene a very good candidate for the CMH4 locus that we have previously identified SSCP analysis and direct sequencing were performed on genomic PCR fragments and on cDNA RT-PCR fragments We found, in two unrelated french families linked to CMH4, a point mutation that changes a consensus CAG splice acceptor site of the cardiac MyBP-C gene to CGG, in all affected individual members This mutation causes either the skipping of the associated exon or the use of a cryptic splice site Both result in a frame shift leading to premature stop codons, they are predicted to produce truncated proteins that should loose their interaction with myosin In conclusion, these data identify the cardiac MyBP-C gene with the CMH4 locus and further demonstrate that FHC is a disease of the whole cardiac contractile apparatus Our data also represent the first report of a mutation that affects a myosin binding protein

5.196

Mutations in the laminin a2-chain gene (LAMA2) cause merosin-deficient Congenital Muscular Dystrophy.

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Congenital muscular dystrophies (CMDs) are severe autosomal recessive muscle diseases About half of the CMD patients present laminin a2 chain (merosin) deficiency in muscle biopsies We investigated the LAMA2 gene for the presence of mutations using illegitime transcription, SSCP analysis and direct sequencing In one family we identified a missense mutation in a splice site (4573-2 A->T) resulting in exon 31 deletion in the transcript and a frame-shift leading to a stop codon in the II domain In another family, a C to T substitution at position 3767 of the cDNA causes a change in the CAA codon for glutamine to a TAA stop codon (Q1241X) in domain IVa By analysing other families we identified other nonsense mutations leading to stop codons (E210X, C867X, Q988X) and deletions of one and two nucleotides resulting in a frame-shift leading to premature stop codons All these mutations leads to truncated proteins lacking the carboxyl terminal G domain, and domains I and II that form the a-helical long arm of the laminin-2 molecule together with the b and g chains The disease is probably due to a disruption of the link between the sarcolemmal cytoskeleton and the extracellular matrix

5.197
Molecular pathogenesis of aspartylglucosaminuria

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Aspartylglucosaminuria (AGU) is a recessively inherited lysosomal storage disease caused by mutations in the aspartylglucosaminidase (ASA) gene AGU disease results in progressive mental retardation starting early in childhood This disease is highly enriched in the genetically isolated population of Finland, where one point mutation, AGU Fin accounts for 98% of disease alleles We have isolated the AGA cDNA and characterized thirteen other mutations from the coding region of AGA Using in vitro expression of wild-type AGA cDNA in different cell systems we have characterized the processing steps of AGA occurring in different intracellular compartments and clarified the role of the post-translational modifications in the formation of the active enzyme molecule Furthermore, we have determined the three-dimensional structure and amino acid residues locating in the funnel-shape active site as well as the catalytic mechanism of the a b heterotetrameric AGA enzyme The knowledge of the three-dimensional structure allowed the prediction of the structural defects of AGU mutations The intracellular consequences of the mutations were also verified by in vitro expression studies, demonstrating that misfolding of the mutated AGA precursor typically results in a deficiency in the proteolytic activation of the enzyme We have recently found the first naturally occurring mutation that hits an active site residue Ser 72 in the AGA polypeptide and results in inactivation of AGA by a different mechanism To further understand the tissue pathogenesis of this disease, we have cloned the genomic DNA of mouse AGA and the creation of the mouse model for AGU disease is under extensive work

5.198
In vitro expression of the neonatal region of the Marfan gene

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Neonatal Marfan syndrome (nMFS) is the lethal form of Marfan syndrome Mutations leading to nMFS are mainly clustered in exons 24-32 of the fibrillin-1 gene (FBN1) We have prepared a FBN1 minigene construct to study the consequences of nMFS mutations in vitro The construct contains exons 24-37 of FBN1 cDNA preceded by a signal sequence of a lysosomal enzyme inserted to a SV-poly expression vector By in vitro mutagenesis we have created several nMFS mutations into this minigene and expressed the constructs in COS-1 cells Pulse-chase experiments are carried out to monitor newly synthesised polypeptides and the cells, medium and extracellular matrix (ECM) are harvested for the polypeptides to be analysed by SDS-PAGE We have also established stable cell lines of the wildtype minigene in HT1080 and CHO cells We will perform medium exchange studies to monitor the level of mutant fibrillin molecules that interfere the processing of the wildtype polypeptides The pulse-chase experiments show that all the minigenes are expressed, their polypeptide products are secreted and incorporated into ECM but there are some differences in the processing of the mutant polypeptides The minigene product also forms "minifibers" demonstrated by rotary shadowing electron microscopy One of the studied nMFS mutations caused a potential new N-glycosylation site to FBN1 polypeptide Using the minigene construct as a model we could demonstrate that excessive N-glycosylation truly represents the molecular pathogenic mechanism in this patient

5.200
Exon 8 skipping caused by a novel acceptor splice site mutation in the hydroxymethylbilane synthase gene

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Acute intermittent porphyria 9AIP) is due to a defect in the gene encoding hydroxymethylbilane synthase (HMB-S) We studied three members of a family (a father and his two daughters) from England with a history of this condition We screened the entire coding sequence of this gene by performing RT-PCR amplifications of its individual exons to check for deletions and insertions Exon 8 was found to be missing from the mRNA and, therefore, the region spanning intro 7 to exon 9 of the genomic DNA was screened by heteroduplex detected was characterised by direct sequencing after generating single-stranded templates by asymmetric PCR The cause of exon 8 skipping was due to a novel G to A transition at the conserved 3' acceptor splice site of intron 7 This base change resulted in the loss of an EcoRII recognition site, which was employed for confirmation The codon at the junction of exons 7 and 8, AAG (lysine), is altered to AAT (asparagine), the last base of this codon coming from exon 9 However, the skipping of exon 8 does not disrupt the translation reading frame bu the mature protein will contain 26 amino acids less

5.201
Physical mapping of the factor H gene family by Mega YAC analysis

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Serum factor H (HF1) plays a central role in the regulation of the alternative complement pathway The HF1 gene is located in band 32 on the long arm of chromosome 1 and according to linkage analysis, 6 9cM centrometric from the regulators of complement activation (RCA) cluster Data from Western and Southern blot analyses infer that there are at least 3 factor H-related proteins comprising a gene family However, the precise location of the HF genes and the function of their protein products are not yet known In order to locate and characterise the various HF genes, we screened the 'CEPH' Mega YAC DNA library with HF1- and HF2-specific PCR primers Five positive YAC clones were isolated, one of which appears to be chimeric The HF genes contained with the different YAC clones were identified by exon-specific PCRs and their integrity was confirmed by restriction analysis and hybridisation of YAC clone DNA, compared to genomic human DNA The results showed that three of the YAC clones are positive for the HF1 gene, whilst one contains the genes for both HF1 and HF2 Since this double-positive YAC is 830 kb in size, our data suggest that these genes are closely linked and supports the hypothesis that one of them may have been creased by a gene duplication event By mapping these HF YACs by pulsed field gel electrophoresis and generating a contig of overlapping clones we intend to generate a physical mpa of the factor H region, clarifying the relationship between serum factor H and the other members of its gene family This work was supported by the DFG (We 1069)

5.202
High resolution mapping of the RCA complex with YACs spanning 1.3 Megabases.

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The human regulators of complement activation (RCAs) comprise a family of membrane-bound and serum proteins which play central roles in the control of the complement cascade The genes for the membrane-bound members of this family, including membrane cofactor protein (MCP), complement receptors type 1 and 2 (CR1, CR2) and decay accelerating factor (DAF), are contained within a 600 kb

segment on the long arm of chromosome 1 (1q32) The gene organisation within the RCA cluster has previously been elucidated by a combination of approaches, the most informative of which has been pulsed field gel electrophoresis (PFGE) mapping of genomic DNA and more recently, a nine YAC contig which spans the region To further characterise the RCA cluster, we screened the 'CEPH' megaYAC library for a single YAC containing all the above-mentioned genes in one segment Using a combination of PCR and restriction fragment analysis of both genomic and YAC DNA, three YACs were isolated which contain the MCP, CR1, CR2 and DAF genes in their entirety Mapping of YAC restriction fragments by PFGE revealed that one is chimeric, whilst the other two are over 1 1 Mb long and contain the whole, intact RCA cluster Both extend over 500 kb into the flanking regions, representing a significant improvement over existing models We are currently generating a YAC contig to extend the map of this region, forming the basis for an integrative approach towards the identification and characterisation of other genes involved in the regulatory control of complement This work was supported by the DFG (We 1069)

5.203
Linkage analysis of Late -infantile neuronal ceroid lipofuscinosis

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The neuronal ceroid lipofuscinoses (NCL) are a group of autosomal recessive neurodegenerative disorders characterised by the accumulation of autofluorescent lipopigment in the CNS Three childhood forms exist infantile (INCL), late-infantile (LINCL) and juvenile (JNCL) A number of variants of LINCL have also been observed The genes for INCL (CLN1) and JNCL (CLN3) have been mapped and identified, and a Finnish variant of LINCL (CLN5) has been mapped to chromosome 13q21 1-q32 The gene for classical LINCL (CLN2) is unmapped and it is clear that at least one further locus will account for this disease in our family resource A systematic search of the human genome has been carried out in 17 families using polymorphic microsatellite markers distributed at approximately 10cM intervals throughout the genome Three hundred markers have been typed but significant linkage has not been detected This suggests that locus heterogeneity may exist A second genome search is underway using homozygosity mapping in a subset of 5 consanguineous families Fluorescently labelled microsatellites and an automated sequencer are being used to allow increased throughput of samples Using a small number of individually more powerful families, evidence for linkage with or without locus heterogeneity will be established more efficiently

5.204
Mutation analysis of NOS1, a candidate gene for infantile pyloric stenosis.

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Infantile pyloric stenosis (PS) is an inheritable complex trait characterised by hypertrophy of the smooth muscle of the pylorus giving rise to gastric outlet obstruction It occurs a few weeks after birth, having an incidence of 1-5/1000 The enzyme neuronal nitric oxide synthase (nNOS) has been implicated in the causation of PS nNOS catalyses the synthesis of nitric oxide (NO) from L-arginine In the gut, NO functions as a neurotransmitter which mediates smooth muscle relaxation The gene for nNOS (NOS1) has been characterised and has a genomic length of 160kb Using two NOS1 intragenic polymorphisms in 27 families evidence was obtained for linkage and transmission disequilibrium in a subset of 13 PS families (Chung et al , in press) We are screening these families for mutations in NOS1 using a combination of SSCP and direct sequencing To date 18 exons have been analysed by SSCP and no significant differences have been observed The promoter region is being analysed by direct sequence analysis We are also using total RNA from a patient cell line to produce first strand cDNA for PCR analysis and direct sequencing Chung, E , Curtis, D , Chen, G , Marsden, P A , Twells, R , Xu, W and Gardiner, R M AmJHum Genet (in press)

5.205
Parental origin of chromosomes in prenatally diagnosed trisomy 21.

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Recent studies using DNA polymorphic markers show that 90-95% of Down syndrome patients receive the extra chromosome from their mother In 80% of cases, nondysjunction occurs during the first meiotic division The aims of our study is to determine the parental origin of trisomy 21 diagnosed during the fetal period and to investigate its relationship to maternal serum hCG and to echographic signs We present our results using 5 CA repeat DNA markers, (D21S215, D21S167, PFKL, D21S1256, D215192) obtained in 62 families with a prenatal diagnosis of trisomy 21 After extraction of DNA, polymorphism sequences were amplified by PCR and identified after hybridization using oligonucleotide probes Out of these 62 families, 54 were informative The origin of trisomy 21 was maternal in 42 cases and paternal in 12 (22 2%) This frequency is significantly high (p<0 001) than that observed at birth (5%) Meiotic origin was identified in 35 cases of maternal origin (22 meiosis I and 13 meiosis II), and in 11 cases of paternal origin, (6 meiosis 1 and 5 meiosis II) Maternal serum hCG was >2 MoM in 50% of paternal cases and in 54% of maternal cases No ultrasound anomalies were seen in 35% of maternal origine cases and in 50% of paternal cases (NS)

5.206
Detection of novel PKD1 gene nonsense mutations in ADPKD families

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Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent genetic cause of renal failure in adults Thus far around ten independent mutations have been found in the PKD1 gene, including deletions, stop, frameshift, and splice mutations We have extracted DNAs of 435 individuals from 126 unrelated Italian and German families (210 members affected) Using heteroduplex and SSCP analysis of exons 35, 36, 38, 44, and 45 in 50 Italian kindreds, we have thus far detected aberrant patterns in affected individuals from 4 unrelated families DNA sequencing revealed in two families different C to T transitions in exon 44 In a third family a C to G transversion was identified in exon 45 All mutations create different premature stop codons RNA studies showed that the message was stable An abnormal SSCP shift in exon 38 was observed in another large family (9 living affected and 15 unaffected members) Further characterization of this mutations (sequencing and Protein Truncation Test [PTT] analysis is in progress) Supported by the Italian CNR Target Projects "Biotechnology and Bioinstrumentation" and "Genetic Engineering", by a Veneto Region Sanitary Research Grant, and Telethon - Italy (Grant no E 253)

5.208

Tri- and tetrameric microsatellite markers on chromosome 3 and 13 maps

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Design of new microsatellite markers would facilitate genetic and physical maps and positional cloning of crucial genes Distribution of 5 motifs was studied among cosmids of ordered chromosome 13 library (ICRF) Half of GACA, GACT, CAC and TCC loci were located in NOR, therefore these 4 motifs would be not efficient for acrocentric chromosome mapping 15 GATG-positive loci were assigned to 13q and used for STS development Three polymorphic markers were designed

FISH	GDB	GATG/ repeats	Gen Bank Accn	Allele number	size,bp	Hetero-zygosity	dbSTS Id
13q12-q13	D13S707	14/22	G05107	6	139-151	0 66	10599
13q14	D13S699	23/42	G05106	5	208-224	0 64	10598
13q32-q33	D13S690	19/39	G05108	6	344-364	0 75	10600

100 NotI-clones of chromosome 3 and 22 overlapping cosmids of 3p21 3 region associated with lung and other carcinomas were screened for 12 repeat motifs Four STSs were designed on the base of CpG-rich sequences surrounding NotI-sites and proved to be not polymorphic, they were mapped using somatic cell hybrid panel Marker positions could be further defined by the use of Mega-YAC library (CEPH), as D13S699 was assigned to YACs 784-G6 and 758-F4, 28 cM from pter-end The project was supported by Russian Human Genome Programme and INTAS Programme (INTAS-94-4053)

5.209

Internal variations at the tetranucleotide tandem repeat HUMTH01 in a Russian population

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Short tandem repeat (STR) loci are gaining the growing popularity for paternity and identity testing But application of these loci in forensic practice requires knowledge on their allele and genotype distribution Allele polymorphism of HUMTH01 locus (tetranucleotide tandem repeat, chromosome localization 11p15 5) was studied among 120 unrelated Moscow Russians PCR-amplified alleles were separated in 12% high-resolution non-denaturing polyacrylamide gels followed by silver staining Allelic ladders with known HUMTH01 alleles were used to analyze PCR products and to determine internal length variations for this locus Six "main" alleles (179, 183, 187, 191, 195, 199 bp in length) were found with frequencies between 0 011 - 0 385 Two alleles (191 and 195 bp) were the most widespread (frequencies 0 241 and 0 385) Additionally to these "main" alleles we detected five "internal" alleles 185, 189, 190, 193 and 197 bp in length through the analysis of 13 individuals As HUMTH01 locus (11p15 5) was included to the several STR panels in USA and Europe it is important to take into account the presence and relatively high frequencies of "internal" alleles in one of the Caucasian populations (Russian) in process of paternity testing and individual identification

5.210

Polymorphism of some loci of dystrophin gene in two populations: siberian and tadjik.

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The RFLP for pERT 87 loci and polymorphism in 3'-untranslated region of the dystrophin gene were studied in Siberia and Tadjikistan using polymerase chain reaction technology The allelic frequences for the site pERT 87-8/ Taq I were a1 - 0 310 , a2 - 0 690 (1, 2 - absence or presence of the cutting site), Het= 0 428 in siberian population(100 independent X chromosomes), and a1 - 0 368, a2 - 0 632, Het= 0 465 in Tadjikistan (57 chromosomes), and for the site pERT 87-15/ Bam HI b1 - 0 290, b2 - 0 710, Het= 0 412 in Siberia (100 chromosomes), and b1 - 0 186, b2 - 0 814, Het= 0 303 in tadjik population (59 chromosomes) Polymorphic (CA)-repeats were analysed in the 3'- end of dystrophin gene In observed group from Siberia population (101 independent X chromosomes tested) allelic frequences were 0 139 (for allele A1), 0 792 (A2), 0 059 (A3), 0 010 (A4), Het= 0 350 In control group from Tadjikistan (54 chromosomes) some peculiarity in 3'-(CA)-repeats was found the frequency of main allele A2 was 0 907, allele A1, which is common among Caucasians (frequency 0 22, as estimated by A Beggs, L Kunkel, C Oudet et al , 1990), was rare one in tadjik chromosomes (frequency 0 037), variant A3 - 0 056, Het= 0 173 High informativity of pERT 87 loci in two population (they were useful in 65% families with DMD patients) and 3'-(CA)-repeat region in Siberia, but low predictive value of tested 3'-region of DMD gene in Tadjikistan were concluded

5.211

A systematic analysis of the mutations of the Uroporphyrinogen Decarboxylase gene in Hepatoerythropoietic Porphyria.

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A deficiency in Uroporphyrinogen Decarboxylase enzyme activity (UROD), the fifth enzyme of the heme biosynthetic pathway, is found in two hereditary diseases, Familial Porphyria Cutanea Tarda (F-PCT) and HepatoErythropoietic Porphyria (HEP) Analyses at the gene level made it possible to differenciate an autosomal dominant disease, (F-PCT) and a most severe form of cutaneous porphyria, with a profound enzyme defect, that occurs early in infancy, and is transmitted as an autosomal recessive trait (HEP) This study describes the identification of the mutated alleles of UROD gene in three unrelated families The mutations were identified by direct sequencing of four exonic fragments that contained the entire coding sequence of UROD gene Two new missense mutations were observed at the homoallelic state P62L (Pro to Leu substitution at codon 62), in a Portuguese patient, and Y311C (Tyr to Cys substitution at codon 311), in an Italian patient In a Spanish family, the previously described G281E mutation was observed in two patients one patient is homoallelic for the mutation and developed a severe form, early in infancy, while the other patient is heteroallelic for the mutation and shows typical symptoms of F-PCT The mutation G281E is the most frequent mutation in Spanish patients The expression in E coli of the newly described mutated proteins was used to assess that the mutations are responsible for the disease Finally, the precise knowledge of the mutations of UROD gene allows genetic counselling and prenatal diagnosis in severe HEP cases

5.212

Primary nocturnal enuresis: Epidemiology and genetic heterogeneity

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Nighttime bedwetting or primary nocturnal enuresis (PNE) affects approximately 15 % of six-year old children Inheritance of the disorder has been shown in previous family- and twin-studies (Oranskij, 1918, Petrovskij, 1934, Frary, 1935, Hallgren, 1957, Bakwin, 1961) A recent epidemiological survey of Swedish children with PNE show that 201 out of 351 affected probands had at least one first degree relative

with PNE (Hjalmås et al, 1995) A I-ratio (incidence in sibs/population) was calculated to less than 4, which indicates a complex pattern of inheritance This puts PNE on the list of relatively common disorders with a genetic predisposition, such as diabetes or atopic disease Linkage analysis of selected families with an autosomal dominant mode of inheritance has provided evidence for a locus at 13q (Eiberg et al, 1995) In an ongoing genome screening for susceptible regions we analysed 16 Swedish such families Linkage to 13q was excluded in twelve, and verified in three families Our combined results from linkage analyses and from epidemiological data suggest genetic heterogeneity with a strong genetic component in about 1/2 of the cases with PNE

5.213
Stargardt's disease; An ancestral recombination deduced in an eight-generation consanguineous family refines the critical region on 1p21-p13

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Autosomal recessive Stargardt's disease (STGD) is characterised by rapidly progressing bilateral loss of central vision and macular dystrophy at low age The gene for STGD has been mapped to 1p21-p13, restricted to a 6,4 cM interval delineated by markers D1S435 and D1S236 (Kaplan et al, 1993) Analysis of recombination events in STGD families of North American and Saudi Arabian origin showed a peak lod score close to locus D1S188 (Andersson K L et al, 1995) Linkage to the same region in late-onset Fundus flavimaticus with macular dystrophy (FFM) support earlier findings (Weleber, 1994) that this is an allelic variant of the same recessive condition (Gerber et al, 1995) We have genotyped five Swedish and Finnish families with typical Stargardts disease Linkage analysis gave a cumulative two-point lod score of 3 18 at D1S435 In one of the families, a consanguineous marriage loop was found eight generations back The affected sibs of the eighth generation were homozygous for marker D1S435, but heterozygus for D1S424 This observation suggests the occurence of an ancestral recombination event in the interval D1S435 - D1S424 Our findings restrict the STGD-gene region to no more than 2 cM centromeric to D1S435

5.214
Byler disease: Evidence for genetic heterogeneity by exclusion of linkage to the 18q21-q22 region in eight Swedish families

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Byler disease, or progressive familial intrahepatic cholestasis (PFIC), has recently been mapped to 18q21-q22 (Carlton et al, 1995) Benign recurrent cholestasis, (BRIC) was confined to the same region using homozygosity mapping (Houwen et al, 1994), which suggests that the two disorders are allelic We performed linkage analysis in eight Swedish families with characteristic features of Byler disease Marker loci D18S69-D18S64-D18S55 and D18S68, previously found to be linked to PFIC and BRIC, were used Multipoint analysis excluded linkage to the region (maxlod < -4 38 at theta=0 03 telomeric of D18S69) Our findings strongly support genetic heterogeneity for PFIC, and further linkage studies are needed in order to map a second locus for the disorder

5.215
Complete genomic organisation of TUPLE1/HIRA, alternative splice products of the same gene

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DiGeorge syndrome (DGS) is one of a number of related syndromes which result from deletions of human chromosome 22q11 TUPLE1 was isolated from a region designated the DiGeorge syndrome critical region (DGCR) and it was proposed that haploinsuffiency for this gene is at least partly responsible for DGS and related abnormalities Another gene was isolated from the same region, whose deduced protein encompasses TUPLE1, but has 207 additional internal amino acid residues The complete protein was renamed HIRA Southern blotting of RT-PCR products produced from RNA of various tissues showed that TUPLE1 and HIRA are different RNA isoforms of the same gene We have analysed the genomic organization of TUPLE1/HIRA This gene spans a region of about 50 kb and has 24 exons TUPLE1 is composed of at least 20 exons, HIRA has an additional four exons All the exon/intron boundaries have been well characterized and intronic primers have been designed flanking each exon A restriction map of TUPLE1/HIRA from cosmids which cover the region has been constructed using the enzymes BamHI, and BglII Knowledge of the genomic organisation of Tuple1 should facilitate mutational analysis of the genomic DNAs from non deleted patients with the CACTH22 phenotype

5.216
Genomic structure of the rBAT gene.

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Cystinuria is an autosomal recessive disorder of amino acid transport, manifesting as three phenotypes (I, II and II) An amino acid transporter gene, rBAT, is responsible for cystinuria and mutation analises and linkage studies have demonstrated the disease to be heterogeneous, rBAT being the defective gene in type I cystinuria The genomic structure of the gene has been established via two strategies i) Construction of two different genomic libraries by subcloning the MegaYAC921B6 (Généthon) containing rBAT, in Lambda-ZAP Screening was performed using rBAT cDNA and different PCR products Positive clones were analysed and sequenced and ii) Generation of genomic fragments by long-PCR using rBAT cDNA derived primers and sequencing We have determined the intron/exon structure of the gene It spans approximately 45 kb and consist of 10 exons ranging from 120 to 438 bp The introns range from 500 to 13000 bp All splice sites conform to the GT/AG rule The promoter region has been further analysed we have found a predicted TATA-box and an Alu repeat spanning over 200 bp nearby, upstream The major transcription initiation site was mapped by primer extension using human kidney RNA (Supported by DGICYTPB93/0738, ICS, MIS and Telethon94E083)

5.217
Genetic studies in CMT/HNPP Spanish families and isolated cases.

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Charcot-Marie-Tooth (CMT) and hereditary neuropathy with liability to pressure palsies (HNPP) are inherited peripheral neuropathies in which 1 5 megabase of genomic DNA in 17p11 2, including the peripheral myelin gene PMP22, has been associated in duplications or deletions causing CMT type 1A or HNPP respectively and suggesting a gene dosage effect We studied a total of 52 families and 9 isolated cases of CMT and 7 families of HNPP in order to delucidate their

duplication/deletion status in 17p11 2 using the markers D17S122 (PCR) and VAW409R3a (Southern) In over 48 non related CMT1 families tested we have found 18 (38 3%) with the duplication, 29 without it and 1 was non-informative For the nonduplication cases, 11 have an autosomic dominant patern of inherithance (AD), whereas 19 were classified only as dominant (D) The remaining 4 CMT families were clinically type 2 and none of them showed 17p11 2 rearrangements, being classified 1 as AD and 3 as D All of the 9 isolated cases analized were CMT1 and none presented the 17p11 2 duplication For the 7 HNPP families we found 1 with the 17p11 2 deletion and 6 were noninformative We are performing sequencing studies in the other reported CMT loci

5.218
A liver form of the DSC1 (Down syndrome candidate 1) protein

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Down syndrome (DS), caused by trisomy of chromosome 21, is a major cause of mental retardation and congenital heart defects The most likely consequence of the presence of three copies of chromosome 21 is the overexpression of several genes, some of which must be responsible for the various phenotypic features of DS The contributions of individual genes to the phenotype can only be assessed by the isolation of each gene followed by expression and functional analyses We have reported a gene isolated from a foetal brain cDNA library which maps to region 21q22 1-q22 2 This gene is highly expressed in brain and heart and may therefore be of potential relevance in DS As part of the further characterisation of DSC1 we now report the genomic structure of the gene and an alternative form of the protein, isolated from a foetal liver cDNA library The liver protein has an alternative first exon which shows high similarity to the C elegans predicted protein F54E7 7 Moreover, this exon is not expressed in brain The liver protein is 26 residues longer than the brain protein DSC1 is organised in 5 exons and all splice junctions conform to the consensus AG-GT motif

5.219
Analysis of the PROS1 gene in families with protein S deficiency and thrombosis: identification of three novel mutations

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To study the molecular pathology of the anticoagulant protein S (PS) deficiency, all the coding regions of the PROS1 gene have been sequenced in 9 Spanish patients presenting heterozygous PS deficiency and venous thrombosis Four had type I or quantitative deficiency and five had type III deficiency (only reduced free PS antigen) Three novel mutations were identified in three patients with type I deficiency 1302-1G->T at the acceptor splice site of intron 10 and two substitutions to cysteine in exon 12 (G441C and Y444C) Since these mutations cosegregate with PS deficiency, were the only abnormalities found and were not present in healthy controls, we conclude that they result in PS deficiency Analysis of the type III deficient families only revealed the presence of the rare PS Heerlen allele of the S460P polymorphism in 4 probands Linkage analysis excluded the PROS1 gene in type III deficiency in two of these families We conclude that while type I PS deficiency is mostly due to mutations in the PROS1 gene, the molecular basis of type III deficiency appears to be more complex Unknown mechanisms might explain the cases in which the defect responsible for the deficiency remains unclear (Supported by FIS-94/0039 and DGCYT PB94-1233)

5.220
Mitochondria and ageing: biochemical and genetic studies in skeletal muscle

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Oxidative phosphorylation appears to decline with ageing and this impairment could contribute to the general decline in physiological functions We investigated the effect of ageing on skeletal muscle mitochondria function from 101 patients, ages ranging between 13 and 94 years, subjected to surgery for femur fractures We found no correlation between individual enzyme activity of electron chain complexes and age Analyses showed that the proportion of deleted mtDNA molecules increase with age but with values always lower than 1% of total mtDNA Only two individuals of 68 and 71 years showed, at low proportion, the A3243G transition typical of MELAS syndrome No individual showed point mutations typical of MERRF or NARP syndromes or of Leber optical athrophy RNA studies showed a decrease in transcription rate associated with age mtDNA vs nDNA quantification revealed that this, and thus the replication rate, increased with age We conclude that muscle mitochondria are biochemically undamaged with age, that the mtDNA alterations that increase with age are insufficient to produce a functional deficit and that decrease in mitochondrial transcription rate is compensated by an increase in the replication rate (Supported by DGCYTPB93-0019, CICYTSAF913/93 and FIS94/1563 AB is depositary of grant MECPF9237289410)

5.221
Six novel PKLR gene mutations identified in patients with hereditary hemolytic anemia from Central Europe

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Direct genomic sequencing of PCR-amplified PKLR gene exons was performed on 28 unrelated patients from Central Europe with hereditary nonspherocytic hemolytic anemia (HNSHA) who had been found to be pyruvate kinase (PK) deficient in their erythrocytes by enzyme assay 17 different mutations including six novel ones were identified among 54 of the 56 alleles at risk 13 of these were missense mutations caused by substitutions of a single nucleotide G787A[new] (Gly263_Arg), G994A (Gly332_Ser), G1006T (Ala336_Ser), G1010A[new] (Arg337_Gln), A1081G (Asn361_Asp), G1127T[new] (Ser376_Ile), G1174A (Ala392_Thr), G1281T[new] (Glu427_Asp), C1454T[new] (Ser485_Phe, C1456T (Arg486_Trp), G1493A (Arg498_His, G1529A (Arg510_Gln), C1594T (Arg532_Trp) The remaining four mutations were as follows a three nucleotide in frame deletion, 1060delAAG (loss of Lys354), a three nucleotide in frame insertion, 1203insAGC (insertion of a Ser after Cys401), a splice acceptor mutation in intron A, 101-1A, and a two nucleotide frame shift deletion, 628delGT[new] We found two mutations to be major ones in the European population These are G1529A and C1456T representing 46 3% and 11 1%, respectively, of all mutations identified in our patients Interestingly, all but one of the 17 English or German patients carried at least one G1529A allele while only one of the 11 Czech or Slovak patients was affected by this mutation

5.222
Cloning and characterization of a yeast-NAM7 gene homolog expressed in human testis.

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Recently we determined the partial sequences of a large series of cDNA selected from a human testis library Analysis of these sequences allowed us to identify new human transcripts homologous to lower species mRNAs (yeast, nematod, Drosophila) The amino acid sequence derived from one of these cDNA exhibits a

significant homology to the S cerevisiæ NAM7 protein Using this cDNA, we screened a human testis library and obtained two cDNA clones that differ in their 3' end Both clones were incomplete at the 5' end However, using 5'-RACE experiment, we obtained a complete sequence The amino acid sequence contains all the helicase specific boxes that have been characterized in the NAM7 protein Northern blot experiments revealed two bands of 6 and 4 kb probably corresponding to the two cDNA clones The smallest mRNA is specifically expressed in the testis while the larger one is present in all the tissues tested with a stronger expression in the testis The spermatogenic stages of expression of the testis specific mRNA is under investigation by Northern blot and in situ hybridization experiments

5.223

Five novel mutations in the Batten disease gene, CLN3

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We have shown that a 1 02Kb deletion in a novel gene is the major mutation underlying Batten disease (Cell,Vol 82, 949-957) This deletion encompasses 217bp of the open reading frame, corresponding to exons 7 & 8 Affected individuals are predicted to have a truncated protein of 181 amino acids, compared to the normal protein of 438 amino acids To ascertain whether exons 7 & 8 are mutation 'hotspots' in the CLN3 gene, we used SSCP analysis and direct sequencing to screen a panel of 38 Batten disease patients (38/76 chromosomes carried the 1 02 Kb deletion) for mutations Three mutations were found in exon 7 two nonsense (C619G & C622G), one missense (T646C leucine → proline) A C→G change was observed 13bp upstream of the intron 6 splice acceptor site, RNA from this individual is being tested to determine if this change affects splicing of exon 7 One mutation was found in exon 8 a 2bp deletion at position 697-698 in the cDNA The two nonsense mutations and the 2bp deletion are predicted to cause severely truncated proteins

5.224

Homozygosity mapping in juvenile myoclonic epilepsy

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There is unequivocal evidence for a genetic aetiology in juvenile myoclonic epilepsy (JME) although the inheritance pattern is complex Linkage studies from two groups have provided evidence for the existence of a gene (EJM1) on chromosome 6p However, studies from our laboratory found no evidence to support this claim (1), possibly indicative of genetic heterogeneity To reduce this risk, we are concentrating our efforts on mapping a gene predisposing to JME in a group of consanguineous Saudi Arabian families Inheritance of the JME trait in this relatively homogeneous population has been shown to be consistent with an autosomal recessive mode of inheritance (2) A genome search is being undertaken in eight JME families employing the technique of homozygosity mapping (3) DNA from affected offspring is being typed initially for 70 fluorescently-labelled polymorphic microsatellite markers spaced at 15cM intervals across the "gene-rich" chromosomes All markers are genotyped using the ABI 373 DNA sequencer and genescan software Markers which show an excess of homozygosity in affected individuals may be indicative of a region harbouring a recessive disease allele which has been inherited through both parents from a common ancestor Such regions will therefore be investigated further using additional markers References 1 Whitehouse WP, et al (1993) Am J Hum Genet 53 652-662 2 Panyiotopoulos CP and Obeid T (1989) Ann Neurol 25 440-443 3 Lander ES, Botstein D (1987) Science 236 1567-1570

5.225

YAC contig map of the candidate region for familial spastic paraplegia (SPG4) on chromosome 2p14-p21

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Familial Spastic Paraplegia (SPG) is a genetically heterogeneous neurodegenerative disorder characterised by spasticity of the lower limbs Currently 6 loci for dominant or recessive familial spastic paraplegia are known SPG1 on chromosome Xq28, SPG2 on chromosome Xq22, SPG3 on chromosome 14q, SPG4 on chromosome 2p14-p21, SPG5A on chromosome 8 and SPG6 on chromosome 15q11 1 We examined linkage in 9 Belgian families with autosomal dominant pure spastic paraplegia with polymorphic markers flanking the SPG3, SPG4 and SPG6 loci With the SPG4 locus conclusive linkage results were obtained in one family while 2 other families showed suggestive linkage Based on our family data the SPG4 gene maps between D2S400 and D2S367, a region of 4 cM In order to facilitate isolation of the SPG4 gene a YAC contig map was constructed A total of 27 YACs were selected through different databases Each YAC was examined with for the presence of STSs and STRs located in the candidate region Seven CEPH megaYACs contained multiple STSs/STRs and 4 of these YACs spanned the entire candidate area The maximum physical size was estimated at 5 Mb based on the insert sizes contained within the overlapping megaYACs

5.227

No mutations of the connexin 43 gap-junction gene in patients with familial visceral heterotaxy.

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Heterotaxy arises from inappropriate lateralization of thoracic and abdominal organs during embryonic development Typical manifestations include complex heart malformations, gastrointestinal malrotation and splenic abnormalities Britz-Cunningham et al recently reported mutations in the cytoplasmic tail of the connexin 43 (cx43) gene in 6 out of 6 patients with heart malformations and defects of laterality Cx43 is a membrane-spanning protein which assembles to form the intercellular channels of gap-junction Evidences for a role of cx43 and its homologues in morphogenesis are beginning to accumulate We searched mutations in a 529 bp fragment of cx43 containing 423 nucleotides of the cytoplasmic tail plus a portion of the 3'-untranslated region This study was done by PCR-Single Strand Conformation Analysis and direct sequencing in patients of 24 families with recurrence of lateralization defects No mutations in the coding sequence were detected in any of the 24 patients studied However, we identified an A-insertion in the 3'-untranslated region of the gene in 1 patient This is the first polymorphism characterized in this gene and we estimated its frequency from 56 unrelated individuals at 0 045 Thus, the relative contribution of cx43 mutations to heterotaxy in humans remains to be clarified

5.229

Identification of expressed sequences in the LGMD2B region of chromosome 2p13

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We have localised a gene for limb-girdle muscular dystrophy to chromosome 2p13 (LGMD2B) Unlike other recessive forms of LGMD which show considerable variability of clinical course all families with LGMD2B so far described have had a generally late onset and relatively slow progression however, muscular dystrophy

with distal involvement (Miyoshi myopathy) maps to apparently the same area of chromosome 2 We have constructed a 42 YAC contig of the region and are assembling a PAC contig across the area Amongst the seven genes mapping to the contig there is apparently complete conservation of the order seen in mouse Recombinations localised the gene to within the region covered by YAC 747-f-5 We have used this YAC DNA as a template for cDNA capture A library of 12,000 clones enriched for muscle sequences in the YAC DNA has been constructed and gridded Screening for muscle genes in the LGMD2B critical region is now under way

5.230
Diagnosis of fetal cells in maternal blood by PCR

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The routine prenatal diagnosis is based mainly on cytogenetic analysis by means of invasive procedures The PCR technique allows molecular analysis of fetal cells, normally found at low levels in maternal blood, in non-invasive approach We performed two types of prenatal diagnosis using PRC applied directly on maternal blood samples a The fetal's Rhesus D (RhD) blood group in cases where the mother's RhD status was negative and their spouses positive, b The fetal sex Checked against the data collected after birth, the diagnosis of the RhD blood group was highly successful (100%) in the third trimester, though less successful (83%) in the first and second trimesters The diagnosis of the fetus by PCR was compared to the results obtained by cytogenetic means For the fetal sex, the results were better predictive than the fetal's RhD blood group 100% in the second and third trimesters and 88% in the first trimester At present the PCR analysis applied to fetal cells in maternal blood might be used only with a backup of traditional analysis since a few false negative results (although no fals positives) are obtained mainly during the first and second trimesters

5.231
Genetic and physical analysis of the chromosome 5 centromeric region

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A highly polymorphic marker has been characterized as localised at the centromere of chromosome 5 It consists of a microsatellite repeat $(CCTTT)_n$ (40 alleles found within the 40 families of the Ceph panel, heterozygosity > 90%) at the 3' end of an Alu repeat embedded within satellite DNA sequences which have only 60% sequence homology with alphoid sequences and which have been localised at the centromere of chromosome 1, 5 and 19 No cross hybridization at high stringency was obtained with the chromosome 5 and 19 alpha satellite specific probe The localisation was confirmed by linkage analysis within the whole panel of Ceph families with polymorphic markers lod scores were > 10 with low recombination fractions (0 03, 0 02 and 0 04 with D5S418, D5S430 and D5S420 respectively) We have started to establish a physical map of the region by PFGE on Ceph families and by using YACs of pericentromeric localisation Sequence similarities, in addition to the alphoid sequences, are shown to exist between chromosomes 5 and 19

5.232
Detection of Apo (B) A-T rich minisatellite specific DNA-binding proteins

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VNTRs (Variable Number of Tandem Repeat) are used as polymorphic markers in DNA genotyping The VNTR located at 3' end of Apo B gene on human chromosome 2p24 consists of an A-T rich core repeat sequence 30 pb long This VNTR is well suited for studying if the mechanism that control the expression of the adjacent gene is depending of the internal structure of repeats which flank the gene and bind to the nuclear matrix by matrix attachment region (MARs) In this study we envisaged the interaction of nuclear proteins binding a potential regulatory domain of the sequence repeat and its ability to activate or to affect transcription of the ApoB gene. Using numerous alleles (n=21 to 48) of various tandem repeats or of different internal structure (Buresi et al Hum Mol Genet 1995 in the press) obtained by PCR, we show, by band shift and overlay procedure, the in vitro detection of two DNA-binding proteinic entities (120 and 75 kDa) which interact specifically with the VNTR These proteins were partially purified by mono Q FPLC and bound (Kd(app)=10^{-10}M) (determined by ELISA with Biotine VNTR) to double strand DNA containing the minisatellite core ATAATTACATAYTTT ATAATTCAATATTTT (Y = C or T) The binding occured with only one repeat

5.233
The gene IFNAR2 belongs to the cytokine receptor gene cluster on human chromosome 21

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We describe the structure and the expression pattern of the IFNAR2 gene, which encodes the second component of the interferon-a/b receptor The IFNAR2 gene maps at 0 5 kb from the CRFB4 gene and therefore belongs to the same cluster of helical cytokines receptor genes as CRFB4, IFNAR1 and IFNGR2 (AF1), mapping in the middle of human chromosome 21 betxeen SOD and GART The IFNAR2 gene encodes three different polypeptydes We show how exon skipping, alternative splicing and differential usage of polyadenylation sites are involved in generating four types of mRNA These mRNAs encode three different proteins One is likely to be secreted and two are transmembrane receptors with identical extracellular and transmembrane domain but divergent cytoplasmic tails of respectively 67 and 251 aminoacids

5.234
Sporadic heteroplasmic single 5,5 kb mitochondrial DNA deletion in a patient with ataxia, hypogonadism and choroid dystrophy

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Over the last few years, 13 cases of patients with a syndrome characterized by ataxia, hypogonadism and choroid distrophy with a autosomic recessive pattern of transmission have been reported We present a patient with these three manifestations and a series of other symptoms affecting several tissues We have performed mitochondrial DNA studies from lymphocytes of the patient and her mother Southern-blot studies revealed the presence of a population of mitochondrial genomes with a single 5 5 kb deletion, that accounted for 42% of the total mtDNA The region containing the breakpoint of the deletion was amplified by PCR and directly sequenced The deletion spanned 5435 bp, is located between nucleotides 8355 and 13790, and is flanked by a 5 bp direct repeat The case reported is the first in which these clinical manifestations are associated with a defect in the mtDNA Thus, we can consider the syndrome in this case to be a new mitochondrial disease (Soported by CICYT SAF 913/93 AND FIS 94/1563 AB is depositary of a grant from MEC PF92 37289410)

5.235
Isolation of new coding sequences from the Down syndrome region.

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Down syndrome (DS) is the most common human birth defect and a major cause of mental retardation The purpose of our work is to isolate new genes with potential relevance to DS, and to understand the relationship between the over-expression of particular genes and the various manifestations of the DS phenotype Seven YAC cloes spanning 3 different regions of HC21 were used to generate 502 cosmid clones To construct a chromosome specific library of ordered and oriented overlapping cosmids, riboprobe and linear PCR strategies were used This high resolution physical map provided the framework to carry out a direct-selection of human foetal brain cDNAs to isolate chromosome 21-specific transcribed sequences A total of 576 micronclones were isolated and arrayed Of these, 104 map back to chromosome 21 by hybridisation to YAC and genomic DNA dot blots and to filters containing gridded cosmids The HC21 specific cDNA clones were sequenced The length of these fragments is 250-600 bp To prove that we have isolated expressed sequences we are performing Northern blots and/or RNA in situ hybridisation for each putative cDNA We are comparing their sequences with other known functional domains or motifs, to provide clues to possible functions

5.237
Quantitative analysis of RNA from patients with Myotonic Dystrophy.

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The mutation associated with Myotonic Dystrophy has been identified as a CTG triplet expansion in the 3+ untranslated region of the DMPK gene This region has also been identified as a potential CpG island for a downstream gene In order to identify potential downstream genes we have trapped exons from a 15Kb region immediately downstream of the DMPK gene One of these exons contains a putative homeo-domain This and two other exons map within the genomic sequence of a recently identified putative homeodomain gene (DMAHP) (Boucher et al 1995 Hum Mol Genet 10 1919-1925) We have obtained muscle biopsies and established fibroblast cell lines from adult onset myotonic dystrophy patients and adult and foetal controls Using RNA from these fibroblasts and biopsies, we have undertaken quantitative analysis of expression of DMPK and its flanking genes (59 and DMAHP) by multiplex RT-PCR Results indicate that the amount of RNA expressed from each of these three genes is not significantly different to the levels observed for the normal control group Using a restriction site polymorphism located within DMPK we have analysed the level of DMPK expression from the DM chromosome compared to the normal chromosome in DM patients Differences in the ratio of the DM to normal allele have been observed in DM patients

5.238
Transcript analysis around DMPK.

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The underlying molecular basis of Myotonic Dystrophy (DM) results from the expansion of an unstable (CTG)n repeat present in the 3+ untranslated region of the DMPK gene In order to explain the multisystemic effects of DM we are currently examining the genomic organization and expression pattern(s) of genes in the vicinity of the DMPK locus The expansion of the triplet repeat may influence the chromatin configuration and the transcriptional regulation of neighbouring genes, resulting in altered expression of these genes In order to identify transcripts around the triplet repeat we have employed exon trapping We have cloned and characterised two genes (59 and 5C-2C) which map upstream of DMPK and two other genes (4W and 20-D7) downstream of DM-PK Other putative genes 9T9 and 23B were also identified The transcriptional orientation of each of these transcripts and putative transcripts has been determined In addition, we have identified a number of exons which map to a region immediately downstream of DMPK These constitute part of a recently identified homeodomain-encoding gene (DMAHP) The partial cDNA sequence of 59 has been extended 5+ indicating that the full length transcript is almost 3 4Kb The putative transcriptional start site and possible transcription factor binding sequences have been found in the promotor region Two cDNAs corresponding to 20D7 have been characterized and 1 5Kb of sequence has been derived from a 2 4kb message DNA database searches of these novel transcripts fail to identify similar sequences and their precise function remains unknown

5.239
Fine Mapping of Genes from the Holt-Oram Syndrome Interval on 12q24.1

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Holt-Oram Syndrome (HOS) is characterised by autosomal dominant inheritance of malformations of the upper limbs and heart The interval containing a gene resposible for HOS has been defined as approximately 6cM between D12S84 and D12S79 We have not been able to reduce this interval as our families show no further recombinants Therefore, our strategy has been to isolate YACs and, subsequently, cosmids from the HOS interval in order to identify genes by exon amplification The number of genes contained within the 6cM interval could be in excess of 60 Thus, we are analysing transcribed sequences on a positional basis More than 200 cosmids have been identified from the HOS interval Small cosmid contigs have been mapped by FISH which allows the positioning of the transcribed sequences Any developmentally expressed gene or any novel gene mapped to the HOS interval is a candidate for HOS and only genes with known roles, such as housekeeping genes can be excluded Hence, we do not regard 4-hydroxyphenylpyruvate dioxygenase (previously mappped to 12q2 and trapped from our contig) an obvious candidate for HOS However, two other transcripts, JTA7-1 and JTQ3-1 have been identified and they are currently being characterised as more valid candidates for HOS

5.240
Physical and transcriptional mapping in Xcen-Xq21.3 in relation with XLMR disorders.

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The proximal long arm of the human X chromosome contains several loci associated with inherited diseases among which syndromic and non syndromic mental retardation conditions We therefore decided to first construct a physical map from this region using YAC contigs and then to integrate a transcriptional map The first goal was achieved by screening three YAC libraries (ICRF, in collaboration with T Monaco, ICI and CEPH, in collaboration with D Le paslier) It resulted in a 18Mb-long YAC contig corresponding to 85 clones analysed with 57 STSs and probes and covering about 70-80% of the region with a single gap
Using these genomic resources, we performed either classical or improved (using Alu-PCR products) cDNA selection experiments and built a "first generation" transcritional map of this region Among the several isolated transcribed sequences characterised so far, 2 correspond to previously known genes PGK1 and ATP7A, 2 were already cloned but unmapped sequences RPS26 and p54^{nrb} and 4 correspond to new sequences XNP, I14, J15 and E2 All these clones are being tested for their potential involvment in genetic diseases assigned to that part of the X chromosome Regarding the XNP gene, an accompanying abstract presents its association to a syndromic mental retardation (ATX-MR)

5.241

A transcription map of the RP3 region: Mutation analysis in candidate genes.

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Locus heterogeneity has been demonstrated for X-linked retinitis pigmentosa (XLRP) with the majority of families linked to a region around the OTC locus at Xp21 1 While analysis of single recombinants in our families indicated a canadidate interval for RP3 between DXS754 and DXS933, deletion patients characterized by us or other groups have pointed to an XLRP locus between the loci CYBB and OTC We therefore constructed a cosmid contig encompassing the entire region Five different transcription units have been isolated from retinal cDNA libraries by direct cDNA selection or by hybridization with evolutionary conserved cosmid fragments One transcription unit appeared to be abundantly expressed in retina with only a faint expression in other tissues as well as evolutionary conserved in mammalian species This candidate gene (SRPX) encodes a predicted protein of 464 amino acids that shows striking homologies to adhesion molecules like P-selectin with which it shares short consensus repeat motifs Microdeletion screening was performed in 40 XLRP families and in a single, clinically well characterized XLRP family, a deletion was observed including exon 1 and the adjacent promoter region No further DNA aberrations were detected and thus microheterogeneity of XLRP cannot be excluded The four other transcripts isolated are currently investigated in detail and will be presented Due to the concept of microheterogeneity, a YAC contig encompassing the region between OTC and DXS993 was constructed to provide a resource for a large scale isolation of further retinal expressed sequences by direct cDNA selection

5.242

Mutation analysis of candidate genes within the chromosome 5 Interleukin cluster in an asthmatic cohort.

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Aim As part of a comprehensive study to ascertain genetic factors underlying asthma, a cohort of asthmatic individuals and families have been investigated for possible pathogenic mutations in so-called candidate genes Patients DNA was extracted from peripheral blood from 30 atopic asthmatics and 30 normal controls Their phenotype was documented via a questionnaire, clinical diagnosis, skin prick tests, total IgE levels and RAST's, lung function, including histamine challenge Laboratory Candidate genes interleukin-5 (IL5), interleukin-4 (IL4) and α-chain of IL-5 receptor, were initially screened with SSCP and Direct DNA Sequencing (DDS) Results (i) All 4 exons of the IL-5 gene as well as the promoter and 3'-untranslated regions were analysed with SSCP, and in some instances DDS, and failed to show any change from the reported normal sequence, (ii) α-chain of IL-5 receptor - no SSCP alterations, (iii) IL-4 promoter - a C to T polymorphism at position 589 from the translation initiation site showed a trend to raised IgE levels in those with, compared to those without the polymorphism ($p = 0.06$) Conclusion Mutation analysis of IL-5, including the promoter and 3'- untranslated regions, and the α-chain of the IL-5 receptor showed no alterations in the asthmatic cohort compared to the normal published sequence A polymorphism of the IL-4 promoter region appears to show an association with the atopic asthmatic phenotype

5.244

Refinement of a STS-based YAC contig map of human chromosome 21p

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We previously constructed a sequence tagged site (STS) based YAC contig map of human chromosome 21p ranging from the centromere to the rDNA cluster at 21p12 In this YAC contig map, two YAC overlaps were established by only one STS To strengthen these overlaps we used 3 new CA repeat markers which were localised to the centromere or the short arm of chromosome 21 by hybridisation and PCR analyses of an extended panel of somatic cell hybrids Two markers mapped in the pericentromeric region of chromosome 21 and were close to D21Z1 on the YAC contig map The third marker was mapped at 21p11 2 In addition, a STS for D21S5 is being developed from probe pPW235D Also, we isolated end fragments of 8 YACs Five terminal sequences of the YAC inserts were determined, one of which is highly homologous to an alpha-satellite repeat sequence From these sequences, STSs were developed to improve the quality of the YAC contig map The refinement of the YAC contig map will allow a more precise size estimation of the region and the construction of a YAC minimum tiling path, which will be used to isolate chromosome 21p specific polymorphism markers Also, the YACs will be used in experiments in aiming at the identification of potential expressed sequences on the short arm of chromosome 21

5.245

Identification by STS PCR screening of a microdeletion in Xp21.3-22.1 associated with non-specific mental retardation.

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X-linked mental retardation (XLMR) is a common and vastly heterogeneous group of disorders which can be roughly categorized as syndromic or nonspecific (MRX) (Neri et al , 1994) Though the prevalence of this disorder is high, large families that allow linkage analysis with significant lod score values are rare Also,the genetic heterogeneity exclude any possibility to pool families and, therefore, to fine map the related disease gene In order to overcome these difficulties and to identify genomic MRX critical regions distal to the DMD gene in Xp21 3-22 1, we have implemented the PCR screening of non fragile X MR patients for the presence of deletions within an interval of assignment The amplification by PCR of 12 markers located between POLA and DXS704 (Ballabio et al 1995) using genomic DNA from 192 MR males led to the identification of one microdeletion which extends from DXS1202 to DXS1065 None of the known genes, POLA, DAX-1, GK and DMD, that map in Xp21 3-22 1 region is affected by this deletion This allelic loss has been found in a 9 year old boy born of healthly non consanguinous parents He presents a moderate mental retardation (IQ 44 at 7 year old) without any dysmorphic or neurological characteristic features This result is in line with the inherited microdeletion associated with non specific mental retardation reported by Fryns'group during the 7th International Workshop "Fragile X and X-linked Mental Retardation" and confirms the presence of a potential locus in Xp21 3-22 1 involved in non-specific mental retardation

5.246
Isolation and mapping of the gene for neural cell adhesion molecule tenascin-R

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Tenascin-R is an extracellular neural recognition molecule expressed by glia and some neuronal subtypes, including horizontal cells in the retina By screening fetal brain cDNA libraries with the mouse tenascin-R gene we have isolated overlapping clones representing the entire 6kb human cDNA Sequence analysis indicates that it shows approximately 80% homology with the rodent gene The gene has been mapped to the long arm of chromosome 1 using tenascin-R-specific primers to amplify DNA from a panel of somatic cell hybrids Analysis of a panel of 93 radiation hybrids indicates that tenascin-R is closely linked to Laminin B2 (LAMB2) in 1q32, in agreement with its map location in mouse The results are consistent with the order cen, tenascin-R, Laminin B2 Phosducin, TAX-1(neural cell adhesion molecule) A microsatellite repeat has been identified in the 3' untranslated region of the gene providing the potential for mapping tenascin-R relative to unidentified loci for several eye disorders in 1q32

5.247
Phylogenetic footprints of the upstream region of the dystrophin gene muscular promoter

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A 750 bp region upstream the -850 site of the muscle promoter of the human dystrophin gene was amplified by inverse-PCR DNA from a yeast strain transformed with the Bp4 YAC (an 850 kb YAC containing the brain and the muscle promoters and the first part of the human gene) was digested with MspI, circularized by ligation and amplified with back-to-back primers The amplified fragment was cloned and sequenced Primers designed on the new sequence were used to amplify the same region from human and other primate DNAs Comparison of the human sequence with a cis-acting factors database evidenced the presence of many putative sites, among them the most significant are two MyoD, two Ap1 and one homeobox Identity with a supposed cDNA sequence of the 5' end of the fragment was discovered by comparison with Genebank database This sequence (EST03785) was isolated from a human foetal brain library, the identity between these sequences suggests that in this region could exist an expressed gene Alignment of the primate sequences evidenced the presence of some highly conserved boxes Phylogenetic analysis is reported Alignment of DNA sequences for the same species in the region of the dystrophin promoter (from -443 to +110) is reported as well

5.248
Cystic fibrosis mutations in bronchiectasis patients

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Sixty patients suffering from bronchiectasis have been analysed for mutations in their cystic fibrosis (CF) genes We have searched for mutations which total 86% of East Anglian CF mutations These include delF508, dell507, G551D, R553X, G542X, R117H, 621+1, G to T and W1282X We have found that seven patients are heterozygous for one of these mutations The clinical phenotypes of the patients are being assessed in detail, and sweat tests are being carried out We have attempted to establish haplotypes for the heterozygous individuals using polymorphic intragenic markers in intron 8 and intron 17B We wish to determine whether the CF genes where no mutation has been found share a common haplotype or haplotypes Preliminary data suggest that they do not These results support other European studies that CF mutations are often associated with bronchiectasis. Important counselling issues are raised by these results

5.249
Hot spot of recombination in a 3.2 Kb region within the CMT1A-REPs sequences in CMT1A and HNPP patients.

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The Charcot-Marie-Tooth type 1A (CMT1A) disease and hereditary neuropathy with liability to pressure palsies (HNPP) are two autosomal dominant neuropathies, associated respectively with a duplication and a deletion of the same 17p11 2 region These two rearrangements are certainly the reciprocal products of a meiotic unequal crossing-over between the two 17 chromosome homologues caused by the misalignment of the CMT1A-REPs, two homologous sequences flanking the 1 5 Mb CMT1A/HNPP monomer unit In order to map the recombination breakpoints within the CMT1A-REPs, a 13 kb restriction map was constructed from EcoRI fragments from the proximal and distal CMT1A-REP Using 17 restriction enzymes, only 3 restriction sites were present in the proximal CMT1A-REP and absent in the distal one, indicating the high homology of these sequences The analysis of 76 duplicated CMT1A index cases and 38 unrelated HNPP French patients showed that the recombination breakpoints occured in 4 regions of the CMT1A-REPs A hot spot of cross-over breakpoints, located in a 3 2 kb region , accounts for 3/4 of rearrangements The corresponding junction fragments can be detected on classic Southern blots

5.250
Genetic heterogeneity of the PBGD gene in Swedish AIP families.

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The porphobilinogen deaminase (PBGD) gene in 28 Swedish acute intermittent (AIP) families, have been investigated by single-stranded sequencing of the 15 exons Seven point mutations in exons 8 and 10, a TG deletion in exon 14, and two splicing mutations in introns 3 and 9 was detected One of the pointmutations (Trp^{198}) was found in 15 families belonging to a large kindred from Arjeplog, in the northern part of Sweden The remaining mutations were identified in one or a few families Two adjacent mutations in exon 10, affecting the first and second base in the Arg^{167} codon, were found in two CRIM-positive families These mutations were also reported in CRIM-positive families from France and England Of special interest is the adjacent mutations in the Arg^{173} codon in exon 10 The mutation affecting the first base was found in CRIM-negative patients while the second base change was found in CRIM-positive families Several of the mutations could be assayed by restriction enzymes cleavage Additionally an assay based on PCR amplification of specific alleles have been developed for the splicing mutation in intron 9

5.251

Fucosyltransferase genes are spread along the genome: FUT7 is located on 9q34.3 distal to D9S1830.

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Synthesis of A, B, H, Lewis and related histo-blood group antigens is catalyzed by different fucosyltransferases Enzymatic acceptor specificity and tissue expression permitted to define two subfamilies of α-2-fucosyltransferases and four types of α-3-fucosyltransferases encoded by specific genes registered as FUT1 to FUT7 We have already assigned FUT4 to 11q21 region (1), the cluster FUT1-FUT2 to 19q13 3 band (2) and the cluster FUT6-FUT3-FUT5 to 19p13 3 interval (2) The last gene cloned (FUT7) encodes an α-3-fucosyltransferase expressed in leukocytes which synthesizes sLe^x antigen, a selectin ligand We localized this gene by PCR assay using somatic cell hybrids which retain rearrangments of chromosome 9 and then by screening cosmid library FUT7 was assigned to chromosome band 9q34 3 telomeric from D9S1830 and ABC2 This gene organization suggests that α-3-fucosyltransferase genes of each cluster derive from an ancestor gene that would has been duplicated and that would evolved independently This cluster organization could be related to tissue expression pattern with respect to embryogenesis stage (3) (1) Reguigne *et al* Cytogenet Cell Genet 66 104-106 (1994) (2) Reguigne-Arnould *et al* Cytogenet Cell Genet 71 158-162 (1995) (3) Candelier *et al* Laboratory Investigation 69 449-459 (1993)

Advanced technologies

6.001
The application of the "β-Globin Strip Assay "to the molecular detection of β-globin mutations in Turkey

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Beta thalassemia (β-thal) refers to a group of inherited hemoglobin disorders, all characterized by a reduced or absent synthesis of the β-globin chains Extensive molecular analysis of the disease over the past years has revealed that clinically the most important forms of β-thal result from point mutations The disorder poses a major health problem in Turkey The average gene frequency is estimated to be two per cent and regions with higher figures are known to exist According to recent studies, carried out in our department, the six most frequent alleles account for about 70 per cent of the disease gene, IVS-I-110 predominating with 40 per cent Each year approximately 600 pregnancies at risk for β-thal are expected in Turkey Advances in the molecular understanding of the disease facilitated the implementation of several prevention programs in combination with significant simplification and automatization of test procedures based on carrier detection and early prenatal diagnosis Among the various non-radioactive methodologies in use, the "β-globin Strip Assay", which is based on the reverse hybridization principle, has proven to be highly efficient in the identification of the most frequently observed β-thal mutations in the Turkish population This study investigates the applicability and reliability of this system in a large number of Turkish patients and evaluates its efficiency on prenatal diagnosis

6.004
Fiber-FISH as a tool in physical mapping; 800 kb contig on 21q22.3

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Positional cloning of disease genes is a laborous task demanding various time consuming techniques High resolution FISH techniques,especially Fiber-FISH (Heiskanen et al 1994) offers convenient, fast and an efficient tool for mapping and ordering cloned sequences in physical maps The benefit of this method is a direct visual analysis of several clones simultaneously APECED (autoimmune polyendocrino-pathy-candidiasis-ectodermal dystrophy) is a recessively inherited autoimmune disease and the disease locus has been assigned on a 500 kb region on chromosome 21q22 3 by linkage disequilibrium studies (Aaltonen et al 1994) This genome region has not been earlier cloned We have applied Fiber-FISH in constructing a contig map of cosmid and PAC -clones over a 800 kb region containing the APECED locus The sizes of the probes and the lengths of overlaps and gaps between probes have also been measured Our results indicate that high resolution Fiber-FISH offers an excellent tool in positional cloning and mapping studies and is routinely applied in our positional cloning projects

6.005
Fiber-FISH: new strategy for positioning and ordering of clones

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The recent development of high resolution Fiber-FISH techniques has significantly increased the power of FISH in physical mapping The hybridization of several clones simultaneously on DNA fibers allows direct visual analysis of clone order, transcriptional orientation and the sizes of gaps and overlaps with a resolution starting from 1kb We have developed a technique where hybridization target is prepared from agarose embedded DNA (PFGE-block) (Heiskanen et al 1994) These PFGE-blocks contain long, intact DNA fibers that can be used for high resolution FISH mapping on a resolution range of 1-500kb Also the physical distances separating the clones can be determined Fiber-FISH was applied to construct a physical map over a 500kb region on 1p32 and to locate a gene responsible for a recessively inherited neurodegenarative disorder, infantile neuronal ceroid lipofuscinosis (INCL), on this map (Vesa et al 1995) This same chromosomal region was studied in small cell lung cancer (SCLC) for the refinement of a chromosomal rearrangement (Mäkelä et al 1992) We have also used the technique for mapping of a collagen gene cluster on 21q22 3 and albumin multigene family on 4q11-13 Fiber-FISH was shown to be an excellent method for efficient physical mapping and this technique is now routinely applied in our physical mapping projects

6.007
Automated construction of radiation hybrid maps using MultiMap.

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A focused effort is underway to further the human physical map through radiation hybrid (RH) mapping RH maps have high density and can include monomorphic markers such as ESTs The mapping process is similar to that of meiotic mapping However, the increase in markers that can be mapped in RHs leads to a scalability problem during map construction Radiation hybrid mapping can be made more efficient, less error-prone and less tedious through automation We have incorporated algorithms for RH mapping into the MultiMap computer program similar to those currently implemented in MultiMap for genetic mapping We have written the RADMAP program which computes multipoint likelihoods in the presence of missing data This computation is rapid and large numbers of markers can easily be analyzed We are testing MultiMap/RH using several sets of computer-simulated data and empiric data from several chromosomes, including 11 and 13 The simulated data allow us to examine various average retention frequencies, numbers of markers and map resolutions Preliminary tests show that MultiMap/RH can construct maps correctly and efficiently MultiMap/RH should prove a useful mapping tool for the physical mapping of humans and other organismal genomes

6.008
PRINS as a method for rapid preimplantation embryo chromosome screening.

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The PRimed In Situ (PRINS) labeling technique constitutes an interesting alternative to FISH for chromosomal identification We have adapted it to preimplantation diagnosis by establishing a semi-automatic, multi-color PRINS protocol The technique was applied to individual blastomeres resulting from morphologically abnormal preimplantation embryos (grade IV) Specific primers for chromosomes 9, 13, 16, 18, 21, X and Y were used in various combinations for double or triple PRINS reactions Using fluorochrome-labeled nucleotides, it was possible to obtain specific labeling of 2 chromosomes in less than 90 minutes reaction In this preliminary study, we obtain a labeling efficiency of 100% on fixed blastomeres Various chromosomal abnormalities (aneuploidy, mosacism, haploidy) were detected The PRINS method is assuredly a well adapted tool for preimplantation diagnosis, because it combines rapidity and specificity The use of PRINS technique may contribute to improve the management of preimplantation diagnosis in a time-frame compatible with IVF procedure

6.010
The Human Genome Data Base - Version 6.0

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The human Genome Data Base has been providing public access to genome mapping information since 1990 Recently, GDB underwent fundamental changes in both database structure and curatorial philosophy Several modifications to the GDB user interface have been made with the recent release of version 6 0 Access to GDB data is now entirely done through World Wide Web query forms Maps in GDB are for the first time viewed graphically, through use of the Mapviewer, a software tool available from GDB The underlying structure of GDB has also been redesigned in an object-oriented framework The new design is more robust and extensible, allowing for richer representation of map data This new design will also allow GDB to keep pace with rapidly changing paradigms within human genome research WWW access has also allowed GDB to move toward a community model of data curation It is now possible for any genome researcher with an Internet connection to obtain an editorial account from GDB, for the purpose of entering data directly into the database In addition, a new bulk submission format has been devised to allow genome centers and larger laboratories to submit large batches of genome mapping data to GDB in an automated fashion To get more information on accessing GDB please contact GDB User Support (email help@gdb org, phone (410) 955-9705, fax (410) 614-0434) For questions about data submission to GDB, contact Data Acquisition and Curation (email data@gdb org, phone (410) 955-9705, fax (410) 614-0434) To access GDB via the WWW, point your WWW browser (i e, Netscape, Mosaic, etc) to http //gdbwww gdb org/

6.011
Prenatal diagnosis of inherited disorders in Russia. Present state and perspectives.

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The current state of molecular and cytogenetic diagnosis of most common inherited diseases in human foetuses of Russia and of FSC is reviewed Basic results of prenatal diagnosis of chromosomal and some monogenic diseases in St Petersburg are presented Over 2000 karyotype analysis of the 1st & 2nd trimester foetuses resulted in 112 cases with abnormal karyotypes (43 - numeral aberrations, 47 - structural and 22 - mosaics) FISH technique for precise identification of chromosomal rearrangements has been applied Molecular diagnosis of the foetuses at risk have been mainly confined to cystic fibrosis (330 cases), Duchenne muscular dystrophy (45 cases), haemophilia A & B (44 cases) as well as phenylketonuria (30), Fragile X-syndrome (5) Basic results of prenatal diagnosis & carrier detection of these disorders in St Petersburg and somewhere else in the Russia are discussed Some peculiarities of allelic polymorphisms and mutation patterns of relevant genes in native populations are briefly outlined The urgent necessity in efficient molecular diagnosis of some other common monogenic disorders (Willibrand's disease, polycystic kidney, Huntington chorea, myotonic dystrophy etc) is stressed Perspectives for implementation of novel technical approaches for more efficient diagnosis of inherited diseases are briefly discussed

6.012
Rapid generation of human chromosomes painting probes from hybrid cell lines: Clinical applications on molecular cytogenetics.

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We have investigated a rapid and efficient procedures in order to generate whole and sub-chromosomal painting probes The human content of hybrid cell lines (human-rodent) has been amplified by Inter-Alu-PCR, according to Liu et al (1993)1 Using this approach, 10 differents total or subchromosomal painting probes are available at the moment for chromosomes 13, 16, 15, 17, 21, 6/22/Xp This rapid approach of probe production can present an alternative to chromosome sorting and DNA cloning These probes are used routinely for pre and post-natal diagnosis in the laboratory to confirm or to specify complex chromosome rearrangements We present the procedures of probes production and validation, illustrated with clinical applications In cancer the hybrid cell lines can help us to clarify the origin of markers and to explain the evolution of chromosomal disorders at different stages of tumor development 1Liu et al, Cancer Genet Cytogenet, 1993, 65, 93-99

6.013
A novel microsatellite DNA marker set for high speed, automated gene mapping

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Linkage mapping with microsatellite DNA marker is a fast and efficient method to localise genes for monogenic and multifactorial diseases Microsatellites are highly variable short tandem repeat polymorphisms which can be amplified by PCR Alleles are separated on polyacrylamide gel electrophoresis based of their fragment size Microsatellites are very frequent and evenly spaced across almost the entire genome By now several thousand microsatellites have been described and different high resolution genetic maps of the human genome have been constructed Disadvantages of this method include a difficult allele pattern of some microsatellites as well as current detection techniques using radioactive labels New technological developments take advantage of fluorescent detection of DNA-fragments on an automated DNA sequencer In this way several different sized microsatellites can be typed on one polyacrylamide gel In addition, the combination of microsatellites labelled with different dyes allows for simultaneous analysis of large numbers of markers We have now established the MDC-microsatellite mapping set, a marker set for autosomal genome screening This set currently includes 333 microsatellites with an average heterozygosity of 79,7 %, ordered into 25 panels of markers which can be analysed simultaneously The length of the genome covered is 3787 cM with an average spacing of 11,45 cM All markers have been selected for robust PCR conditions and clear allele patterns We have also established a genotyping facility which applies the MDC-microsatellite mapping set on an automated DNA-sequencer to different gene mapping projects A streamlined operation which makes extensive use of parallel sample amplification as well as a standardised fragment analysis allows for high throughput of genotypes Currently we can produce aprox 800 genotypes/day In addition a software module, LINKRUN, developed at the MDC allows for direct verification of mendelian segregation of the genotypes obtained as well as automated linkage analysis using LINKAGE programs Using this procedure we have so far mapped several monogenic disease genes at an average of 6 weeks/project This facility is also open to outside projects

6.015

A simple and rapid postnatal technique for the DNA diagnosis of Down syndrome and other aneuploidies

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The differential diagnosis of Trisomy 21 Down Syndrome comprises one of the largest referral entities of Clinical Cytogenetics Laboratories Currently this involves karyotyping following in vitro culture of blood lymphocytes, an expensive skilled and time consuming process In 1994, Mansfield described a simple, quick and inexpensive method to identify Trisomy, using the polymerase chain reaction to amplify polymorphic short tandem repeat (STR) markers Using the tetranucleotide repeat polymorphisms D21S11 and IFNAR we have applied this technique to successfully analyse non-invasive mouthwash samples from 42 cytogenetically verified Down Syndrome cases and 50 normal controls We have made use of the ABI gene scanner, coupled with flourescent primers to give a clear, rapid and relatively inexpensive DNA screen

6.016

Studies on radiation hybrid mapping

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A decade ago pulsed field gel electrophoresis promised to revolutionize gene mapping, but failed to provide long range connectivity Today radiation hybrids compete with projection of the genetic map for connectivity and with STS content maps, contigs, and FISH for resolution of order and distance Our multiple pairwise analysis allows for preferential retention (usually proximal to the centromere), monosomy or disomy of donor chromosomes and error filtration Omission of these refinements loses a large fraction of the information in several examples We have implemented analysis in conjunction with a location database, which automatically provides a trial map constrained to arm length in megabases This gives maximal connectivity and succeeds where an alternative method partitions the map into clusters with unspecified distances However, map order and distance are sensitive to assumptions about retention, polysomy and error, and Bayesian calculations on a simplified model have little relation to reality With current radiation hybrid data the accuracy of map location is no better than 1 Mb, and so it is doubtful that radiation hybrids will replace alternative methods However, they provide an efficient albeit approximate location of expressed sequence tags (ESTs) and other markers with low heterozygosity that will be refined as other physical methods approach connectivity The programs ldb+ and map+ and the location database in which they operate are available on Internet (http //cedar genetics soton ac uk/public_html)

6.017

ldb: a location database of the human genome

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The location database comprises a suite of software for high resolution integrated map construction and a comprehensive human genome database The software consists of map+ for linkage non-disjunction and radiation hybrid mapping and ldb+ which integrates maps, provides the interface to map+, constructs partial physical maps from clonal data and provides connectivity for physical maps by projecting the covering genetic map onto a physical scale The database component comprises the most comprehensive integrated maps currently available covering the whole genome with locations for more than 6,300 loci Many locations have been obtained through mapping more than 520,000 pairwise lods (available in ldb) with map+, these derived from the CEPH 7 1 and LODSOURCE databases Integrated summary maps for each chromosome have been constructed by combining map+ generated partial maps with maps from the literature or online databases More than 420 partial maps are now available in ldb. The World Wide Web provides an exciting global interface to ldb The WWW site (http //cedar genetics soton ac uk/public_html) has, in the space of a few months, become established as an essential internet resource Features include a search of ldb for information relating to a locus initiated by a hypertext link The output provides details of flanking loci in each partial map, the 10 largest lod scores in the database and locus-content details of the relevant CEPH-Genethon YACS together with a hypertext link to the GDB entry

6.019

Improved software for automated gene localization studies on an ABI automated DNA sequencer

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Recently it has become possible to analyze fluorescently labeled DNA markers on an Applied Biosystems (ABI) automatic DNA sequencer, and specialized software packages called Genescan and Genotyper have been developed by ABI to perform data processing However, we experienced a number of problems using the most recent version 1 1 of the Genotyper software To solve these problems, we developed two new software modules, Linkage Designer and Linkage Reporter Linkage Designer is able to automatically carry out several procedures that are inefficient or missing in Genotyper, including allele binning (defining size-intervals for the different alleles), converting fragment sizes into allele numbers, checking of Mendelian inheritance and generation of the linkage file format The Linkage Reporter module integrates lod scores and intermarker distances into chromosome maps showing the regions where linkage is excluded Linkage Reporter can also be used in traditional radioactive marker analysis These computer programs are freely available for non-profit research organizations In conclusion, Linkage Designer and Linkage Reporter represent a significant improvement for data analysis in automated gene localization studies

6.020

Fragmentation vectors for the direct subcloning of YAC into BACs

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The use of large insert Yeast Artificial Chromosome (YAC) DNA libraries has facilitated the analysis of the human genome by its ability to allow the construction of chromosome long contiguous arrays Despite this ability, YACs are disadvantageous in constructing fine structure maps and in locating genes because of their large insert sizes and since manipulating YACs in a yeast genetic background is difficult With these considerations in mind we are developing vectors which allow the direct subcloning of YACs into BACs (Bacterial Artificial Chromosomes) Since BACs are easier to manipulate they allow the construction of high resolution contig maps which are important for efficient isolation of genes The vectors are designed as fragmentation vectors containing Alu or LINE sequences as targets for homologous recombination The Lysine-2 gene is used as a selective marker, allowing direct use of these vectors with the AB1380 yeast strain Furthermore these vectors also contain the complete BAC vector sequence flanked by a multiple cloning site for (rare cutter) restriction enzymes, allowing the recovery of BACs from a fragmented YAC after restriction digestion, circularisation and transformation in the appropriate E coli host cells In order to access the feasibility of these vectors, they will be used on a chromosome 14 YAC clone 788H12 This YAC contains the presenilin-1 gene (S182) known to carry mutations leading to early-onset Alzheimer's disease

6.021
YAC and BAC based vectors for gene transfer into mammalian cells.

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We are developping vectors allowing to transfer 10 - 1000 Kb of DNA into mammalian cells We constructed a YAC-based vector that contains a putative human origin of replication, a MAR element, and loxP sequences for circularisation using the Cre recombinase, assuming that circular DNA may replicate episomally in mammalian cells We are presently transferring constructs containing the CF cDNA or the entire CF gene into cells in culture with the cationic lipid DOTAP or poly-Lysine - derived proteosomes However, only small amounts of intact YAC DNA can be purified We are therefore devising strategies to package gene constructs as BACs into filamentous phages, which have been shown to transferinfect cells The system is composed of two vectors, one containing the transgene and the f1 ori for packaging, and one carrying a recombinant M13 gene III The aim is to produce circular DNA that is protected by the phage capsid until it enters the cell Targeted gene delivery can be achieved via receptor-mediated endocytosis by displaying recombinant paratope at the surface of the capsid Project supported by the Association Française de Lutte contre la Mucoviscidose

6.022
INFOBIOGEN : a resource centre for the medical and scientific European community.

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INFOBIOGEN (bioinfo@infobiogen fr) is a resource centre supported by ten french public bodies, mainly CNRS, INSERM and AFM. Its goal is to provide biological databases of interest and means to access them This task includes the management of a collection of databases and computational analysis tools, users' support and training courses As a ressource centre, INFOBIOGEN puts also some efforts on research activities, specially on Information Management with projects such as VIRGIL ("Automatic Generation of Links between Heterogeneous Genomic Databases", Proceed Internat IEEE Sympos on Intelligence in Neural and Biological Systems, pages 78-83 May 1995) The centre is responsible for collecting genomic data from French and South-European research groups to edit the GDB (and clinical disorder databases) mainly when attending to chromosome workshops Moreover two databases are developed and managed at INFOBIOGEN GENDIAG and GENINFO The training programme will be continued with a special focus on the medical community which now has a better access to the network and consequently to bioinformatic resources The activities of INFOBIOGEN will be presented at the ESHG meeting with an emphasis on new developments

6.023
Construction of a murine model of the human Charcot-Marie-Tooth disease type 1A, by microinjection of YACs in mouse oocytes.

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Charcot-Marie-Tooth disease type 1A, is a peripheral neuropathy caused, in most cases by the duplication of a 1 5 Mb region from the proximal short arm of the chromosome 17 The major gene involved in this disorder is PMP22, which codes the synthesis of a myelin protein Thus this disorder is caused by the over-expression of this protein in patients It is interesting to note that clinical signs of the disorder are occurring late (usually after 30 years) and that homozygotes (carrying four copies of the gene) are more severely affected We decided to construct an animal model in order 1- To have more informations on the physiopathology of the disease 2- To assay different strategies to reduce the expression of the gene, which could lead to gene therapy For this purpose a 550 Kb human YAC, encompassing the proximal part of the region duplicated in CMT1A (including PMP22 and its regulatory elements) has been microinjected in mouse oocytes 7 founder mice carry the PMP22 gene with a variable amount of the rest of the YAC In three of them, fingerprinting and STS content demonstrated that no gross rearrangement of the YAC DNA has occurred All of them have had litters and are transmitting the transgene Three lines are showing a neuropathic phenotype The severity is correlated to the number of integrated copies Data will be presented, demonstrating that we have now a model as close as possible to the human disease

Expressed sequences in the human genome

7.001
The nephronophthisis (NPH1) critical region on 2q13: Constructing a 6.4 Mb integrated map of YAC and PAC contigs and assigning candidate genes

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Nephronophthisis, the commonest genetic cause for renal failure in children, is an autosomal recessive cystic kidney disease A gene for nephronophthisis (NPH1) was mapped to 2q13 (Genomics 22 296, 1994, Genomics 25 360, 1995) We constructed a complete YAC contig of 6 4 Mb in the NPH1 region by sequence tagged site (STS) content mapping of 29 CEPH mega-YAC clones, assigning 14 polymorphic and 6 non-polymorphic STS markers to the contig A partial transcriptional map was set up containing 13 expressed genes (ESTs), some of which may be candidates for NPH1 Analysis of recombinant NPH1 families defines D2S340 and LA2C as flanking NPH1, confining the NPH1 critical region to an interval of <4 8 Mb The construction of a partial contig of >18 P1 related artificial chromosome clones (PACs) allowed for high resolution mapping of the STS/EST markers The total marker density was 1 STS/EST marker/100kb The contigs and transcriptional map provide the basis for identification of the NPH1 gene and will alleviate other mapping efforts in the 2q13 region (The contribution of marker LA2C by Dr M McAlleer, Oxford, and the contribution of DNA from family 28 by Dr C Antignac, Paris, is gratefully acknowledged)

7.002
Identification and characterization of a new human gene (21-GARP) located in the Down Syndrome Critical Region of Chromosome 21 and differentially expressed in fetal heart and skeletal muscle.

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In an effort to identify genes candidated for the pathogenesis of congenital heart defects (CHD) that affect 40% of Down Syndrome patients we are performing cDNA selection using a panel of YAC clones that cover a region of chromosome 21 between the markers D21S55 and D21S15 and mixtures of cDNA synthetized from fetal heart poly A+ RNA Here we describe the isolation and characterization, using for cDNA selection the YAC clone 3H1y21, of a new human gene differentially expressed in fetal heart and in fetal and adult skeletal muscle mapping in the CHD Critical Region of chromosome 21 The complete sequence was obtained from several overlapping cDNA clones and contains an open reading frame of 528 bp coding for a putative protein of 176 aminoacid residues The predicted protein contains a proline-rich domain that is in agreement with the consensus sequence for SH3 binding domain and is particularly rich in glutamic acid residues (25%) Because of this last feature we named the new gene 21-GARP (Chromosome 21 - Glutamic Acid Rich Protein) Due to its expression pattern and its localization, the 21-GARP gene might play a role in the pathogenesis of CHD and muscolar hypotonia in Down Syndrome

7.003
Subtractive cDNA hybridization: an approach to identify genes responsible for the phenotypical manifestations of human disease.

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One of the major goals of the human genome project is the identification of all transcribed sequences Several groups work on the identification of so called 'expressed sequence tags' (ESTs) of various human tissues Tissue-specific gene expression specifies cell fate and function Of special interest is the differential expression of genes in processes like cell differentiation and malignant transformation One approach to address this task is the generation of subtracted cDNA libraries Usually for this kind of approach great amounts of RNA are required We are interested in the pathology of the hereditary cancer syndrome Neurofibromatosis type 1 The main features of this disease are multiple neurofibromas and pigmentation anomolies named café au lait spots Primary cultures of human melanocytes serve as a model system for the benign lesions of the disease As these cells grow very slowly we developed a PCR-based protocol working with lower amounts of input RNA Results obtained with this new method applied to melanocytes from NF1 patients in comparison to melanocytes from healthy donors will be presented In general this method should be useful to define the differences in gene expression responsible for the phenotypical manifestations of human disease

7.004
Small-scale, specific cDNA sequencing projects can usefully complete data provided by massive sequencing centers

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One of the most prevalent aspects of the ambitious human genome project is its approach by cDNA strategies To date, the widely-used, single-pass partial sequencing technique has lead to the identification of more than 300'000 ESTs, isolated from more than 200 cDNA libraries which represent a wide variety of human tissues and developmental stages Genes that are expressed at very low levels or only in marginal spatio-temporal conditions are however not likely to be detected by random approaches Here we present the preliminary results of a small-scale cDNA sequencing project After construction of a 6 week-old human embryo cDNA library, 4000 clones were plated and 250 of them, representing the lowest-abundance transcripts, were selected Partial sequencing showed that about 30% of them were unknown in databases thus confirming the interest of selecting poorly transcribed genes to minimize redundancy In addition, all unknown cDNAs were regionally mapped on chromosomes by metaphase in-situ hybridization, thus providing new markers for both genetic and transcriptional maps Finally, several cDNAs that showed high homology with tumour or growth-related genes already described in other mammalian species were selected for more complete analysis, including full-sequencing and expression patterns

7.005
Transcription mapping of Human chromosome 16p12.3-12.2.

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As part of a European Consortium on the physical and transcriptional mapping on the short arm of Human chromosome 16, contigs of chromosome 16 cosmids have been used as the genomic resource for transcript identification in 16p12 3-12 2, a region defined by 5 hybrid intervals We have been focusing on the hybrid intervals CY11-Cy15, which consists of 15 contigs containing 69 cosmids Each cosmid has

been analysed for the presence of CpG islands by digestion with Not I, AscI, EagI and BssHII To identify unique fragments, EcoRI restricted cosmids have been hybridised with Human placental DNA We have used these results to select 21 cosmids from this region which we have used in Direct cDNA selection and Exon Trapping experiments We are currently analysing over a thousand selected cDNA clones or trapped exons by sequencing and hybridisation with chromosome 16 clones So far at least 12 cosmids contain sequence represented in the selected cDNA library, Exon Trapped library or in the Human Foetal Brain cDNA library

7.006

Direct Selection of expressed sequences from a 1.2 Mb region of human chromosome 4q12-13.

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We have applied 'direct selection' methodology to the identification and characterization of transcribed genes (cDNAs) encoded by a 1 2 Mb YAC isolated from human chromosome 4q12-13 The application of this methodology is a continuation of initial work (1) on the development and application of direct selection technology to human chromosome 5q12-21 The region of chromosome 4 analyzed in this study encodes the genes for a novel inflammatory cytokine family (chemokines) which includes the IL-8, GRO, BTG (4q12-13) genes The chemokine superfamily are structurally subdivided into two groups, C-C and C-X-C, depending on wheither the first two cysteines of the mature protein are adjacent or separated by a single amino acid residue The group clustered on 4q12-21 are members of the alpha chemokine family while the beta chemokine group, represented by the Rantes gene, are clustered on 17q11-12 In this study we were interested in isolating additional cDNA's of 'immunological origin' from 4q12-13 cDNA was prepared from mRNA isolated from activated (LPS,PHA,PMA and calcium ionophore) peripheral blood lymphocytes A 1 2 MbYAC clone was isolated from the CEPH YAC library by PCR screening with IL-8 specific primers This clone was subsequently shown to encode the entire IL-8/GRO/BTG locus A number of selection criteria were improved upon, including optimization of cDNA to YAC ratio's used in both primary and secondary selections and YAC repetitive DNA blocking by COT1 DNA The abundance of the IL-8/GRO/BTG following two rounds of selection were between 1 and 3% of selected cDNA's 96 of these clones were further screened by hybridization and direct sequencing Following the elimination of mitochondrial, ribosomal and ALU repeat clones, 15 clones, unique by BLAST database searches, were identified The majority of these clones show mitogen induced or activated expression profiles, are human in origin and map back to the YAC (1) Morgan, J G , et al (1992) Nucleic Acids Res 20 5173-5179

7.008

Human chromosome 5q13 harbours a novel conserved heavy metal transporter gene with a somatostatin receptor domain (SHMT1).

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The cDNA clone ck2 3 was isolated from a human colon expression library using a bacterial maltose-binding protein-specific antiserum This cDNA probe revealed specific hybridization signals on genomic DNA of all species tested including the slime mold Dictyostelium discoideum The human ck2 3 sequence was assigned to chromosome 5q13 by FISH Northern blot analyses on various cell lines identified mRNA isoforms of 3 8 and 4 7 kb T7-promoter-directed in vitro transcription/translation of the 50 kDa deduced open reading frame (ORF) of the ck2 3 cDNA gave rise to bands of higher m w , suggesting spontaneous formation of oligomeric molecular complexes Data base searches of the deduced ORF of ck2 3 revealed several regions with up to 76% homology at the aminoacid level to mitochondrial membrane proteins encoded by the yeast genes zrc1 (the zinc-cadmium resistance protein) and cot1 (the cobalt uptake protein) Conserved domains constitute two clusters of 4 and 2 transmembrane regions interrupted by stretches containing poly-histidine residues for the coordination of metal ions Additionally, a conserved peptide domain at the C-terminus is prone to intraluminal interactions with G-proteins In contrast to the yeast genes zrc1 and cot1 the polypeptide chain of ck2 3 contained a conserved somatostatin receptor type 2-homologous domain, which renders ck2 3 (SHMT1, i e somatostatin-dependent heavy metal transporter protein) likely to represent a constituent of somatostatin-regulated signal transduction pathways Funded by Wilhelm Sander Stiftung, 094 001Ba

7.009

Construction of a transcription map of the cri-du-chat critical region

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The cri-du-chat syndrome is a classic syndrome characterized by a deletion in the 5p, cytogenetically, and a typical cat-like cry and other symptoms, clinically Based on our collection of more than 100 patients with different deletions from various countries, we have narried down the critical region to 5p15 2, between loci D5S721 to D5S24 A high-density YAC contig was constructed with 18 DNA markers Now we are constructing a transcription map by screening for expressed sequences from YAC, BAC and cosmid clones in this region by means of both exon-trapping and direct cDNA selection 11 differenct cDNA libraries were used for cDNA selection The candidate cDNA fragments were analyzed by genomic origin, tissue-distribution and sequencing The range of insert size was around 0 3 - 1 0 kb These cDNA clones provided the resource for identification of the gene(s) responsible fro cro-du-chat syndrome (Supported by NSFC, DMC and UNESCO)

7.014

Functional analysis of the FACC Protein in vitro.

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In Fanconi anaemia (FA) the increased frequency of spontaneous chromosomal aberrations and hypersensitivity to DNA crosslinking agents have usually been interpreted as evidence for a defect in the repair of DNA lesions This certainly would explain the increased predisposition to malignancy in FA patients However, there are alternative explanations, such as the inefficient detoxification of oxygen radicals, a defect in regulation of the cell cycle or a mutation in a DNA processing enzyme not primarily involved in DNA repair Unfortunately, the molecular cloning of the first Fanconi anaemia gene, FAC, has so far been surprisingly little help in the elucidation of the basic defect in cells from these patients To further characterise the function of the FACC protein, we have constructed a full length fusion protein with glutathione-S-transferase which can be used to isolate the protein from E coli In a first series of experiments we have examined the ability of this fusion protein to bind to DNA in vitro A 500bp DNA fragment containing the Lac-operater sequence was used and DNA binding was monitored by two different mobility-shift analyses, one using polyacrylamide gels and one using high-resolution agarose gels The GST-FACC fusion protein, but not GST alone or bovine serum albumin, is clearly able to bind DNA in both these assays Furthermore, an artificial mutant, lacking the last third of the FACC sequence is not able to bind DNA When the DNA fragment was treated with psoralen and long wave UV light to create interstrand crosslinks and again used in mobility shift analyses There was no obvious difference in affinity of the fusion protein for DNA, and the crosslinked DNA could be efficiently displaced by native DNA Clearly if this binding is also relevant in vivo, it is not primarily involved in recognition of this type of DNA interstrand crosslink The results will be discussed with respect to the identification by others of DNA-binding motifs in the FACC sequence (Liebetrau et al , 1995, J Med Genet 32 669-670) and the demonstration of a cytoplasmic localisation for the FACC protein (Yamashita et al 1994, PNAS 91 6712-6716)

7.015

Human cystathionine β-synthase gene: genomic organization and evidence for at least two distant promoter regions.

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Cystathionine β-synthase (CBS) enzyme of the sulfur amino acid metabolism is encoded by a gene located in 21q22 3 It is overexpressed in Down's syndrome and its deficiency characterizes homocystinuria We have studied the cDNA isolated from adult liver mRNA and the gene isolated from a cosmid library The gene contains 17 exons with a rather complex 5' UT region In this region there are two exons 1(E1a and E1b) which are differentially used for CBS expression in various human tissues Moreover we have found that the CBS gene encodes for not only two mRNAs having in their 5'UT region either E1a or E1b, but also for a third mRNA having E1c Exons/introns boundaries sequences have been determinated and correspond to consensus gt-ag rules Preliminary results on CBS promoters will be discussed

7.016

Human embryonic mRNA expression maps by differential display.

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A comprehensive map of differentially expressed genes in early human development would be a powerful tool for the dissection of normal embryonic development with potential implications for the understanding of human congenital malformations and malignancies Differential display reverse transcribed polymerase chain reaction (DDRT-PCR) has emerged as a general method to identify and isolate genes that are differentially expressed In order to evaluate whether we can use DDRT-PCR for systematic characterisation of genes expressed in different tissues at various stages of human development, we have started assessment of the methodological validity and reproducibility By DDRT-PCR analysis of various tissues (i e liver, heart, adrenal gland, kidney, intestine, eyes and thoracic) from a single human foetus, reproducible apparently tissue specific fragments as well as fragments common to all tissues were observed 29 cDNA fragments was excised from the gel, sequenced and compared with known sequences to verify the identity of the tissue specific fragments and whether the common fragments represents 'housekeeping' genes

7.017

Mapping of chromosome 9 ESTs

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108 expressed sequences isolated from human skeletal muscle and brain cDNA libraries were previously assigned to chromosome 9 (Auffray et al, 1995, C R Acad Sci 318, 263-272) using a panel of monochromosomal hybrids So far, forty-three of these ESTs have been localised to one of four regions of chromosome 9 using additional somatic cell hybrids as part of attempts to add to the transcription map of chromosome 9 and to identify chromosome 9 disease genes, particularly TSC1, one of the genes involved in tuberous sclerosis Progress is as follows, Region and No ESTs shown, 9p 5, 9cen - 9q22 10, 9q22 - 9q34 1 (ABL) 18, 9q34 1(ABL) - 9qter 10 For selected ESTs cosmids were obtained by amplification of a chromosome 9 cosmid library (LL09NC01) in PCR pool format kindly provided by Dr Bart Janssen More precise mapping and integration into cosmid contigs will be presented

7.018

Isolation of human genes transcribed in yeast artificial chromosomes (YACs)

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The insertion of large segments of genomic DNA into YACs offers an invaluable tool for the analysis of specific chromosomal regions Overlapping YAC clones containing almost the entire human genome are now available, and major efforts are underway to characterise the transcribed sequences within these clones Several methods are currently being used for this purpose, such as cDNA selection, however, the task of finding expressed genes amongst the majority of non-coding regions is still quite arduous In order to isolate human transcripts from YAC clones we have established a procedure based on the expression of such genes in yeast As a first step, we have shown that human sequences are indeed transcribed in the host system The experimental approach we use involves the PCR amplification of known human genes contained within the human genomic insert Primer pairs are chosen which span different exons within the investigated gene such that the spliced molecules can be clearly distinguished from the larger PCR products representing DNA sequences For the isolation and characterisation of unknown genes from YAC clones, we are using the "differential display of mRNA" technique YACs with different, or overlapping human insert DNA, are compared in order to differentiate yeast and human transcripts Candidate PCR products, amplified from reverse transcribed mRNA, which appear to be differentially displayed amongst yeast cells with different YACs, are isolated After recovery from acrylamide gels, the DNA is reamplified, cloned and sequenced The human origin of the amplified bands is shown by hybridisation of the bands of interest to dot blots and pulse field gel blots The efficiency of the method will be discussed

7.020

Chromosomal localization of the human glucosidase I gene to 2p12-p13 by fluorescence in situ hybridization

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The processing of N-linked glycoproteins is initiated by the action of glucosidase I, which cleaves specifically the distal -1, 2 linked glucose residue in the Glc3-Man9-GlcNAc2 oligosaccharide precursor after its "en bloc" transfer from Dol-PP to the nascent polypeptide chain Glucosidase I has been purified from calf liver, pig liver and yeast A probe synthesized by MOPAC with pig liver firststrand-cDNA was used to screen a human hippocampus cDNA library, resulting in the isolation of several glucosidase I-specific clones and allowing the reconstruction of a full-length cDNA of 288 1 bp length The oligonucleotide sequence showed no homology to other processing enzymes cloned so far Furthermore, screening of a genomic human placenta library yielded two glucosidase I-specific clones which covered the entire coding sequence of the protein These clones were biotinylated as probes for fluorescence in situ hybridization on metaphase spreads prepared from human peripheral blood lymphocyte cultures treated with 5-bromodeoxyuridine (BrdU) Signals were detected immunohistochemically using fluorescein isothiocyanate conjugated to avidin Hybridization consistently gave positive signals on the short arm of chromosome 2 in the region of 2p12-p13

7.021
The study of homeobox gene expression in human diploid cell strains of embryonic and postnatal origin.

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Homeobox genes have been show to play essential role in embryonic development of all multicellular species including Homo sapience Using the RT-PCR method, we studied the expression of homeobox genes in three embryonic diploid strains derived from human first trimester embryos, and in two postnatal diploid strains derived from donor (aged 1 year and 28 years) as well as in cells of 7-years old patient with progeria All six cell strains were derived from skin biopsies of forearm The following intron spanning primer set was used

upstream-primer - 5'-CGGATCTACCCCTGGATGC-3'
downstream-primer - 5'-CGCCTGTTCTGGAACCAG-3'

Clones with the Bgl II restriction site were selected for sequencing mRNA transcripts were found for four homeobox genes HoxA7 (3 clones), HoxB4 (36 clones), HoxB5 (49 clones), HoxC6 (3 clones) in embryonic cells while only mRNAs for gene HoxB5 (43 clones) were presented in postnatal cells

7.022
Characterization of the expression profiles of human gene transcripts in muscle and brain by quantitative hybridization of high density CDNA arrays

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We have conducted an integrated approach for the analysis of human skeletal muscle and brain cDNA libraries by collecting sequence and mapping data and by characterizing the expression profiles of the corresponding gene transcripts We have developed an efficient technology for large scale and systematic analaysis of gene expression through the collection of hybridization signatures, allowing the identification of differentially expressed genes High density filters have been produced on which inserts of cDNA clones were spotted and the membranes were hybridized with radiolabeled complex probes based on reverse transcription of mRNA An image analysis software was adapted to automatically quantitate the hybridization signal for each spot We have applied this method to study the expression profiles of 1000 clones from the muscle library representing over 900 distinct gene transcripts Hybridizations with complex CDNA probes derived from various tissues provided differential expression profiles of the transcripts, allowing assessment of their tissue specificity Novel gene transcripts with ubiquitous or muscle-restricted patterns of expression were identified The methodology is being applied to study the expression profiles of 12 000 clones from a normalized infant brain cDNA library in order to determine the tissue-specificity of the corresponding genes

7.023
Analysis of eight CAG/CTG trinucleotide repeat-containing loci as candidate genes for several mental and neurological disorders.

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Abnormal expansion of genes with trinucleotide repeat polymorphisms has been found to be responsible for a number of neuropsychiatric disorders We investigated eight brain-expressed loci with trinucleotide tandem repeats, obtained from cDNA clonotecks (GeneBank dbEST) DNA samples from peripheral blood of schizophrenia (12 samples), essential tremor (8 samples), families spastic paraparesis (11 samples) patients, 26 control DNAs and 14 DNA samples from brain tissue of schizophrenia patients were analysed On the base of PCR-technique followed by polyacrylamide gel electrophoresis we examined all of these loci for allele polymorphism For 7 loci there were not found any polymorphisms for trinucleotide repeat area A total of 6 EST 04896 alleles (138-153 bp in length) were found A significant excess of homozygosity was observed in the DNA samples from brain tissues of schizophrenia patients in comparison with the DNAs from peripheral blood

7.024
Transcriptional mapping of human chromosome 16p12.1

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As part of a European Consortium contributing to a physical and transcriptional map of the short arm of chromosome 16, we are constructing a transcriptional map of human chromosome 16p12 1 This region at present consists of 125 cosmids, representing about 50-60% coverage of the region Our initial strategy involved identifying cosmids that contained CpG islands and isolating unique restriction fragments that do not hybridise to human placental DNA So far, 32/89 (35%) cosmids have been identified as containing CpG islands and 56 unique fragments have been isolated Computer-aided analysis on derived sequences is being used to predict exons and identify any sequence homologies Potential coding fragments are then used for hybridisation-based cDNA screening Fragments from one cosmid have identified sequences from three separate cDNAs, two that have no homology to any known genes in the databases (D16S3247E/D16S3248E) and the previously known protein kinase C beta gene (PRKCB1) Direct cDNA selection and Exon Trapping have been carried out on CpG island-containing cosmids and initial analysis of cloned libraries is underway We also aim to use shotgun sequencing on Fugu rubripes cosmids that contain known genes, to identify other genes that may map within close proximity

7.025
Structural organisation of the Batten disease gene CLN3

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Batten disease is a neurodegenerative disorder of childhood The disease gene, CLN3, has been isolated and the complete 1689 bp cDNA sequence described We have characterised the complete genomic structure of the CLN3 gene by a combination of sequencing of plasmid subclones of a cosmid containing the whole gene, and gap-filling by PCR The gene consists of 15 exons spanning approx 15 kb The exons range in size from 47 to 344 bp with all splice donor and acceptor sites conforming to the GT/AG rule, and introns range from 73 bp to 4 2 kb Determination of the site of transcription initiation by primer extension analysis revealed a major start site 170 bp upstream from the translation initiation site We have obtained 1 2 kb of sequence in the 5' flanking region The putative promoter contains a number of potential binding sites for transcription factors (such as Sp1 and AP-2 motifs) No TATA or CAAT box is apparent, consistent with features of a housekeeping gene We are currently performing comparative sequencing in mouse, chicken and the pufferfish Fugu

7.026
The PEX gene is mutated in nine patients with X-linked hypophosphatemic rickets

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The gene PEX is a candidate gene for the X-linked hypophosphatemic rickets (HYP), it codes for a protein with homologies to endopeptidases (Nature Genetics, 1995, 11, 130-136) We screened for mutations 9 of the 17 exons identified in the gene PEX in a population of 19 families We amplified exons, selecting primers in the intronic flanking sequences Using SSCP on different gels at different temperature, we identified 9 mutations From the 5' region to the 3' region of the published cDNA, we detected the following mutations In exon 3, one transversion changed the amino acid 74 from a cystein into a serine This mutation concerns the third conserved cystein in the CDNA sequence of the endopeptidases family In exon 4, one transition changed the amino acid 135 from a leucine into a proline One deletion of the exon 10 was detected We determined the intronic flanking sequences of exon 10 and 11, two additional SSCP variants were identified in exon 10, and one in exon 11 as well Three variants were detected by SSCP in exon 15 In conclusion, it can be assumed that mutations in the PEX gene actually account for the disease of X-linked hypophosphatemic rickets

7.027
Identification of two novel genes expressed in brain and localized at the WAGR locus.

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Identification of genes involved in brain development is an initial step toward understanding the mental retardation of WAGR syndrome (Wilms' tumor, Aniridia, Genito-urinary abnormalities, mental Retardation) We have described a genic map of chromosome 11 (1) based on eSTSs (expressed Sequence-Tagged Sites, 2) This genic map has been integrated with the cytogenetic, genetic and disease maps This integration reveals the presence of 2 eSTSs, distal to the brain-derived neurotrophic factor (BDNF) gene, within a region implicated in the mental retardation of WAGR patients (3) We describe the primary structure of the 2 corresponding transcripts and the analysis of their expression pattern The cDNA clones have been fully sequenced with the view of characterizing the coding sequence Sequence analysis reveals that these 2 cDNAs are incomplete at their 5' end and correspond to unknown genes Expression studies show that the corresponding mRNAs are 6 4 and 7 kb long and are prominently expressed in brain and fetal brain The satio-temporal study of expression and the cloning of complete cDNAs are underway (1) Rosier *et al*, Genome Res 5 60-70 (1995) (2) Houlgatte *et al*, Genome Res 5 272-304 (1995) (3) Fantes *et al*, Genomics 25 447-461 (1995)

Section 8: Genes with trinucleotide expansions and their proteins

8.001
FRAXA, FRAXE and linked microsatellite markers in a large population of boys with learning difficulties and their mothers.

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We are undertaking a population based survey of boys aged 5-18 with learning difficulties together with their mothers We are determining allele size at the FRAXA and FRAXE loci and four closely linked microsatellite markers The maternal X chromosome not inherited by the son serves as a control To date we have tested over 1000 boys and 700 mothers With respect to FRAXA we have found five full mutations, one premutation and thirty six intermediate alleles among the boys, and no full or premutations and fourteen intermediate alleles in the control chromosomes For FRAXE we have observed no full mutations, one premutation and ten intermediate alleles among the boys and no full or premutations and two intermediate alleles among the control chromosomes For both FRAXA and FRAXE we found marked linkage disequilibrium between allele size and haplotype of the closely linked markers Our results demonstrate that the frequency of FRAXA mutations may be very much less than formerly supposed, while FRAXE mutations must be very rare indeed

8.002
Long PCR-formatted protocol for myotonic dystrophy expansion detection

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The DM expansion varies from 50 to about 4000 CTG units, with about 98% of DM patients showing an expansion less than 2500 Southern blot analysis, after restriction enzyme genomic digestion (e g EcoRI, BglI and BamHI) is the method of choice for studying the DM mutation We defined a PCR formatted protocol involving the amplification of the CTG repeat in DM patients using DNA amplification, gel-electrophoresis and oligo-specific hybridization The PCR assay utilizes 10 units of AmpliTaq DNA polymerase and an equal number of units of Taq extender PCR additive in 30 cycles of reaction of 10s at 94°C, 30s at 62°C, 8 min at 70°C Using this protocol we were able to amplify up to 2500 CTGs starting from 15 pg of genomic DNA This method is specific, sensitive, rapid, and can be used for detecting very large DM alleles in single cells Work supported by Italian Telethon grant N 511

8.003
Molecular study of 40 fragile X families from the north of Spain.

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Objective Fragile X syndrome is caused by a mutation involving expansion of a CGG trinucleotide repeat in the FMR1 gene This study was undertaken in 1991, after the description of this mutation The objective was to determine the accuracy of direct molecular diagnosis in detection of index cases, female carriers and prenatal diagnosis *Patients* Seventeen known fragile X families previously studied cytogenetically and twenty-three families found, screening retarded males They are all living in the North of Spain although their origins are diverse *Methods* Hybridization with the StB12 3 probe (provided by Mandel) in EcoRI+EagI double digests blots Linkage analysis with the microsatellite DXS548 PCR analysis of the CGG repeat Detections were all accomplished with a chemiluminescent reaction, using a modification of the DIG system protocols from Boeringer-Mannheim *Results* A total of 242 individuals at risk were analyzed,including 60 affected males, 46 full-mutated women, 52 premutated women, 3 normal transmitting males and 81 unaffected Six prenatal diagnosis were performed, of wich four predicted affected foetus and pregnancies were terminated *Conclusions* Molecular diagnosis of the FMR1 CGG repeat in conjunction with cytogenetic and clinical analysis, are the most important tools to detect and prevent mentally retarded individuals Supported in part by spanish grant (FIS number 94/0857)

8.004
Western blot analysis of the FMR1 protein (FMRP) and single stranded conformational polymorphism (SSCP) analysis of the FMR1 gene, in mentally retarded males with the lack of trinucleotide expansion in the FMR1 gene.

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The fragile X syndrome is one of the most common forms of inherited mental retardation It is usually caused by an expansion of a trinucleotide repeat (CGG) in the 5'UTR of FMR1 However, a few patients with the clinical phenotype of fragile X syndrome, have been found, who have deletions or point mutations in their FMR1 genes As naturally occurring mutations in the FMR1 gene could be very informative in the elucidation of the function of FMRP, we wanted to search for mutations in the FMR1 gene in mentally retarded males without the trinucleotide expansion in the FMR1 gene We have analyzed FMRP expression in transformed lymphocytes from four patients with mental retardation and macroorchidism, by western blot analysis using an anti-FMR1 antibody (J -L Mandel) In contrast to transformed lymphocytes from classical fragile X patients which did not show expression of (or very low levels of) FMRP, the expression pattern of all four patients was similar to the wild type control We have also analyzed FMR1 by SSCP We have investigated exon 5 from 40 patients and found a shift in mobility in three of them Further investigations of these three patients will be done and reported

8.005
Low percent mosaicism for normal size alleles in a fully mutated Fragile X fetus

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We report evidence of low percent mosaicism for normal alleles in a Fragile X male fetus A false negative result was initially obtained with a Pfu PCR assay showing a faint band of normal size after amplification of amniotic cell DNA, while linkage analysis indicated that the fetus carried the maternal Fragile X chromosome More DNA was obtained with cordocentesis from fetal blood for a conventional Southern blotting which demonstrated a completely methylated full mutation (D=1 4-2 6 kb) with no detectable normal size alleles An additional radioactive PCR was then performed allowing accurate detection and sizing of normal alleles of the CGG repeat at the FMR1 gene 5' end The premutated mother shows a normal allele of 20 repeats, while the fetus displays a whole set of alleles ranging from 18 to 55 repeats with some extra bands of approx 80-90 repeats, but the absence of any signal at 20 repeats rules out maternal contamination Thus, CGG repeat instability seems able to generate minor cell lineages with reduced triplet number down to the normal range and this also underlines the importance of Southern analysis for prenatal diagnosis in Fragile X families

8.006

Analysis of Germline Variation at the FMR1 CGG Repeat Shows Variation on the Normal-Premutated Borderline Range.

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In order to characterize the dynamics of CGG repeat instability at the fragile X syndrome locus (FMR1 gene), we have used small pool PCR to estimate the mutation rate within germline (sperm) and somatic tissue (leukocytes) of two normal males, one carrying the most common 29 CGG repeats allele, the other carrying a borderline normal-premutated allele of 55 repeats Large contractions and moderate expansions of the repeat were evidenced in sperm and blood for the 55 repeats allele while almost no variation was evidenced in sperm or blood with the 29 repeats allele Somatic blood DNA exhibited fewer expansions and contractions than sperm Contractions were more frequent than expansions and all the expansions were found in the +4 to +10 repeats range, while most of the contractions were found in the -10 to -30 range, suggesting that a subset of contractions and expansions results from distinct mechanisms These results also suggest that the dynamics of the CGG repeat could be partly due to germline instability within the high normal or premutated ranges

8.007

Regression or gene conversion of an amplified to a normal CTG repeat in myotonic dystrophy.

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One of the peculiar features of myotonic dystrophy (DM) is anticipation, which is caused by the intergenerational size increase of an amplified CTG repeat in the 3' untranslated region of the DM gene Only in a minority of cases intergenerational contraction of the CTG repeat has been observed, and this almost exclusively in paternal transmissions We report here the transition of an amplified repeat of 105 CTG triplets in a mildly affected father to a normally sized repeat of 5 CTGs in his son, who is asymptomatic at the age of 11 years The son inherited the disease-associated paternal haplotype with informative markers within 200 kb at both the proximal and distal side of DM None of the intragenic markers, however, was informative As the father's wild type DM allele also has 5 CTG repeats, both gene conversion and repeat regression are possible mechanisms to explain the observed transition of 150 to 5 repeats in this family

8.008

Differences in the characteristics of soluble and membrane bound forms of huntingtin

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Huntington's disease is an autosomal dominant neurodegenerative disease associated with an expansion of a (CAG)n repeat The repeat gives rise to a polyglutamine stretch close to the N-terminus of a 350kDa protein, huntingtin, which has no known function and few homologies We have been investigating huntingtin with the aim of elucidating its normal and pathological function We have previously found that huntingtin extracted from mammalian brain exists in both a soluble and membrane-bound form and that the relative distribution of the two forms is species dependent On non-denaturing-PAGE the soluble form of huntingtin migrates with an Mr of 300-400kDa The membrane-bound form can be solubilised using Triton X-100 and migrates on non-denaturing-PAGE with an Mr of 700-800kDa indicating that huntingtin exists as a dimer or part of a larger complex when membrane-bound Solubilising synaptosomal membrane pellets in a range of Triton X-100 concentrations from 0 1-1% indicates gradual solubilisation of huntingtin in a large complex from the membranes, starting at 0 4% Triton X-100, with the complex becoming smaller at increasing Triton X-100 concentrations until by 0 9% Triton X-100 the 800kDa complex is left which does not undergo further molecular weight reductions The characteristics of these complexes are being investigated

8.010

Molecular analysis and clinical evaluation of a large italian family expressing the FRAXE fragile site.

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Expression of the folate-sensitive fragile site FRAXE, 600 Kb distal to FRAXA, seems to be associated with mild mental impairment, even if published reports concerning the involvment of FRAXE in mental handicap are still controversial We studied an 8 yr-old child, displaying mild mental retardation and learning disabilities A preliminary cytogenetic analysis performed in order to detect the common fragile site in Xq27 3 showed expression of the fragile site in 17% of the metaphases However molecular analysis of FMR1 gene did not evidence any amplification in the CGG trinucleotide region, ruling out that the proband carried the fragile X syndrome In order to establish whether or not trinucleotide repeat expansion at the FRAXE locus might be responsible for the expression of the fragile site at Xq27 3, molecular analysis was carried out by Southern-blot with OxE 20 probe (kindly provided by Dr K Davies) and PCR by using primers flanking the CGG repeat region Both these tests confirmed the amplification of FRAXE locus DNA analysis was then extended to ten other family members and allowed the identification of .i) two male cousins showing mild mental handicap, one with full mutated allele and the other with a premutation/full mutation pattern, ii) an apparently normal male cousin with a premutated allele, iii) a full mutated allele in the apparently normal proband's mother and in two proband's maternal aunts Results so far obtained by molecular analysis are consistent with the clinical evaluation on the different family members and supports the hypotesis that amplification of the CGG repeats at the FRAXE locus may be involved in mild mental retardation

8.011

DNA analysis of patients with apparently sporadic Huntintgon's disease (HD).

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The diagnosis of HD is uncertain in the absence of a positive family history ("sporadic cases"), particularly when atypical features are present We analysed IT15 gene in 88 patients Among them we selected 25 patients with a negative (both parents living and healthy or died without neurological and psychiatric disorders over the age of 65) or a doubtful family history (one or both parents death in young age, a vague family history of neurological or psychiatric disorders or suicide) Clinically, patients were classified as having 1 clinically probable (progressive chorea with dementia) or 2 doubtful HD (incomplete or atypical clinical picture) All the patients underwent neuroimaging studies and neuropsychological testing An abnormal number of repeats (42 to 85) was found in 14 patients Mutation analysis confirmed the diagnosis of HD in 63 6 % of patients with clinically probable HD and in 43 7 % of patients with clinically doubtful HD DNA analysis confirmed the diagnosis even in patients with very atypical clinical features (such as unilateral or focal distribution of choreic dyskinesias, sudden onset, additional neurological dysfunction), while it allowed to exclude the HD diagnosis in patients with typical clinical manifestations suggestive of HD

8.012

Microdeletion of 486 bp involving the CGG repeat of the FMR1 gene caused by an intra-repeat loop mechanism due to misalignment of GTT tandem repeats at chi-like elements at both breakpoints in mosaic with a full mutation

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Nearly all patients manifesting fragile X syndrome demonstrate methylation and large expansion of the CGG repeat in the 5' UTR of exon 1 of the FMR1 gene Only singular cases have been reported to be due to large deletions Due to the size of the deleted contig, fine mapping of the breakpoints is lacking and so far a mechanism responsible for the deletion has not been identified Recently, clustering of the 5' breakpoints of five microdeletions proximal to the trinucleotide repeat has been observed However, the underlying mechanism of these deletions is still unknown We identified a fragile X patient mosaic for a full mutation and a microdeletion The microdeletion spans 486 bp involving 168 bp upstream from the CGG repeat, the entire CGG repeat, exon 1 and 138 bp of the first intron In contrast to previous reports, the 5' breakpoint does not fall into the hotspot region The proximal breakpoint 5' GTGGTT/T 3' as well as the distal breakpoint 5' GTTGTT/GG 3' can be characterized as chi-like elements and are flanked by direct tandem repeats Strikingly, 35 bases out of 90 bases downstream the proximal breakpoint are complementary to 35 bases out of 59 bases upstream the distal breakpoint favoring formation of a stem loop by intrastrand base pairing Furthermore palindromes are found stabilizing the stem loop by formation of four secondary loops Subsequent excision of the stem loop during replication could lead to the deletion Mosaicism of full mutation and microdeletion in the DNA of the patient's leukocytes indicates the mitotic origin of the microdeletion Since the EagI restriction site in the 5' flanking region is not deleted and the microdeletion has been demonstrated to be unmethylated (EcoRI/EagI restriction, probe StB12 3), it can be concluded that the deletion is not derived from the methylated full mutation but from an unmethylated premutational allele We postulate the microdeletion to be generated by an intra-repeat loop mechanism due to slipped mispairing at GTT repeats at chi elements flanking both breakpoints This is the first report providing molecular data and sequence analyses elucidating a possible mechanism of a mitotic microdeletion of the FMR1 gene

8.013

Deletions within the FMR 1 gene.

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Fragile X syndrome is the most frequent heritable cause of neurodevelopmental disability, caused by inactivation of the FMR 1 gene Inactivation is caused by expansion of a CGG repeat in the 5' untranslated portion of the FMR 1 transcript In regard to the CGG unstable triplets at the FMR 1 locus, individuals are divided into three groups according to the number of repeats 6-50 - normal, 52-200 premutation, >200 - full mutation Only several patients have been described with deletions involving the FMR 1 gene The deletion size ranged from 1,6 kb to 9 Mb We present here two families with small deletions within FMR 1 gene Family 1 A 4 year old male with typical features of the syndrome was subjected to cytogenetic studies which revealed a normal male karyotype with no evidence of fragile X expression DNA was isolated from peripheral blood by conventional methods Following digestion with EcoRI and EagI, Southern blot analysis with probe Ox1 9 revealed abnormally light fragment about 2 0 kb in contrast to the normal 2,8 kb fragment There was no evidence of abnormal methylation The same analysis were carry out on DNA from great-grandmother, grandmother, mother and mentally retarded uncle who has shown expression of the fragile X in 16% of cells The full mutation was revealed in all female relatives, but the uncle had a smaller deletion (2 2 kb fragment) than the proband Family 2 Proband with features of the syndrome revealed a male karyotype with r(22) Molecular analysis have shown premutation in mother and deletion in patient (2 4 kb fragment) These findings confirm the instability of the expanded repeats which can be excised and cause deletions within the gene This work was supported in part by the Russian Human Genom Project,

8.014

Single-strand conformation polymorphism (SSCP) analysis of the FMR1 gene.

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Fragile X syndrome is the main cause of familial mental retardation This disease is caused in 95% of cases by a CGG expansion in the 5' untranslated portion of the FMR1 gene The remaining 5% include other molecular defects affecting this gene Several deletions of FMR1 and flanking sequence have been reported Moreover, three different point mutations within the gene resulting in fragile X phenotype have been described to date SSCP analysis of exons 2 to 17 of FMR1 was performed in a selected sample of 31 male patients without the CGG expansion The selection was based on the Hagerman's clinical checklist, where these patients scored 16 or higher. The SSCP analysis was performed using a non-radioactive detection technique (silver staining) and polyacrylamide gel electrophoresis Anomalous conformation migrations were observed in exons 2, 5, 8, 10, 11, 15 and 17, and PCR products were sequenced in order to identify the underlying base changes A G→A synonymous mutation in exon 5 was detected in two unrelated patients and an intronic CA duplication in exon 15 was detected in four patients and in 10% of screened control samples The rest of anomalous migrations are currently undergoing characterization (Supported by FISS 93/0004-02)

8.015

Mutation and activation patterns in female carriers of fragile-X full mutation

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Direct detection of the fragile X mutation supposes a great advance in diagnosis of this syndrome However, predictive prognosis of phenotype of heterozygous full-mutation carriers remains unsolved A controversy has recently arisen on the correlation or not of the repeat size with intellectual function in females, as previously determined for the activation status of the FMR-1 gene Here we show our results with a sample of 27 female carriers of full mutation Mutation usually occurs as a set of distinct bands in each individual, where sizes were carefully determined previous Pst I digestion of the DNA samples Densitometric analysis of the lanes was used in order to estimate the relative proportion with which appears each increment In addition, the activation patterns of normal X chromosomes were determined from Eco RI + Eag I Southern blots We could confirm a slight decrease of the averaged increment with age However, we were not able to find any correlation between intellectual handicap and averaged size, intra-individual variability of the increments or activation status So, our conclusion is that current methods have no prognostical use for female carriers of full mutation

8.016

The genotype-phenotype relationship in individuals with 30 to 40 (CAG)n repeats in the Huntington's disease gene.

Rubinstein, David C[1], Leggo J, Coles R[1], Curtis D, Cassiman JJ, Taylor R, Almqvist E, Hayden MR, Harper PS, MacMillan JC, Dode C, Kaplan J-C, Chotai K, Krause A, Temlett JA, Curtis A, Jackson A, Novelletto A, Whittaker JL, Differ A-M, Biancalana V, Mandel J-L, Graham C, Morrison P, Connarty M, Ross CA, Sherr M, Losekoot M, Simpson SA, Dodge A, Craufurd D

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Abnormal (CAG)n expansions in the IT-15 gene are associated with Huntington's disease Most large analyses that defined the limits of the normal and pathological size range employed PCR assays which included the (CAG)n repeats and a (CCG)n tract which was thought to be invariant Many of these experiments found an overlap between the normal and disease size ranges Subsequent findings that the (CCG)n tract varies by at least 5 repeats suggested that the limits of the normal and disease size ranges should be re-examined using assays that excluded this polymorphism Since patients and unaffected individuals with (CAG)n lengths close to the overlap range are rare, a consortium was assembled to provide DNA from 177 normal and affected individuals with $(CAG)_{30-40}$ All samples were reanalysed in Cambridge using an assay specific for the (CAG)n repeats Six asymptomatic individuals aged 78, 79, 87, 90, 90 and 95 had 36 or more repeats and six patients with Huntington's disease had 36 repeats The case details of two exceptional cases from this series will be presented a 95 year old asymptomatic man with a (CAG)n repeat length in the pathological range and a 55 year old woman with HD-like symptomes but 34 (CAG)n repeats

8.017

Different size distributions of CAG repeats in sporadic and familial Huntington's disease and transmission studies for repeat sizes between 36 and 40 CAG repeats suggest a stepwise origin of HD in Scotland.

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Using primers specific for the CAG repeat in the IT15 gene we have typed 617 HD chromosomes from 297 HD families and 2418 normal chromosomes Confirmation of diagnosis has been requested in 121 individuals from definite HD families with expansions present in two generations (familial) and 121 cases with no proven family history (sporadics) A repeat size in the affected range (36-96 CAG) was seen in 119 of the familial and 65 sporadic cases When CAG repeat size distributions for the confirmed sporadic HD and familial groups were compared, a significant excess of repeat sizes in the 36 - 40 range was seen in the sporadics (p=0 00005) Data for CAG repeat size variation on transmission were obtained In the normal range [9 - 35], we observed 3 changes on transmission from 449 meioses, all in the high normal range [33 - 35] For 133 transmissions from 93 HD patients (56 paternal, 77 maternal) we saw 83 size changes Only 23% of HD chromosomes showed an increase of greater than one CAG repeat We found a particularly striking 5 generation family with individuals separated by up to 9 meioses and all affected members had repeats in the range 36 - 40 Unlike others, in Scotland we see relatively few dramatic increases even within the affected range We suggest that in our population HD arises predominantly by gradual incremental mutation of alleles in the high normal range rather than dramatic increases

8.018

Comparison of the myotonic dystrophy(MD) associated CTG repeat in five different population of former USSR.

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MD is associated with an increased number of CTG repeats in the 3' nontranslated region of the myotonic protein kinase gene (MP-1)(19q 13 3) CTG repeats in normal subjects are highly variable, with the copy number ranging from 5 to 37 Pattern of repeats alleles was studied by PCR in the five populations of former USSR (Russian, Moldavian, Uzbek, Georgian and Azerbaijanian) Heterozygote frequencies were 88%, 87%, 87%, 86%, 72% respectively) The frequencies of the CTG allelles in studied populations were compared with published data Own modification of the method X2-like statistics originally described by C Hirotsu was applied Method for grouping rows (copies of the repeat) and columns (populations) into homogeneous subgroups was used for the statistical analysis Frequencies of the CTG repeats in Russian, Azerbaijanian, Uzbek and Georgian populations have no statistical significant difference and could be treated altogether as homogeneous subgroup (RuAzUzGe)

All squared distances among populations become statistically highly significant as shown in the Table

	RuAzUzGe	Japanese	Negroid	Moldavian	European
RuAzUzGe	0	42 5	50 9	56 9	71 9
Japanese		0	59 2	54 2	83 6
Negroid			0	68.2	96 5
Moldavian				0	83 4
European					0

Critical values of the Wishart distribution W (4,6,0 001) = 28 3

8.019

The clinical and molecular spectrum of spinocerebellar ataxia 3 and Machado-Joseph disease

Stevanin, Giovanni[1], Cancel, G[1], Didierjean, O[1], Abbas, N[1], Benomar, A[2], Julien, J[3], Serdaru, M[1], Agid , Y[1], Brice, A[1], Durr, A[1]

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Purpose Evaluation of instability of the CAG expansion and correlation with clinical variability *Methods* 173 patients with autosomal dominant cerebellar ataxias (ADCA) or related disorders were screened for the SCA3/MJD mutation *Results* 126 patients from 34 families carried an expanded CAG repeat in the MJD1 gene There was a combined effect of the number of CAG repeats of the expanded and the normal allele on age at onset, which accounted for 70% of its variability The length of the CAG repeat influenced the clinical presentation Small expansions were associated with abolished reflexes, peripheral neuropathy in the lower limbs, and decreased vibration sense Large expansions were correlated with pyramidal signs The frequency of other signs such as supranuclear ophthalmoplegia, amyotrophy or sphincter/swallowing difficulties were determined by disease duration and not by the size of the CAG expansion Transmission of the expanded CAG repeat was unstable with variations ranging from -8 to +5 The mean expansion was larger in paternally than in maternally transmitted alleles Analysis of sperm DNA revealed marked gonadal mosaicism with larger as well as smaller expanded alleles than in blood DNA *Conclusion* SCA3/MJD is the most frequent unstable mutation responsible for ADCA type I The CAG expansion is not the only factor which influences onset and clinical presentation

8.020

Linkage disequilibrium analysis of MJD1 closely linked markers in Machado-Joseph disease patients of different ethnic origins

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Machado-Joseph disease (MJD) is an autosomal dominant spinocerebellar degeneration originally described in families of Azorean-Portuguese ancestry We conducted a study of linkage disequilibrium on 118 individuals, from 66 kindreds of different origins, to test the hypothesis that its present world distribution resulted from the spread of an original founder mutation We detected association between the MJD locus with marker alleles at loci D14S291, D14S280, D14S1050 and D14S81 *All patients, except one Chinese family, shared allele 3 (237 bp) at* D14S280 Specific haplotypes were found in patients originating from either Flores or São Miguel, the two Azorean islands with the highest disease prevalence Our present results suggest a founder effect in these MJD families, though we cannot yet rule out completely that more than one founder mutation are segregating with the same D14S280 allele

8.021

Cataract patients as potential carriers of myotonic dystrophy mutations

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Cataract is a part of the phenotypic expression of the mutations in the myotonic dystrophy (DM) gene, and might be the only clinical symptom in patients with minimal type of DM. In order to investigate if patients with cataract might present a potential pool of premutations in the DM gene, we conducted a study in the Istria region, Croatia Previously, the high prevalence of DM (27/100000) was found in this region A group of 48 unrelated cataract patients with no obvious precipitating factor and a control group of 48 unrelated age matched individuals from the same region without cataract were screened for CTG amplification by the PCR method. In the cataract patients group we identified three mutations (6 3 %), while no mutation was found in the control group On the basis of our study of population with a high incidence of DM, and considering a high prevalence of the cataract in the general population, we conclude, that patients with cataract might present a potential reservoir of the DM mutations

8.022

Polyglutamine expansion as a common pathological epitope detected in Huntington's disease, in Spinocerebellar ataxia 1, 2 and 3 and in Autosomal dominant cerebellar ataxia type II.

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Polyglutamine expansion in proteins is responsible for five human neurodegenerative disorders Spino-Bulbar Muscular Atrophy (SBMA), Huntington's disease (HD), Spinocerebellar Ataxias type 1 (SCA1) and 3 (SCA3) and DentatoRubral-PallidoLuysian Atrophy The target proteins become pathological when their polyglutamine stretch (containing typically between 10 and 35 gIns) is expanded over 37 gIns Expression of these proteins is not restricted to the affected tissue and the mechanism whereby the expanded polyglutamine-containing proteins causes selective neuronal death has not yet been elucidated Here we show that a monoclonal antibody is able to recognize the expanded polyglutamine-containing proteins in HD, SCA1 and SCA3 patients, while the normal proteins are not detected. For the pathological proteins, the signal intensity increases with the length of the expanded polyglutamine Our results strongly suggest that the antibody detects a unique conformation which requires a minimun length of polyglutamine Using this mAb, we also detect specific pathological proteins, expected to contain polyglutamine expansion, in Autosomal Dominant Cerebellar Ataxia type II (ADCA II) and in SCA2 This antibody is a remarkable tool for understanding the pathogenesis of known glutamine-repeat diseases, and for characterizing and cloning new proteins/genes involved in other such disorders

8.023

Methylation of the CGG repeat and expression of FMRP

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The fragile X syndrome is caused by an expansion of the CGG repeat in the 5' UTR of the FMR1 gene Expansion of the repeat above 200 repeat units induces methylation of the CpG island, which results in the lack of transcription We described a male fragile X patient with a full mutation in 99% of the cells One percent showed a premutation of 160 repeat units The lung tumor of this patient showed a premutation, which is most likely derived from a lung cell containing a premutation Surprisingly, although the EagI and BssHII sites were methylated, FMRP was detected in the tumor Methylation of both restriction sites has thus far resulted in a 100% correlation with the lack of FMRP, but these results suggested *that the CpGs in these restriction sites are not essential for regulation of FMRP* We present data about methylation of restriction sites during different stages of the fetal development Villi and fetal tissues were studied and the correlation between methylation of the CGG repeat and expression of FMRP was determined

8.024

A fragile X program in The Netherlands: implications of screening for the fragile X syndrome among 3559 mentally retarded individuals.

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A DNA-diagnostic program for the fragile X syndrome was introduced in several regional institutions and special schools for mentally handicapped individuals (n=3559) For 1160 individuals (33%) a diagnosis was available After oral and written information 70% of the parents/guardians gave consent for testing in 1679 individuals with mental retardation of unknown cause A brief physical examination was done and a bloodsample was taken for FMR1 gene analysis Besides 33 already diagnosed fragile X patients, 11 patients were newly diagnosed suggesting that a quarter of the fragile X patients are not yet diagnosed All newly diagnosed patients (n=11) were in a "high risk phenotype" group except for one female patient Evaluation of the phenotype apparently improves selection of individuals for FMR1 studies Attitudes and expectations were studied in 1090 consenting parents/guardians through pre- and post-test questionnaires Parents/guardians of newly diagnosed patients were interviewed Also 435 non-consenting parents/guardians were evaluated for their motives The acceptance of introducing new diagnostic techniques is high both among parents and institutional/school staff The consenting parents would opt for further investigations after exclusion of the fragile X syndrome, nonconsenting parents were reluctant to learn more about the cause of the mental handicap in their family

8.025

Genetic differentiation of autosomal dominant cerebellar ataxias by direct DNA analysis.

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Autosomal dominant cerebellar ataxias (ADCAs) are a clinically and genetically heterogeneous group of neurodegenerative disorders ADCA type I combines late-onset cerebellar ataxia associated with ophthalmoplegia, optic atrophy, dementia and extrapyramidal signs It is known five main subtypes of the ADCA type I Other type is cerebellar ataxia associated with pigmentary retinopathy (ADCA type II) There are many difficulties to distinguish the subgroups in ADCA type I patients using clinical features Mapping of six ADCA loci on chromosomes 6p23-24, 12q23-26 1, 14q32 1, 16q24-ter, 11cen, and 3p12-21 1 may improve differentiation of this disorder In tow subtypes of spinocerebellar ataxia (SCA1 and SCA3) the mutations consist of CAG repeat expansion We performed the screening of numerous sporadic ADCA cases for the search of the expanded alleles SCA1 (6p23-24) was diagnosed in 37% ADCA patients Using direct DNA analysis we did not find SCA3 (14q32 1) in the examined Russian patients

8.027

The clinical and molecular spectrum of spinocerebellar ataxia 3 and Machado-Joseph disease

Stevanin, Giovanni[1], Cancel, G[1], Didierjean, O[1], Abbas, N[1], Benomar, A[2], Julien, J[3], Serdaru, M[1], Agid , Y[1], Brice, A[1], Durr, A[1]
[1]Fédération de Neurologie and INSERM U289, Hôpital de la Salpêtrière, Paris, France [2]Hôpital des Spécialités, Rabat, Morocco [3]Hôpital de Haut Lévêque, Bordeaux, France

Purpose Evaluation of instability of the CAG expansion and correlation with clinical variability Methods 173 patients with autosomal dominant cerebellar ataxias (ADCA) or related disorders were screened for the SCA3/MJD mutation Results 126 patients from 34 families carried an expanded CAG repeat in the MJD1 gene There was a combined effect of the number of CAG repeats of the expanded and the normal allele on age at onset, which accounted for 70% of its variability The length of the CAG repeat influenced the clinical presentation Small expansions were associated with abolished reflexes, peripheral neuropathy in the lower limbs, and decreased vibration sense Large expansions were correlated with pyramidal signs The frequency of other signs such as supranuclear ophthalmoplegia, amyotrophy or sphincter/swallowing difficulties were determined by disease duration and not by the size of the CAG expansion Transmission of the expanded CAG repeat was unstable with variations ranging from -8 to +5 The mean expansion was larger in paternally than in maternally transmitted alleles Analysis of sperm DNA revealed marked gonadal mosaicism with larger as well as smaller expanded alleles than in blood DNA Conclusion SCA3/MJD is the most frequent unstable mutation responsible forADCA type I The CAG expansion is not the only factor which influences onset and clinical presentation

8.028

Trinucleotide repeat sequences within the transcription unit of a gene act as cis regulatory element and cause transcriptional repression

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To investigate whether triplet repeat sequences can act as cis regulatory element and cause transcriptional repression in addition to the proposed polyglutamine mediated disruption of gene function, we have constructed a series of plasmid each carrying different length of (CAG/CTG)n sequences in the N-terminal region of a reporter gene and the clones were designed in such a way that either a polyserine or polyalanine tract is incorporated instead of polyglutamine to rule out the possibility of glutamine tract mediated inactivation of protein function The expression of the reporter gene E coli decreases with the increase of (CAG/CTG)n repeat length However, insertion of a 126 bp DNA fragment containing 12 CTG repeats at the same locus in pBluescriptIISK+ had no effect on gene expression in vivo indicating cis regulation by expanded triplet repeat sequences RNA analysis shows that CAG/CTG sequences when present within the transcribed region of a gene can act in cis and affect transcription elongation in vivo Here we also report studies on plasmids which arose from clones containing 60 or more CTG repeat units in-frame and in the N-terminal region of the reporter gene in plasmid pBluescriptIISK+ as a result of spontaneous deletion and point mutations when propagated in E coli for several generations These mutations result in the generation of variable lengths of triplet repeat sequences The occurrence of point mutations within the triplet repeat length and the stabilization of the triplet repeat length at a critical threshold appear to be correlated We observe that the deletions always occur in multiples of the triplet repeat and the point mutations are always CTG to CTT transversions These mutations do not change the reading frame of the gene and also leave the flanking sequences unaltered The micro variability in triplet repeat length apparently finetunes the gene expression in E coli The formation of putative hairpin/slippage structure by long CTG/CAG repeat sequences and its implication for transcriptional regulation are discussed here

Clinical Genetics

09.001
Probability of finding an allele identical by descent in relatives with an autosomal recessive disease

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Although patients with autosomal recessive diseases most often do not have affected relatives outside their own sibship, sometimes families are encountered with affected relatives other than sibs (e g first cousins) Intuitively one assumes that these affected relatives will share at least one mutation identical by descent, but it turns out that this is not always the case (see for instance Park et al J Med Genet 1995, 32. 401-402) The question therefore arises how frequently relatives other than sibs share one mutation as a result of descent from a common ancestor This can be computed by using the formula $Dq/(Dq+(1-D)q^2)$ or $D/(D+(1-D)q)$ In this formula D is the probability that the allele passed on by one of the related parents to his affected child is the same (identity by descent) as the allele passed on by the other related parent

Table Probability of finding an allele identical by descent in relatives with an autosomal recessive disease

Parents are	Frequency of mutant alleles (q)			
	0 01	0 02	0 03	0 04
Sibs (D=0 25)	0 971	0 943	0 917	0 893
First cousins (D=0 0625)	0 870	0 769	0 690	0 625
Second cousins (D=0 015625)	0 613	0 442	0 346	0 284

9.002
Characterization of 83.5% of CF alleles in Greek CF patients and phenotype of novel mutations.

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To determine the type and frequency of Cystic Fibrosis (CF) mutations in Greek population, 236 patients were screened by DGGE analysis DNA samples showing a shift in mobility were sequenced Nine mutations accounted for 71 8% of the CF alleles in Greek patients, including AF508 with a frequency of 53 6% and a novel mutation E822X (1 05%) located in exon 13 (a C>T nucleotide change) resulting in the substitution of Glu for STOP at codon 822 Another 30 mutations accounted for another 11 70% including 2 novel mutations one identified in intron 14a (2752-26A>G), constituting a splicing defect with creation of an alternative cryptic acceptor splice site and one identified in exon 6a (Y247X), a STOP codon mutation probably resulting in a truncated protein

The phenotype of the patients with the novel mutations are shown in the table

Genotype	sex	Cl^-conc mmol/l	PI/PS	FVC%	FEV%
Y247X/AF508	M	81 2	PI	45	39
2752-26A>G	M	43-56	PS	-	-
E822X/3272-26A>G	F	92	PS	98	92
E822X/621+1G>Ô	M	100	PI	-	-
E822X/?	F	104	PI	59	61
E822X/AF508	M	136	PI	68	76
E822X/ R1070Q	M	97 5	P	-	-

9.004
An association of epidermolysis bullosa hereditaria simplex and muscular dystrophy in a woman.

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We report a case of a 25 year old female patient presenting with epidermolysis bullosa simplex in asso- ciation with muscular dystrophy No other member of the woman's kindred is known to have had either epidermolysis bullosa (EB) or muscular dystrophy, or a combination of the two There are three previous reports of other kindreds with such an association of diseases with autosomal recessive inheritance. It is suggested that the observed disorder is actually a new, distinct form of hereditary disease combining epidermolysis bullosa with muscular dystrophy and caused by a single gene inherited in an autosomal recessive fashion

9.005
Genofond of Central Asian region: current status and trends of development

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Republic of Tuva is a reserved region in a geographic center of Asia, feably connected with other regions of Russia Tuva is a typical arid country with population of 300000 We performed the complex study of Tuva population including epidemiology of inhererted diseases and congenital malformations, gene frequencies characterisation of erythroid and serum proteins, RFLP analysis of nuclear and mitochondrial genomes, estimation of intensivity of natural selection, inbreeding and migration. It was revealed higher values of inbreeding comparing with other Siberian populations, extended reproduction with high childhood mortality, relatively high Crow index value (I=0 70) caused by the prevalence of differential fertility component (If=0 43) The main factor of population genetic parameters dynamics is, probably, the ethnic consolidation of Tuvinians on backgroung of continuous emmigration of Russians from Tuva These processes may cause the change the spectrum of inhererted diseases in studied population

9.006
"Hygiene of marriage" concept (to the 130th anniversary of publishing "Improvement and degeneration of human race" by Vassily Florinsky)

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Development of genetics as a scientific discipline starts from 1865 when two paradigms were formed - biometry and mendelism This year in Russia V Florinsky put forward a "hygiene of marriage" concept, which had a strong eugenic intention Essence of this concept is clear from the contents of his book (I) Changeability of the human type, (II) Heredity as a main reason for changing and impruving a man, (III) Conditions promiting the changeability of the human type (taste and demand for the known qualities, influence of the outward living conditions, rational marriage, influence of blood marriage) This book and name of its author are unknown for the genetisists and science historians in the West In Russia ideas of V Florinsky considered as a source of medical genetics Florinsky presented vast material on epidemiology of inhererted diseases and congenital malformations in ethnic groups with different inbreeding levels and formulated physiological laws of inheritance Florinsky hygiene of marriage and Galton's eugenics were born in the same year and dealt with the same subject - impruving of the human race - or, in modern terms, genetic counceilling

9.007
DNA polymorphisms associated with coronary artery disease and serum lipid levels

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The purpose of this study was to identify DNA markers associated with coronary artery disease (CAD) and/or serum lipid levels in Russian population Polymorphic loci within genes involved in lipid metabolism were analysed using polymerase chain reaction Xba1 (X), Msp1 (M) and EcoR1 (E) restriction fragments length polymorphisms (RFLPs) of the apolipoprotein B (APOB) gene and Pvu2-RFLP of the lipoprotein lipase (LPL) gene Allele, genotype and haplotype frequencies were determined in 96 male patients with CAD proven by angiography and in 119 unrelated men, free from clinical signs of CAD (control group) The results showed that the frequency of M+ allele (presence of the cutting site) was significantly higher among patients than in control group Some MX and ME haplotypes of paired APOB polymorphic loci were also associated with disease status Variance analysis and estimation of allele effects demonstrated significant differencies in total triglycerides and LDL-cholesterol levels in individuals with different APOB EcoR1-RFLP alleles and genotypes, while no associations with serum lipid levels could be demonstrated for the LPL gene polymorphism Therefore, we conclude that genetic variability within the APOB locus may contribute to the development of CAD in certain extent in Russian population

9.008
Consanguineous marriage: An emerging issue in Community Genetics within Australia.

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Marriage between close biological relatives is strongly favoured in the majority of Middle Eastern Countries During the last three decades substantial numbers of migrants from this region have settled in Australia With the exception of a study on the Lebanese community in Sydney during the 1980s little information is available either on the prevalence of consanguineous unions among migrants or their outcome A sample of patients of Middle Eastern origin who attended Westmead Hospital, Sydney from 1990 - 1994 was compared with an Australian born control group The coefficient of inbreeding (F) was calculated for both groups and the reason for referral compared A number of generally rare autosomal recessive disorders such as Werdnig-Hoffman disease, metachromatic leucodystrophy, cytochrome-c-oxidase deficiency and dihydropteridine reductase deficiency were diagnosed in the children of parents of Middle Eastern origin In addition a considerable number of children with dysmorphic features and/or developmental delay in this sample group were thought to have an autosomal recessive disorder in view of the degree of consanguinity present and the presence of affected sibs in some of these families Other differences between the two sample groups and issues involved in the counselling of such families will be discussed

9.009
Epidemiology of monogenic disorders in the Tomsk region populations.

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Data on the prevalence of monogenic hereditary diseases were obtained and analyzed in ten rural populations of Tomsk region situated in West Siberia Total number of inhabitants of these populations is about 991000 In the course of clinical examination were detected 870 families with 1458 patients They included 130 families with autosomal recessive, 127- with autosomal dominant, and 25 - with X-linked forms of hereditary diseases Segregation analysis proved the correctness of the material subdivision, according to the mode of inheritance Totally 50 nosological forms of Mendelian diseases were found Among them neurological forms and rare syndromes were the most frequent Autosomal recessive disorders were presented by the following forms Smith-Lemli-Opitz and Bowen-Conradi syndromes, Fanconi's anemia, Tay-Sachs disease Crouzon, BBB, CCA syndromes and others were found among autosomal dominant disorders The role of certain genetic processes in the distribution of hereditary diseases pattern in the studied populations is discussed

9.010
Ovarian failure in female patients with the Nijmegen breakage syndrome

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Nijmegen breakage syndrome (NBS) is a rare autosomal recessive condition, classified as ataxia telangiectasia related disorder The major symptoms include microcephaly, distinct facies, chromosome breakage, immunodeficiency and enhanced predisposition to malignancy Among the 23 affected NBS children identified by us, there were 9 girls, two of whom reached pubertal age Observation of the latter revealed lack of development of genital organs, breasts, pubic or axillary hair One patient died from B-cell lymphoma at the age of 15 years The second is currently 17 years old and still presents infantile sexual characteristics and amenorrhea. Small, homogenic ovaries and infantile uterine were found on repeated pelvic ultrasonography Markedly elevated plasma levels of LH (30 0 and 25 0 IU/l) and FSH (106 0 and 108 0 IU/l) and, very low estradiol levels (below 5 5pg/ml) found in annual intervals indicate ovarian dysfunction In light of these findings, the same parametrs were evaluated in defrosted plasma samples from the deceased girl, with similar results (LH 30 0 IU/l, FSH 167 0 IU/l) From our observations and those of Conley et al (Blood,1986,67·1251-1256) who reported on similar hypergonadtropic hypogonadism in a 21-year-old NBS female, it appears that ovarian dysgenesis or hypoplasia should be included in the clinical spectrum of NBS

9.011
Cerebro-Oculo-Facio-Skeletal (COFS) syndrome in a 3 years old boy

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Here we report a case with Cerebro-Oculo-Facio-Skeletal (COFS) syndrome, a rare autosomal recessive disorder characterized by postnatal growth retardation, mental retardation, hypotonia, microcephaly, ocular abnormalities, facial dysmorphism and skeletal anomalies Our case was 3 years old boy and the first child of the parents, who were non-consanguineous marriage Motor and mental retardation, hypotonia, microcephaly, cataract, micrognathia, large ears, camptodactyly, rocker-bottom feet deformities were detected by his physical examination Cytogenetic analysis was performed and his karyotype was shown 46, XY, parents karyotypes were normal The present study, we will discuss and compare of this rare and interesting syndrome with other similar conditions

9.012

The case report of the stillbirth with Roberts-SC phocomelia syndromes.

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Roberts-SC phocomelia syndome is a rare autosomal recessive disorder characterized by perinatal and postnatal growth retardation limb reduction abnormalities and craniofacial anomalies Mitotic figures from Roberts-SC phocomelia patients display unusual phenomenon known as premature centromere seperation (PCS) Analysis of mitosis in Roberts-SC phocomelia patients have revealed abnormal mitotic event in metaphase duration and/or anaphase progression We here report a six months stillbirth male with Roberts-SC phocomelia syndrome involving about 55 % random premature centromere seperation of all chromosomes Severe growth retardation, hydrocephaly, tetraphocomelia, hypertelorism were detected by ultrasonography, after that cordosynthesis was performed for cytogenetic analysis during about six months pregnancy After two weeks fetus was death Intrauterine dead baby was delivered in sixth month of pregnancy This case was the second pregnancy of the family Th first one was also stillbirth female in seventh month of pregnancy and had severe limb deformities, hydrocephaly, growth retardation as described by the family Their parents were not relatives but their uncestors were from the same willage Mother d enied any drug usage or radiation during her pregnancy Parents' cytogenetic analysis were normal We reviewed and compare our clinical and cytogenetical finding with the features described in the literature

9.013

Interchange trisomy resulting from the unbalanced segregation of a maternal (1;7) translocation.

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We report a reciprocal translocation t(1,7)(p22,q21 2) from which 3 1 segregation was observed The proband was referred because of recurrent miscarriages A subsequent six week gestation miscarriage inherited the unbalanced interchange trisomy of the maternal translocation, 47,XY,der(1)t(1;7)(p22,q21 2),+der(7)t(1,7)mat, resulting in trisomy for chromosome 7 The rarity of interchange trisomy particularly when chromosome 18 or 21 is not involved is discussed, as is the risk of uniparental disomy when an apparently balanced translocation is seen at prenatal diagnosis

9.014

Coloboma, mental retardation, hypogonadism and obesity: critical review and updated nosology of the so-called «Biemond syndrome type 2»,

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Biemond syndrome type 2 (BS2) is classically regarded as a recessively inherited condition (MIM 210350) featuring mental retardation, eye coloboma, obesity, polydactyly, hypogonadism, hydrocephalus and facial dysostosis Clinically, the disorder is closely related to Bardet-Biedl syndrome Few cases have been reported, most of them before 1970 We present clinical data on 4 mentally retarded sporadic cases fitting the diagnosis of BS2, three with coloboma, obesity, and hypogenitalism, and one young girl with coloboma, and polydactyly Revision of the literature reveals striking clinical heterogeneity among the patients suggested to have BS2 We propose a new nosology of those cases, and delineate several new clinical forms Purported BS2 cases may be divided in 1) Bardet-Biedl syndrome with probably fortuitous coloboma or aniridia, 2) BS2 sensu stricto (recessively inherited syndrome of sexual infantilism, short stature, coloboma and preaxial polydactyly without obesity), 3) « new » dominantly inherited form of colobomatous microphthalmia occasionally associated with obesity, hypogonadism and mental retardation, 4) Rubinstein-Taybi syndrome, 5) unclassifiable, early lethal syndrome resembling Buntinx-Majewski syndrome, 6) « new » coloboma-zygodactyly-clefting syndrome The latter two syndromes may result from undetected chromosomal anomaly

9.015

A new Autosomal Dominant dysmorphic syndrome of cleft lip, cleft palate and inguinal hernia.

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A family with a new autosomal dominant syndrome of facial dysmorphism, cleft lip and cleft palate is presented The proband, a ten year old male, was born with bilateral cleft lip and cleft palate which were subsequently repaired He had congenital left inguinal hernia requiring hernioplasty He has minor learning difficulties, but continues to be at normal school On examination, he is well built with proportionate tall stature; his height being > 97th centile for his age He has long hands and feet, but measurements are not suggestive of marfanoid habitus His fingers and toes are spindle shaped with widening at the middle interphalangeal joints There is mild cutaneous syndactyly appreciable in hands His great toe show peculiar valgus deformity Craniofacial features include macrocephaly (head circumference > 97th centile), hypertelorism, epicanthic folds, downslanting palpebral fissures, flat nasal root, coarse look with thick and everted lower lip, a transverse groove on chin and posteriorly rotated simple ears The proband's father and elder brother have similar body habitus, phalangeal abnormalities and craniofacial features Both had unilateral cleft lip, cleft palate and inguinal hernia at birth The proband's elder brother has more marked phalangeal abnormalities and craniofacial dysmorphism with additional features of synophrys and large ears suggesting variable expression in this syndrome Results of further laboratory and imaging workup will also be presented

9.016

Trials of the Model for complex inheritance: IDDM and asthma

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The model for complex inheritance specifies a single logistic parameter It is the only model under which paternal and maternal transmissions to sibs of specified phenotypes are independent In available data on recurrence risks and identity by descent it is significantly more powerful than alternative methods Additivity of loci on the logistic scale is not disproven. IDDM typifies a clear dichotomy between normal and affected, with most of the information in affected x affected pairs Asthma and correlated atopy represent more complicated phenotypes that are essentially quantitative, and extreme discordant pairs are most informative We will present applications to IDDM as a benchmark (kindly provided by June Davies and John Todd) and to Wessex data on asthma, comparing the nonparametric approach with a two-locus disease model

9.017

Novel mutations in HERG, the human eag-related gene, in long QT syndrome (LQTS) support its role as the LQTS 2-gene

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The long QT syndrome (LQTS, MIM192500) is an autosomal-dominant inherited cardiac arrhythmia with a heterogenous genetic background Recently, two of a least of four causative genes have been identified One of these, the human eag-related gene (HERG), which is located on Chromosome 7q35-q36 (LQTS 2), encodes a cardiac potassium channel We investigated 15 unrelated patients with congenital LQTS by direct sequencing of PCR amplified genomic DNA isolated from lymphocytes The HERG regions which were predicted to encode the major functional regions between the expressed S1 domain and the cyclic nucleotide binding domain, were analyzed In two patients, new mutations in a highly conserved region of HERG were identified A single base pair deletion (delT1671, nucleotide numeration beginning with the methionine start codon) resulted in a premature stop codon within the S5-domain The likelihood for a causal relation of this defective HERG allele and the clinical disease was established by the subsequent identification of the mutation in two additional affected family members and its absence from two unaffected members In another LQTS patient, a nonsense mutation in codon 611 (Tyr 611 End) was found The patient's family was not available for study Both mutations resulted in truncated proteins and a loss of important functional domains including the channel pore and the nucleotide-binding domain Our findings of additional mutations in LQTS patients provide further evidence for an important role of this gene in the disease's formation Moreover, the finding of two mutations upon sequencing of only a minor portion of the HERG gene in fifteen individuals demonstrates that mutations in this gene are likely a frequent cause for LQTS

9.018

An autosomal recessive oculo-skeletal-abdominal (OSA) syndrome

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We have examined two adult sisters born to healthy, unrelated parents, presenting with a syndrome of ocular, skeketal, and abdominal defects They were born at the 38th week of a uncomplicated pregnancy, with a birthweight of 3,000 g Concordant clinical findings are short stature, downward slanted and short palpebral fissures, epicanthus inversus, dystopia canthorum, ptosis, high arched eyebrows, small mouth, gothic palate, rounded and prominent chin, and partial agenesis of the abdominal muscles Skeletal defects include thick cranial theca, platybasia, hypoplastic posterior cranial fossa, occipital horn, scoliosis, sacral schisis, large semicircular canals and vestibulum Discordant findings are empty sella, radioulnar synostosis, schisis of the atlas, and hypoplastic 12th rib in the older sister, and Arnold-Chiari malformation, short 5th metacarpals and fusion of carpal bones in the younger sister Mental development is normal The clinical manifestations of these two sisters have not been reported previously, although some facial and abdominal features overlap those observed by Carnevale et al (Am J Med Genet , 33 186,1989) in two mentally retarded brothers The recurrence of this condition in sisters born to unaffected parents suggests an autosomal recessive inheritance Work supported by the EC concerted action "European Working Group on Genetics of Hearing Impairment"

9.019

The frequencies of some autosomal recessive diseases in Birmingham, UK

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Birmingham is a multiracial city in the West Midlands of approximately 1 million, with 15-16,000 births annually 10% of its children are of Afro-Caribbean descent, 11% Indian, 13% Pakistani and 2% Bangladeshi These percentages are much higher than in the rest of the NHS region We have studied the frequency of some autosomal recessive diseases in these ethnic groups In a prospective cohort study of about 5,000 unselected babies followed from birth for five years, children of Pakistani couples who were consanguineous (70% of Pakistani couples) had a 16-fold increase in the incidence of recessive diseases These were of heterogeneous aetiology, but a third were associated with severe mental retardation and a third with neurological problems In Birmingham between 1981 and 1994, phenylketonuria was diagnosed (by neonatal screening) in 13 Europeans from 11 families, one Afro-Caribbean/Arabic child and one Pakistani child Over the same period, galactosaemia was confined to European children (7 cases, 3 to extensively inbred Irish travelling families), and tyrosinaemia type I to Pakistani (8) and Arabic (1) children (8 families known to be consanguineous) Phenylketonuria was less common in Birmingham than in the rest of the region (1 20,800 v 1 15,600) while tyrosinaemia was more common (1 23,100 v 1 91,900), These findings illustrate the interplay between gene frequency and parental consanguinity in determining disease frequency in different populations

9.020

Psychosis, ataxia, dementia and epileptic seizures in a large family.

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Ten affected subjects have been identified in a family with Calabrian origin, genealogically reconstructed in a kindred of more than 400 people The disease segregates in autosomal dominant manner over three generations The illness is characterized by age at onset spanning a large range (from 17 to 50 years) wwith prominent psychotic symptoms as first manifestation Tremor in the hands, distonic movements and disartria follow, together with dementia The illness progresses slowly and in the advanced state patients show considerably increased tone in their limbs, anartria and generalized epileptic seizures Molecular genetics excluded Huntington's Disease and Spino-Cerebellar Ataxia type 1 The global clinical picture suggests Dentato-Rubral-Pallido-Luysian Atrophy Molecular genetics is in progress

9.021

Phenotypic description of FAD families with 146Met->Leumutation in S182 gene.

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Two large Italian families (FAD4 and Tor1 1) have been fundamental in the isolation and cloning of S182 gene, whose mutations cause early-onset Familial Alzheimer's Disease FAD4 and Tor1 1 families carry the same 146Met->Leu mutation, demonstrating their common origin, as already supposed Their roots have been traced in Calabria (Southern Italy) until the XVII century One hundred five is the total number of affected subjects in these families They constitute a powerful sample to characterize the phenotypic picture of AD due to S182 146Met->Leu

mutation, showing early onset and remarkable presence of psychotic symptoms, myoclonus and epileptic seizures

9.022

Development of pineal astrocytoma in an "unaffected" mother of a proband with neurofibromatosis 1: should NIH criteria be revisited?

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Neurofibromatosis is a heterogeneous group of disorders with at least two phenotypically and genotypically distinct entities NF-1 and NF-2. To aid the diagnosis, the NIH consensus group developed minimal diagnostic criteria for each type Our experience indicates that a strict adherence to these criteria occasionally leads to mis- or under-diagnosis of NF A female proband was diagnosed with NF-1 based on the presence of 12 cafe-au-lait macules and bilateral axillary freckling Both parents were unaffected by NIH criteria However, the mother had 3 cafe-au-lait macules The family was informed that the proband was a de novo mutation Subsequently, at age 30, the mother developed neurological symptoms leading to identification of malignant astrocytoma in the pineal region which showed transient improvement with gamma knife treatment However, new cerebral and spinal tumors, believed to be metastases, were later identified and the patient died An attempt is being made to obtain tissue for molecular study
Due to the extreme rarity in the general population of pineal astrocytomas and increased frequency of astrocytomas in NF, we believe that the mother's course is most likely due to NF mutation and not a coincidence Thus, we question whether NIH diagnostic criteria ought to be revisited

9.023

Evaluating comparative genomic hybridization (CGH) as a strategy for preimplantation diagnosis of unbalanced chromosome complements.

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An alternative to the prenatal diagnosis of genetic disease is preimplantation diagnosis This involves genetic analysis of 1-2 cells biopsied from preimplantation embryos, generated using conventional in vitro fertilisation (IVF) techniques The embryos are maintained in culture and only transferred back to the mother if the biopsied cells prove to be normal Fluorescent in situ hybridization (FISH) has been used to detect specific chromosomes in preimplantation embryos This has allowed the preimplantation diagnosis of unbalanced chromosome complements as well as the sex of the embryo The vast majority of embryo cells biopsied for preimplantation diagnosis are in interphase, making normal karyotyping impossible and limiting the number of chromosomes that can be successfully analysed by conventional FISH We are trying to overcome these limitations by using comparative genomic hybridization (CGH) CGH is a FISH technique which, in a single hybridization, allows the copy number of all 23 pairs of chromosomes can be assessed Using DNA extracted from blood and fibroblasts we have successfully detected trisomy 21 and trisomy 18, as well as amplifications and deletions in tumour samples A significant quantity of DNA from the specimen (100 ng) is required for CGH To satisfy these requirements we are performing whole genome amplification on single cells prior to labelling and CGH Alternative strategies for overcoming the significant technical difficulties of single cell CGH will be compared and discussed

9.024

Preimplantation diagnosis of autosomal aneuploidy using multicolour fluorescent in situ hybridisation (FISH)

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One of the commonest reasons for requesting preimplantation diagnosis is repeated spontaneous or induced abortion due to chromosome imbalance caused by a parental translocation or gonadal mosaicism We have developed the use of locus-specific YAC/cosmid probes to detect chromosomes 13, 14, 15, 18 and 21 in interphase nuclei using a standard two day multicolour FISH protocol Preliminary tests with these probes on lymphocytes and single blastomeres have demonstrated the reliability of this technique Preimplantation diagnosis was attempted for four couples with poor reproductive histories, two with Robertsonian translocations, t(13,14) and t(13,21), one with a reciprocal translocation t(6,21) and another with suspected gonadal mosaicism for trisomy 21 Three IVF cycles were carried out in total with biopsy of 2 cells from day 3 post-insemination embryos As we have detected high levels of mosaicism in early human embryos, only those embryos from which two biopsied cells could be scored as normal were transferred One patient was cancelled due to ovarian hyperstimulation syndrome, one patient had no chromosomally normal embryos and two patients each had one embryo transferred, one of which resulted in a biochemical pregnancy All four couples are willing to undergo further preimplantation diagnosis cycles

9.025

Detection of β thalassaemia mutations using SSCP analysis: An approach to preimplantation diagnosis

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β thalassaemia is the world's most widespread genetic disorder and represents a major health problem Although the current management has succeeded in prolonging the patients' life, economical and psychological factors make it difficult for many patients to benefit from it Furthermore, prenatal diagnosis is not feasible in many countries due to social and religious objections to pregnancy termination Preimplantation genetic diagnosis (PGD) is an alternative procedure for prevention and would be preferred by several ethnic groups To enable PGD of β thalassaemia by direct detection of the mutant B globin genes at the single cell level, we employed nested PCR to amplify the region of interest in the gene This was performed on various types of single cells isolated from different individuals known to be carriers of β thalassaemia mutations To detect mutations we applied SSCP analysis and silver staining as a sensitive, nonradioactive, direct and rapid method A total of 297 single cells from thalassaemic patients with different mutations were amplified and analyzed The number of cells that amplified was 282 (90%) Contamination was observed only in 4 6% of the negative control tubes The total running time of PCR and SSCP analysis is approximately 8 hours We believe that the combination of nested PCR and SSCP analysis is reliable and represents a suitable procedure to be followed for PGD

9.026

Prenatal Diagnosis of four Chromosome Anomalies from Transcervical Cell Samples

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Prenatal diagnosis of fetal chromosomal abnormalities was performed by the analysis of transcervical cells (TCCs) retrieved by aspiration and lavage from four pregnant women between seven and thirteen weeks of gestation, prior to termination of pregnancy Diagnosis of fetal trisomies 18 and 21 and fetal triploidy was achieved by performing fluorescent in situ hybridisation (FISH) on whole TCC samples, and confirmed by the same procedure performed on placental material Fetal triploidy was also confirmed by FISH and the polymerase chain reaction performed on clumps of trophoblastic cells isolated from the endocervical samples In another case TCC samples were obtained from a pregnancy with dizygotic twins The TCC samples, analysed by interphase FISH, were found to contain two populations of cells, one with two X chromosomes and one Y, the other with one X and two Y chromosomes Further investigations of maternal and fetal cells revealed that the mother had a translocation 46,XX, t(Yqh,15), while both fetuses were chimeras with different ratios of XX+Yqh and XY+Yqh cells in all tissues analysed, probably as a result of early interchange of embryonic cells These results suggest that a minimally invasive approach, based on the collection of endocervical samples, may be employed for the prenatal diagnosis of selected inherited disorders

9.027

A epidemiological, clinical and genetic study of congenital deficiencies of limbs

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Limb Deficiencies (LD) are congenital malformations characterized by missing or severe hypoplasia, total or partial, of the skeletal components and soft parts of a limb LD are useful markers of the action of human teratogenes They are frequently associated with other malformations forming specific entities, frequently with genetic etiology Therefore, the epidemiological, clinical, and genetic study of the LD, within a temporally clearly defined geographically population, is an interesting area yielding theoretical and practical applications Between 1985 and 1992, in the Iasi area of Romaina different types of congenital abnormalities were found in 1,747 children (16 97%) from 102,974 born children For the study a group of 42 newborn infants with LD was selected The classification and diagnosis of LD used a system derived from Frantz and O'Rahilly's classification (1961) Within this group, LD represented 0 41 or 1 2452 newborn infants There have been no major seasonal or annual variations Our values are smaller than in other studies (0 50-0 55) reflecting probably a real smaller incidence compared to other regions This hypothesis uses LD obvious at birth as a base, thus reducing errors in detection Other epidemiologic parameters of LD within the group are similar with other international studies Thus, the Finger Deficiencies (28 6%) and Terminal Transverse Deficiencies (21 4%) are the most frequent morphological types, males (62%) are more frequently affected than females (38%), upper LD is twice as frequent as those of the lower limbs, and left unilateral are more frequent than bilateral 57% of LD are associated with other muscle-skeletal and visceral abnormalities (renal and cleft lip-palate) Three syndromes were identified EEC, Roberts and Acro-Renal The high percentage of association requires that attention should be payed to syndrome identification in order to improve the quality of genetic counselling

9.028

Syndrome of Hypotonia, Psychomotor Retardation, Seizures, Delayed and Dysharmonic Skeletal Maturation, and Congenital Fibre Type Disproportion : a new case.

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We describe a 4 years old male, first child of healthy urelated parents, who presents marked non progressive hypotonia, severe psychomotor retardation, myoclonic epilepsy, delayed bone age and congenital fibre type disproportion at muscle biopsy We believe that clinical and radiological features of our patient are very similar to those of three patients recently reported by Quazi et al (J Med Genet 1994; 31 405-409) The following table summarizes clinical and laboratory findings of our patient compared to previous report

Patient	1	2	3	4*
Age at evaluation	4 year	3 5 years	3 years	4 years
sex	M	M	M	M
B-W	3185	3690	3380	3470
hypotonia	+	+	+	+
milestones	delayed	delayed	delayed	delayed
retardation	severe	severe	severe	severe
seizures	-	+	+	+
constipation	+	+	+	+
lenght (centile)	20	25	80	50
weight (centile)	90	50	95	75
OFC (centile)	50	50	60	25
open mouth	+	+	+	+
long filtrum	+	+	+	+
high arched palate	+	+	+	+
prominent nasal root	+	+	+	+
hypertelorism	+	+	+	+
EEG	abnormal	abnormal	abnormal	abnormal
EMG	N	N	N	N
muscle biopsy congenital fibre type disproportion	+	+	+	+
CK	N	N	N	N
bone age	delayed	delayed	delayed	delayed

1,2,3 patients described by Quazi; 4* our patient

Male sex of our patient supports the possibility of an X-linked mutation

9.029

Prenatal Diagnosis of Unmapped Genetic Syndromes

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A large number of genetic syndromes which are characterized by multiple congenital anomalies (MCA) cannot be identified prenatally by means of specific laboratory tests However, ultrasound may detect structural anomalies which, though unspecific, indicate a broad range of possible MCA syndromes which have different prognosis During 1994 we have followed 27 pregnancies at risk for genetic non chromosomal MCA syndromes including Fryns (2), X -linked arthrogriposis(2), AD oloprosencephaly(2), fetal acynesia sequence, SLO, Stikler, Aicardi (2), Marshall, Silverman-Handmaker, Platispondilic Chondrodisplasia, Hereditary Hypomyelination (2), Meckel (2) , Roberts, Mesomelic dysplasia, Fraser (2), AR dyaphragmatic agenesis, multiple pterigium syndrome (2), cleft lip-palate-syndactily, ablepharon-macrostoma syndrome In the same period we have followed 15 pregnancies without specific genetic risk, complicated by fetal MCA Serial ultrasound scans alone of the first group allowed the identification of 7 affected fetuses between the 13 and 28 week of gestation and no false negative

cases occurred To attain correct prenatal identification of genetic syndromes in the second group of affected fetuses we devised a diagnostic algorithm which includes fetal blood sampling for cytogenetic analysis and emathological evaluation followed by echocardiography and serial sonograms Presumptive diagnosis was corrected in 1 case but retrospective analysis of the data acquired prenatally would have allowed identification of the correct syndrome involved in 12 cases We believe that previous clinical experience plays a major role in the correct diagnosis of genetic syndromes during pregnancy

9.030
DOO-DOORS Syndrome: Report of Three Italian Patients

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We report on two patients affected by DOO syndrome (deafness and onycho-osteodystrophy) and one patient affected by DOORS syndrome (deafness,onycho-osteodystrophy,mental retardation and seizures) DOO and DOORS syndrome, firstly described by Cantwell in 1975, appear to be distinctive clinical entities with different mode of inheritance· autosomal dominant mutation for the DOO syndrome and autosomal recessive for the DOORS. The following table summarizes main clinical manifestations of our patients Case n

	1	2	3
sex	M	M	M
age at diagnosis	5 years	10 months	7 years
Consanguineity	-	-	+
Neurosensorial deafness	+	+	+
Mental retardation	-	-	+
Seizures	-	-	+
Asymmetric face	+	-	-
Ptosis	-	-	+
Squint	-	+	-
Retinal coloboma	-	-	+
onychodystrophy	+	+	+
Absence/hypoplasia of hands distal phalanges	+	+	+
Triphlangial great toes	-	-	+/-
Absence/hypoplasia of feet distal phalanges	+	+	+
Elevated 2-oxoglutarate	-	-	-

These findings confirm the marked clinical variability and genetic heterogeneity of DOO-DOORS syndrome which have been described also in 20 previous patients The differential diagnosis between these two syndromes, which can be based on the presence of mental retardation and seizures in DOORS, is critical to genetic counselling and to assessing the recurrence risk Cantwell R J Hum Genet 26 261-265,1975

9.032
Evidence of Cystic Fibrosis Among Adults With Diffused Bronchiectasis

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Cystic fibrosis (CF) is an autosomal recessive disorder usually diagnosed before 16, on clinical symtoms and elevated sweat chloride levels (SCL) Diffused bronchiectasis (DB) is a constant major sign of CF in the childhood Conversely DB in adults is often associated with other conditions, such as kartagener syndrome, tuberculisis, α1 antitrypsine deficiency but in other cases the causes of DB remain undeterminated The aim of this study is to look for CF mutations in adults with DB without known etiology Ninety four patients with DB were referred to pulmonary unit DB was confirmed by chest X ray and computed tomography of the lungs Patients presenting kartagener syndrome, post-tuberculosis bronchiectasis or other etiologies were excluded All patients had the same medical This group of patients has been compared with two hundred and seventy nine patients from the general population The most frequent CF mutations in the French population (ΔF 508, ΔI 507, 1717-1G→A, G542X, G551D, R553X, W1282X, N1303K) were analysed by polymerase chain reaction (PCR) and allele specific oligonucleotide (ASO) using INNO-LIPA CF2 kit (INNOGENETICS) Ten patients among 94 and four control among 279 were heterozygote for ΔF 508 mutation (X2 = 16,49 , p < 10-3) DNA of ΔF 508 patients was then tested by denaturing gradient gel electrophoresis (DGGE) for each of 27 exons of the CF gene This study is still on going To date, 4 patients carry a second mutation (D1152H, Y569C, R668C, 3849 + 10kb) This result shows the evidence of CF among adults with DB event when SCL were normal

9.033
Modification of Beutler's fluorescence spot test and its application to large-scale screening of severe glucose-6-phosphate dehydrogenase (G6PD) deficiency in Turkish population.

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Severe glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the clinically significant inherited metabolic disorders It causes acute hemolytic anemia induced by specific drugs and food, and neonatal jaundice Beutler's fluorescence spot test is a qualitative assay for screening severe G6PD deficiency Our primary objective was to reduce its cost without losing its specificity, sensitivity and validity parameters, and to carry out large-scale screening in Turkish population The reagent of spot test was prepared in bulk The original volume of test reagent(100 ml) and blood (10 ml) were reduced by %50 Then it was standardized internally and externally by using heparinised fingerstick blood Not only its cost became %50 lower, but also the number to be screened was doubled Sample population from all over Turkey was 12,500 men 220 men were found as a severely G6PD deficient The average frequency of severe G6PD deficiency in southeast Anatolia, Aegean and Mediterranean regions of Turkey is % 1 76 There were apparent differences among the locations such as Adana(n=829, % 2 65) Ankara(n=710, % 0 99), Hatay(n=550, % 6 73), Izmir(n=178, % 4 49), Urfa(n=746, % 3 08). Those who are severe deficient were given drug and food list and information booklet to prevent possible acute hemolytic crisis in their future life This high frequency indicates that severe G6PD deficiency is very common in Turkish population, and therefore population screening at birth should urgently be put into practice

9.034
Multiple familial lipomatosis with polyneuropathy, an inherited dominant condition.

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A 22-year-old man had polyneuropathy, facial dysmorphia, atopia and multiple lipomatosis His mother had neuropathy as two of her first cousins, but she had not lipomatosis The proband's grand mother had multiple lipomatosis as her own mother and a sister of her mother, but these relatives had not neuropathy Familial mutliple lipomatisos is a rare genodermatosis inherited as an autosomal dominant trait and charaterized by the development of a variable number of slowly growing lipomas of various size in a widespread distribution involving the trunk and the limbs. Hereditary motor and sensory neuropathy are frequent disorders difficult to classify Especially difficult to classify are those disorders which have, in addition to the sensory/motor neuropathy, signs affecting other systems This family is an example of a dominant syndrome the principal features of which are multiple lipomas and polyneuropathy Although a random association of two independant

traits could have occured, another possible explanation for this previously unrecognized association of multiple lipomas and polyneuropathy could be a contiguous gene syndrome caused by a chromosome deletion, chromosome 12, where abnormalities in patients with lipomas were found, is a likely site for this deletion in our family

9.035
Autosomal dominant intestinal atresia

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We present a family manifesting a variety of small bowel atresias in an autosomal dominant fashion The index case was found to have multiple jejunal atresias in association with apple peel mesentery and biliary atresia His father, paternal aunt and paternal uncle had all presented with high small bowel atresias Previously reported familial occurrences of small bowel atresias have all been consistent with autosomal recessive inheritance This report is the first to document autosomal dominant inheritance of small bowel atresias

9.036
Effect of the «Triple Test» on trisomy 21 incidence at birth: the South Belgium experience

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Maternal screening with the « triple test » in the second trimester for fetal Down syndrome has gained a wide acceptance in South Belgium during the 4 last years The practice of triple test, in Belgium, is not supported by any global project Access to the test is free and offered through several private laboratories besides ours (which screens about 1/3 of all pregnancies) However, all cytogenetic analyses are performed in only registered Centers, allowing complete ascertainment of diagnosed fetuses/newborn Between 1984 and 1994, 329 newborns (29 8 ±8 7 / year) and 134 fetuses (12 2 ±8 6 / year) were found Annual incidence of DS was stable during this period ($p < 0.12$), even when birth rates were corrected for prenatal diagnosis During the 1984-1990 period (pre-triple test), the rate of prenatally detected cases was 19 5% (corrected 14 5%) In 1992-1994 period (triple test in use), the rate increased to 51 1% (corrected 42 2%), significant at $p < 0.05$ Our data suggest that a large use of triple test, in our region, is the most likely explanation for the dramatic increase in DS prenatal diagnosis

9.037
Molecular analysis of 21-hydroxylase deficiency heterozygous carriers in two groups of patients with Turner syndrome.

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By means of evaluation of 17OH progesteron levels with the ACTH stimulation test, we have previously observed (1) a high frequency (29 6 %) of possible heterozygous carriers of 21 hydroxylase deficiency in patients with Turner syndrome (16 out of 54) The surrenalic 21 hydroylase is an enzyme involved in the metabolic pathway of adrenal steroids synthesis and its deficiency causes about 95% of the congenital adrenal hyperplasia (CAH) This observation has been confirmed in an another group of Turner syndrome patients (29 4 % , 10 out of 34) from Bologna We decided to investigate in all these patients the presence of the most common mutations of the coding gene CYP21B which cause different levels of enzyme impairment The availability of examining the complete families from the first group of Turner patients permitted a more accurate analysis of the patterns of inheritance We performed an hybridization using oligonucleotides specific for normal and mutated sequences after specific amplification of the CYP21B gene, which is localized in the HLA class III region All the patients and the relatives were characterized for HLA class I, II and III We observed the presence of some of these mutations, confirming previously described association such as the substitution Val281-Leu on the HLAB14, DR1 haplotype (2) Our analysis will be fulfilled by sequencing in order to explain some interesting findings and look for new possible mutations still undetected 1)Larizza et al , Clinical Endocrinology, 40, 39, 1994 2)Dondi et al , European Journal of Immunogenetics, 21, 341, 1994

9.038
Polycistic ovary syndrome (PCO)and CYP21B gene mutations : a molecular study

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Two different groups of hyperandrogenic women with clinically defined PCO from Italy (N=48) and from Slovenia (N=48) were screened by means of the ACTH stimulation test in order to evaluate their 17-OH progesteron values A serum increase of this steroid reveals an alterated activity of the surrenalic 21 hydroxylase enzyme (1) whose deficiency is the main cause of congenital adrenal hyperplasia (CAH) Different degrees of enzyme impairment are known to be caused by different mutations in the coding gene CYP21B which maps in the HLA class III region, near C4B gene We observed a high frequency of supposed heterozygous carrier of this enzymatic deficiency in both PCO groups (38 6 % in the Italian group and 16 6% in the Slovenian one) Thus we investigated the frequency of the most common mutations which cause CAH, when occuring in homozygous status (2), by means of hybridization with specific oligonucleotides for the normal and the mutated sequence after specific amplification of the CYP21B gene All the patients had been fully characterized for HLA class I, II and III (C4 and Bf) polymorphisms In the Italian group we observed the presence of mutations at the codon 307, codon 318, codon 218, codon 30 and intron 2 In the Slovenian group the most frequent mutation seems to be a 8bp deletion in exon 3 The sequencing analysis will check this observations and perhaps show other undetected mutations 1)New et al J Clin End and Metab , 57, 320, 1983 2)Speiser et al , J Clin Invest , 90, 584, 1992

9.039
Prenatal diagnosis of pulmonary agenesis and microphthalmia syndrome

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A syndrome consisting of bilateral pulmonary agenesis, microphthalmia, eventration of the diaphragm and vascular structural defects of the heart was previously reported (Am J Med Genet Supplement 3 379-382, 1987) We present a prenatal diagnosis by ultrasonography at 22 weeks gestation with the same constellation of findings, confined only to the the right side of the body Pathological examination revealed right diaphragmatic hernia, agenesis of right lung right pulmonary artery and vein as well as bilateral cystic microphthalmia (predominantly to the right side) The ophthalmologic findings were verified by a through pathological dissection Chromosomes were normal This is the second report of this syndrome but the first one that was diagnosed prenatally The possible etiologies of this syndrome are 1 A genetic syndrome with variable expression 2 A developmental disruption defect with unknown etiology in an early embryonic stage that caused the above findings It may be that in our case the cells that were damaged created later the right side of the body and therefore the predominant damage in the present case was confined to this side Further delineation of the possible etiologies will be discussed

9.040

Angelman Syndrome -The experience of one genetic clinic

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Over the last 5 years, 18 patients seen in our clinic, were diagnosed as having AS Previously no cases were recognised in this clinic The clinical findings ranged between mild phenotype to severe clinical involvement 16 of the cases were sporadic and two were familial The age at diagnosis ranged from 1 year to 24 years and 10 of the patients were females No cases of methylation mutation were found but the other 3 genotypic classes were represented One patient, the offspring of a balanced t 15/22 carrier mother had an unusually mild phenotype with a considerable vocabulary He was found to have a paternal UPD Two affected siblings, a male and a female had no molecular defect, but shared the same maternal haplotype Although the "incidence " of AS in our clinic seems unusually high, it reflects the increased awareness towards this syndrome over the recent years, and the buildup of clinical experience

9.041

Detection of a female Charcot-Marie-Tooth type 1A (CMT1A) patient with different mosaicism patterns in different tissues concerning the duplication in 17p11.2: a casereport

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With a prevalence of 1 2500 the CMT disease is one of the most commonly inherited diseases in human It includes several subtypes of disease, defined by different clinical features and candidate genes Patients suffering from the subtype CMT1A have - in most of the cases - a 1,5Mb duplication on chr 17p11 2 including the genelocus for PMP22 (peripheral myelin protein 22) In some cases without duplication point mutations within this gene are found We describe the case of a female patient clinically diagnosed as Charcot-Marie-Tooth (CMT) type 1 A duplication of the 1,5Mb region on chromosome 17p11 2 has been detected by EcoRI/Southern blot analyzes with the probe pNEA102 (kindly provided by JR Lupski, Houston) Two colour inteharpse FISH (fluorescence in situ hybridization) analyses with the specific probe pVAW409R1, located in the duplicated region and a control probe (located on 17q11.12) revealed a mosaicism In peripheral blood lymphocytes and cells of the buccal mucosa the percentage of interphase nuclei with duplication in 17p11 2 was lower (49% and 51%) than in interphase nuclei extracted out of hair root cells or paraffin embedded nerve tissue (66% and 74%) Either this finding could be due to an event during early embryonic development leading to different mosaicism patterns in different tissues, or one could postulate a growth advantage of cells without duplication, which gets visible only in cells proliferating throughout whole lifetime The mechanism how a mosaicism in CMT1A disease may occure, remains still to be discussed Chimerism, mitotic unequal crossing over or homologous recombination between the CMT1A-REP elements within the duplicated region may be possible models Acknowledgement TL holds a Herbert Quandt Stiftungs fellowship, KDB, BR and HG are funded by the DFG

9.042

Charcot-Marie-Tooth (CMT) disease and tomaculous neuropathy (HNPP): results of a collaborative German and Belgium study

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In early 1993, the Institute of Human Genetics and the Department of Neurology in Erlangen started a collaboration concerning the clinical, genetical and biological pathogenesis of hereditary motor and sensory neuropathies, mainly Charcot-Marie-Tooth disease and tomaculous neuropathy We collected over 300 DNA samples from different centers The ongoing analysis of DNA, blood cells and serum as well as tissue material like sural nerve biopsies confirmed the crucial role of alterations of known candidate genes like peripheral myelin protein 22 (PMP22), myelin protein zero (P0, MPZ) and connexin 32 (Cx32) in the pathogenesis of these peripheral neuropathies Gene expression studies on sural nerve biopsies and other tissues allow a better understanding of the function of PMP22 in neural and non-neural tissues Further evidence for the postulated non-sister chromatide exchange during spermatogenesis leading to the 17p11 2 duplication and deletion is given A recombination analysis of 13 Chr 17p11 2 deletions revealed evidence for heterogenous structures of the HNPP deletions A breakpoint analysis of the 50 identified Chr 17p11 2 duplications and deletions will give us more insight into the recombination mechanisms leading to the most common mutations in CMT disease and tomaculous neuropathy Intrafamiliar phenotype variations will be discussed Acknowledgement we wish to thank the following scientists for their contribution Valentijn, LJ, Amsterdam, Netherlands, Toyka, KV, Wurzburg, F R G , Christen, H-J, Göttingen F R G , Wolf, D, Hameister, H, Vogel, Ulm, F R G , Hausmann, R , Dumser, T, Prols F, Erlangen, F R G , Kunath, B, Leipzig, F R G , Hillenbrand, R, Schachner, M, Zurich, Swiss, Sostarku, M, Zagreb, Kroatia, Cawthon, R, Salt Lake City, USA, KDB, BR and HG are funded by the DFG, TL holds a Herbert-Quandt-fellowship We wish to thank C Gehring, K Thoma and K Kammler for excellent technical assistance

9.043

A new point mutation in the peripheral myelin protein 22 (PMP22) associated with Charcot-Marie-Tooth diseases.

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We have studied the peripheral myelin protein gene PMP22 in a large family with Charcot-Marie-Tooth disease type 1 (CMT1A), in which the typical duplication of the PMP22 gene on chromsome 17p, responsible for CNT1A, was not found Neverthe less, analysis of linkage in this family was compatable with PMP22 being responsible for the disease Sequencing of DNA and cDNA of affected members showed a missense point utation G^{368} T in the exon 5 of PMP22, which is redicted to determine a valine for glycine substitution at codon 107, a position which maps to the center of the PMP22 protein putative transmembrane domain III Using sequence-specific oligoncleotide probes (SSOP), we found the point mutation in all affected CMT1A subjects but neither in healthy members of the family nor in control 314 chromosomes, thus evidentiating that the G^{368} T point mutation is not a polymorphism Since the G^{368} to T point mutation is located in the splice consensus sequence, we performed the sequencing of cDNA from lymphoblastoid cell lines of one affected and one healthy memeber of the family The same point mutation G to T was found in the affected subject but not in the healthyrelative Molecular analysis suggests that in this family CMT1A disease is due to the G^{368} T missense mutation

9.045
Direct quantitative radioimmunoassay determination of platelet serotonine, in a group of autistic patients and a control group.

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Autism is a non specific syndrome, and the expression of many different diseases Cook in 1990 pointed out that the most consisting biochemical finding in autism has been that over 25% of patients with autism are hyperserotoninaemic and that this was also found to be familial Some authors postulate that a consisting high level within a family would mean an association with genetic factors or a predisposition to autism Concentration of platelets serotonin has been extensively studied over the years, but there are almost no papers of values measured by direct RIA methods These are sensitive, simple and a fast way to analyse a great number of samples with good results We studied a group of 30 autistic patients (25 males and 5 females), 8 patients (3 males and 5 females) with severe mental retardation and some autistic behaviours, that had been classified as autist some years ago, 2 normal sisters of a index case and a control group of 23 matching age and sex The results showed that the serotonin levels of autistic patients were significantly higher that the control group (p=0 000) There were no significant difference levels between sex (p=0 117,p=0 518) and age (p=0 703,p=404) in all patients and control group Also no significantly difference between autistic patients and mental retarded patients with "autistic behaviour" (p=0 706) In 86% of autistic patients there was a hyperserotoninaemia (media 86 69/ SD 40 36, controls media 49 54/ SD 16 49 mmol/dl) Some of the patients with high serotonin levels and severe behaviour problems are going to initiate medication with fluoxetine It also is presented the evaluations between serotonin levels in our patients with known aetiologies and the ones described in the literature

9.046
Coffin Siris like phenotype in a patient with de novo 46,XX,del(9)(q31.2-q33.2) karyotype

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The Coffin Siris syndrome is a multiple congenital malformation/mental retardation syndrome of unknown cause It is characterised by low birthweight, hypotonia, sparse scalp hair, coarse face, thick lips, large mouth, hypodontia, hirsutism and characteristically a short fifth finger with hypoplastic nails The inheritance pattern is unclear Recurrences within families have been documented We describe a female with the combination of low birthweight, hypotonia, mental retardation, sparse hair, coarse face, large nose, full lips, hypodontia, and cupped ears Moreover she has short stature, hirsutism, small hands with very short fifth fingers with hypoplastic nails, and small feet Family history and pregnancy are unremarkable The Coffin Siris syndrome was strongly suggested in our patient Cytogenetic analysis of peripheral blood lymphocytes however showed an interstitial deletion of the long arm of chromosome 9, the karyotype being 46,XX,del(9),(q31 2q33 2) in all cells Because of the striking resemblance of our patient with patients with the Coffin Siris syndrome, a comparison is made between published cases of Coffin Siris syndrome and cases with this chromosome 9 deletion Cytogenetic investigation in cases of clearcut Coffin Siris syndrome is needed to establish whether the syndrome could be a microdeletion syndrome located on the long arm of chromosome 9

9.048
Mosaic 45,X/46,XX in a patient with Craniofrontonasal syndrome.

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Craniofrontonasal dysplasia is characterised by hypertelorism, coronal synostosis with brachycephaly, joint anomalies, down slanting palpebral fissures, clefting of nasal tip, longitudinally grooved fingernails and other anomalies Although the most accepted mode of inheritance is X-linked dominant , published pedigrees are not according with autosomal dominant, autosomal recessive, X-linked dominant or X-linked recessive inheritance We report a female 38 years old with characteristic phenotype, moderated mental retardation (IQ 87) and precocious menopause Her mother and her sister have hypertelorism but no other manifestations of the syndrome are present The cytogenetic study showed a mosaic 45,X/46,XX in the patient and a normal karyotype in mother and sister We have found in the literature one case with a terminal deletion of Xpter-p22 2 while her mother and sister had normal karyotype

9.049
Clinical , cytogenetic and FISH findings in a family with a reciprocal translocation t(4 q;21 q) and Crouzon syndrome t(4 q;21 q) and Crouzon syndrome

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Families with Down syndrome with translocation and breakpoint in or near the Down chromosomal region are rare The Crouzon craniofacial dysostosis (CFD) is an autosomal dominant disorder of craniofacial development characterized by premature craniosynostosis, hypertelorism, maxillary hypoplasia and shallow orbits with exorbitism We present a 3- generation family with reciprocal translocation 4q35,21q22 1 and breakpoint proximal of the Down chromosomal region (D21S55,D21S65) Four individuals have balanced translocation t(4q,21q) and Crouzon syndrome with varileab phenotype expression The anomaly was first picked up in a girl with Down syndrome who had Down syndrome to and Crouzon syndrome and the unbalanced translocation resulting in partial trisomy 21 and partial monosomy 4 The translocation was identifed by cytogenetic analysis of GTG-banded metaphases and molecular cytogenetic (FISH) studies Genes for Crouzon syndrome have been reported at chromosomal regions 10q25-26, 7p13 and 4p16 (Adelaidetype) Our results suggest that either the gene for Adelaidetype craniosynostosis of the short arm of chromosom 4 is inherited with the translocation chromosom or a new candidate gene for Crouzon syndrome is localized at or near chromosomal breakpoint 4q 35 or 21q21 1 in this family

9.050
Cytogenetic and fluorescence "in situ" hybridization in a case of Angleman syndrome.

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This report studies a cytogenic and fluorescence "in situ" hybridization of a family in which a female child showed all the main characteristics of Angleman syndrome (AS) Her kariotype revealed a translocation that involves the chromosome 15 and originates a deletion of the Angelman region Several members of her family appeared to be carriers of the same translocation, but showed no symptoms Their karyotypes showed a marker chromosome, that was not present in the female with AS Fluorescence "in situ" hybridization revealed that the marker chromosome corresponded to material of chromosome 15 The present study is in agreement

with the suggestion that genomic imprinting is one of the mechanisms involved in AS

9.051
Gaucher disease mutations in Italian patients.

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Gaucher disease is characterized by marked clinical and biochemical heterogeneity also found in more than 50 mutations identified within the glucocerebrosidase gene We here reported molecolar characterization of most Italian patients Mutation analysis of 70 unrelated patients (47 type 1, 9 type 2 and 14 type 3) was realized using polymerase chain reaction (PCR) followed by non isotopic single strand conformation polymorfism (SSCP) combined with restriction fragmentation analysis The mutations N370S (33 58%) and L444P (32 86%) account for 93 Gaucher alleles (66 44%) The 370 mutation was detected more frequently It was identified in heteroallelic form in 37 of 47 type 1 patients Homozygosity was detected in 7 type 1 patients The 444 mutation was present in heteroallelic form in 5 of 8 type 2 patients Homozygosity was found in 9 of 14 type 3 patients, that varied in severity of neurological symptoms Also one patient with severe non neuropatic manifestation presented the 444 mutation in homozygous form The rare mutations recNciI, G6490→A and D409H were demostrated in 6 alleles (4 28%) Four new mutations were characterized on five mutated alleles (3 56%) Thirty six alleles (25 72%) remained to be characterized The molecular characterization of patients is of great importance for clinical genetics and therapeutic aspects

9.052
Screening for mutations in Italian patients with Glycogen Storage Disease type Ia.

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Glycogen Storage Disease type Ia is an autosomal recessive inherited disease caused by the deficient activity of microsomal glucose-6-phosphatase, an enzyme expressed in the liver, kidney and intestine A recent report of full-length G6Pase cDNA (Lei et al Science, 262 580-583,1993) indicated the possibility of a diagnosis without a liver biopsy and offers the opportunity of a prenatal diagnosis To date, different mutations have been reported R83C and Q347Z that appear to be the more frequent, 130Z, R295C, G222R, V166G and ΔF327 We are currently studying 22 patients each exon was PCR amplified and a screening by SSCP analysis was performed, deviant SSCP patterns were sequenced to fully characterise mutations Two patients resulted homozygous for the common R83C mutation, two other patients are compound heterozygotes for the same mutation and Q347Z, Q347Z has been found in homozygosity in four patients, two of whom were brothers, and in heterozygosity in two other patients We have also found a novel mutation in exon 5 S298P in homozygosity in a patient whose parents are related This mutation is due to a T>C transition at nucleotide 971 which causes a serine>proline substitution in the protein

9.053
A 9bp deletion in the Arylsulfatase A gene of two unrelated Metachromatic Leukodystrophy patients

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Metachromatic leukodystrophy (MLD) is an autosomal recessive inherited disorder caused by deficiency of the lysosomal hydrolase arylsulfatase A (ASA) Three clinical forms of MLD are distinguished according the age of onset of symptoms, a late infantile, a juvenile and an adult form Using single strand conformation polymorphism analysis (SSCP) and sequence analysis we found a 9bp deletion (2320del9) in exon 8 of the ASA gene of two unrelated patients, a late infantile and a juvenile, both heterozygous for this mutation The deletion causes a Ser-Asp-Thr loss at codons 406-408 Interestingly, in both patients the deletion lies on a pseudodeficiency (pd) allele The late infantile patient is heterozygous for the pd allele, the juvenile patient is homozygous for the same allele The late infantile patient was shown to be heterozygous for the 609A mutation, the most common among late infantile MLD patients We suggest that in the late infantile patient the clinical phenotype is caused by the described molecular alterations (2320del9-pd / 609A) In the juvenile patient the described molecular alterations let us make the hypothesis that an unknown X mutation (2320del9-pd / X-pd) is a mild one, so to cause a juvenile phenotype

9.054
Development of an ARMSTM screening test for the detection of 12 common European mutations of the CFTR gene.

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Cystic fibrosis (CF) is the most common severe autosomal recessive disorder of the Caucasian population, with an estimated incidence of 1/2500 and a carrier frequency of 1/25 Since the discovery of the cystic fibrosis transmembrane conductance regulator (CFTR) gene in 1989, over 500 mutations have been identified Most of these mutations are rare and are not routinely screened for We have developed a two tube multiplex ARMS (Amplification Refractory Mutation System) test which detects 12 CF mutations prevalent in the European population The primary application of the test is screening for individuals who may be carriers of one of these mutations In addition, the test gives genotype information on DF508, the most common mutation Genomic DNA is added to both tubes, amplified by ARMS PCR and the reaction products separated by gel electrophoresis The presence or absence of a diagnostic product band of a defined size indicates whether or not a particular mutation is present in the sample The test was validated using a large panel of typed DNA samples and was shown to be very reliable It is simple to perform and highly suitable for routine use in clinical diagnostics

9.055
Prevalence and geographical distribution of Huntington's disease in Devon and Cornwall (S.W. England)

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Parallel prevalence studies were carried out in the counties of Devon and Cornwall during the twelve month period up to 01/09/95 for the estimation of service provision needs Ascertainment was by examination of genetic and medical records, distribution of family questionnaires, direct enquiry to neurology, psychiatry and social service departments, family doctors, specialist Huntington's disease (HD) units, and lay support groups 126 symptomatic individuals were identified in a population of 1 52 million, giving a prevalence of 8 4 per 100,000 An additional 18 people have had positive predictive tests The study revealed a wide disparity in the geographical distribution of HD Plymouth (urban) had a prevalence of 13 5/10^5, and North Devon (rural) 5 6/10^5 Five (9 6%) of the 52 Plymouth kindreds accounted for 34 5% of affected individuals, suggesting a founder effect related to a stable population with a Naval base A similar founder effect was observed in North Devon Founder effects were not convincingly demonstrated elsewhere The overall prevalence is among the highest in the UK and compares with other areas which have similar stable populations

9.056

Hydrocephalus, severe cerebellar hypoplasia, and brain stem calcification - a new autosomal recessive syndrome.

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Two fetuses conceived by a non-consanguineous couple are described Both were terminated following abnormal ultrasound examination, the first (female) at 20 weeks gestation, the second (male) at 18 weeks Both had normal head circumferences and similar brain pathology consisting of 1) dilatated ventricles and attenuated cortex showing neuronal necrosis with foamy macrophages and prominent vascularity, 2) aplasia/hypoplasia of the cerebellum with highly disorganised tissue, and 3) extensive necrosis and dystrophic calcification of the ventral aspect of the brain stem In Case 2 a tiny cerebral aqueduct was noted Micrognathia and a duplex right urinary collecting system were noted in Case 2 but otherwise no internal organ abnormalities were seen in either case Case 2 showed a normal male karyotype A review of the literature shows that these sibs differ from similar cases by 1) the distribution of the necrosis/ calcification, 2) the lack of evidence for a proliferative vasculopathy or cortical migration defect, and 3) pattern of inheritance They appear to represent a previously unreported central nervous system malformation syndrome and autosomal recessive inheritance is likely

9.057

Stickler syndrome type 2 and linkage to the COL11A1 gene

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Stickler syndrome (hereditary arthro-ophthalmopathy) is an autosomal dominant condition characterised by severe short sight, arthritis, deafness, flat facial appearance, cleft palate and small chin It is the commonest inherited cause of retinal detachment There is genetic heterogeneity, with about two thirds of cases caused by mutations in the gene encoding type II procollagen (COL2A1) We have recently shown that Stickler syndrome can be sub classified on the basis of vitreo-retinal phenotype type 1 families with a characteristic congenital vitreous anomaly show linkage without recombination to markers at the COL2A1 locus, type 2 families with different congenital vitreo-retinal phenotypes are not linked to COL2A1 We have now investigated linkage to other candidate genes in a large type 2 family with vitreo-retinal, articular, oro-facial and audiometric features of Stickler syndrome A maximum lod score of 2 7 at zero recombination was obtained with the marker D1S223 which is 2cM from the COL11A1 locus on chromosome 1 Linkage to COL2A1, COL5A2, COL9A1, CRTL1 and COL11A2 was excluded Brunner et al (Human Molecular Genetics 1994,3,1561 - 1564) have recently reported linkage to COL11A2 in a Dutch pedigree with systemic features of Stickler syndrome but without ocular involvement Both COL11A1 and COL11A2 are expressed in cartilage, but on the basis of studies of bovine vitreous it is likely that only the a1(XI) chain encoded by COL11A1 is present in vitreous This would be consistent with the hypothesis that mutations in the genes encoding collagen XI can give rise to manifestations of Stickler syndrome, but of these only mutations in COL11A1 will give the full syndrome including the vitreo-retinal features

9.058

The risk of a pregnancy trisomic for chromosome 21. Maternal Age related risk in over 7000 affected pregnancies.

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While the maternal age related risk has largely been derived from population studies before 1980, this data has been used for counselling for a number of years It is necessary to see if these rates have changed over time while allowing for the impact of prenatal diagnosis The NDSCR accumulates reports of diagnoses of trisomy 21 from the clinical cytogenetic laboratories of England and Wales Data accumulated on over 7000 registrations over the past seven years allows a new measure of single year risk that includes necessary corrections for those cases diagnosed prenatally that might not have reached term From the data, a new regression curve with a very good fit to the data has been calculated Below maternal age 25, the overall risk of a Down birth is below 1 1500 rising to 1 500 at age 35 The plateau in risk starts at about age 45 with a risk of close to 1 50 of a Downs birth It is estimated that the revised curve will make little change to the risk factors derived from serum assay except in women over 40 where there is a distinct plateau in the data

9.059

Twinning in Down syndrome

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Among about 7400 pregnancies registered with the NDSCR, 108 were multiple pregnancies In four cases the affected fetus was known to be one of triplets and in twelve cases the twins were concordant for DS and sex It is clear the registration of multiple pregnancies is incomplete yet in this population, the frequency of multiple pregnancies is at least 1 69 This is slightly higher than the 1 78 for all maternities in England and Wales in 1993 but the latter excludes deliveries before 24 weeks Among the concordant Downs twin pairs, possibly monozygotic, the sex ratio was 2 0 (4FF 8MM) The sex ratio (M/F) of the affected discordant twins where known was unexpected, 0 85 compared with 1 25 for all Down's births however the sex ratio of the normal sib where this was known was 1 05, identical to the general population birth ratio Further details about multiple births in the registered population are being sought

9.060

Oliver-McFarlane syndrome in two sibs.

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We present a 21 year old girl and her 18 year old brother with Oliver-McFarlane syndrome The features of this syndrome are chorioretinopathy, hypogonadotropic hypogonadism, trichomegaly (long eyelashes) and thin scalp hair Mental retardation is often present To our knowledge there are only 10 patients decribed in the literature The parents are healthy, non-consanguineous and of Indian origin There are three elder healthy brothers The two affected sibs have the four above mentioned symptoms and are mentally retarded, the girl more severely than the boy Additional investigations showed cerebellar hypoplasia on MRI, unusual EEG pattern (non-reactive constant alpha rhytm), absent ankle tendon reflexes Both karyotypes were normal Moreover the girl was obese and she had renal insufficiency The Oliver-McFarlane syndrome has some overlap with the Bardet-Biedl syndrome where retinitis pigmentosa, genital hypoplasia, obesitas, mental retardation, polydactyly and sometimes renal insufficiency are seen Polydactyly is absent in our patients and the trichomegaly and thin hair is pronounced which are both absent in the Bardet-Biedl syndrome and typical for Oliver-McFarlane syndrome

9.061
Myopathology in 2 patients with Noonan syndrome.

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Noonan syndrome is a well known autosomal dominant condition, characterized by typical facial appearance, congenital heart defect and short stature The congenital heart disease most often is a pulmonary valve stenosis Approximately 20% of the patients, however, are born with hypertrophic obstructive cardiomyopathy (HOCM) We describe two patients with Noonan syndrome who had progressive HOCM from birth, and in whom abnormal histopathological changes in striated musculature were detected In both patients an increased density of muscle spindles was found in biopsy To our knowledge there is no known disorder associated with such an increase in muscle spindle density in skeletal muscle Histopathologic changes in heart muscle in relation to Noonan syndrome have been reported An extent of myocardial disarray has been described, as is found in classical familial HOCM In one of our patients histologic examination of the heart muscle revealed also this myocardial disarray Here we report the first Noonan syndrome patients with progressive HOCM and additional abnormal histopathological changes in striated muscle

9.062
A two step clinical score for Williams syndrome: application to 27 new cases.

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Williams Syndrome (WS) is a clinical condition characterized by peculiar facial dysmorphisms, short stature, mental retardation, typical neurobehavioural attitude and congenital heart disease (SVAS above all) In 1993 Ewart et al found that affected WS patients bear a submicroscopic deletion spanning at least 114 Kb including the elastin gene at 7q11 23 In 1995 a simple clinical score based on six traits was proposed by Lowery et al We analyzed by FISH with the ONCOR probe for the WSCR 27 patients in which a clinical diagnosis of WS was hypotized Aim of our study was to correlate the results of FISH analysis with those obtained by applying a new two-step clinical score The first step, "facial score", is based on the presence/absence of 18 minor anomalies, the second, "total score", combines the results of the facial score to six extrafacial traits The final score indicates the probability for a patient to be affected with WS FISH analysis demonstrated hemizygosity of the WS-specific region in 20/27 cases (74%) The total score was fully concordant with the FISH results, being positive in 20/20 (100%) deleted patients and negative in 9/9 (100%) non deleted patients The facial score alone was rather predictive too

9.063 Need to check this against original
Correlation between sural nerve biopsy findings and PMP 22 gene duplication or deletion

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From our data file of more than 4,000 sural nerve biopsies we are in the process of retrospectively analysing selected, glutaraldehyde or formalin fixed, paraffin embedded sural nerve and muscle biopsies from 96 suspected cases with dominantly inherited motor and sensory neuropathies, type IA (CMT 1A), and 88 suspected cases with tomaculous neuropathy in respect to their proportional DNA content at 17p11 2-12, *i e , at the coding region of the peripheral myelin protein* (PMP) 22 gene Three of four corresponding exons are amplified by the polymerase chain reaction (PCR) with the use of genomic DNA (extracted from the tissue) and primer sets 1-3 (Roa et al 1993 N Engl J Med 329 96) mapping to the appropriate exons 1-3 Since marker 5G7 (D17S458) is not duplicated in CMT 1A (Chance et al 1993 Cell 72 143) it is used as a reference primer Seven cases have thus far been evaluated using these methods The number of preserved, degenerated and regenerated, demyelinated and remyelinated nerve fibers (onion bulb formations) with or without axonal atrophy, with or without abnormally thick myelin sheaths (hypermyelination) correlated with the clinical severity of the neuropathy Deletions and reduplications, however, were not detected in all cases studied suggesting point mutations that will be investigated subsequently According to Gabre??ls-Festen et al (1995 Acta Neuropathol 90 645) a more severe degree of neuropathy is apparent in cases with point mutations only

9.064
Quantitation of the telomeric and centromeric SMN genes by Solid Phase Minisequencing. Implication for carrier diagnosis of SMA.

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Childhood onset spinal muscular atrophy is one of the most common recessive disorders affecting 1 in 6 000 live birth A candidate gene, the survival motor neuron (SMN) gene has recently been cloned The SMN gene is present in two almost identical copies on chromosome 5, a centromeric and a telomeric version The telomeric copy has been shown to be deleted in more than 95 % of SMA patients Deletions of the telomeric copy can be detected by either Single Stranded Conformation Analysis (SSCA) or by introducing a restriction site into the PCR product Deletion analysis is therefore rather simple and can be use for both pre- and post-natal diagnosis However this method cannot be used for carrier diagnosis, We have used the Solid Phase Minisequencing Method to measure the ratio between the centromeric and the telomeric copies of SMN The two sequences differ by one single base pair in exon 7 Using primers spanning this site, and allowing incorporation of either 3H-dTTP or 3H-CTP in the minisequencing reaction, the ratio of incorporation can be determined, which is reflecting the ratio of the different copies of SMN in the sample

9.067
Unexpected phenotypes and genotypes

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Clinical examination represent the major determinant in establishing the diagnosis of a genetic condition However, since most syndromes have been described for the first time in the last three decades, dynamic data concerning the evolution of the phenotypes are often lacking or incomplete. When this variable is absent it may hamper clinical recognition of a well-known syndrome and conversely lead to overdiagnose syndromes that will eventually be discarded when taking into account clinical course The potential burden of these misdiagnoses may be considerable for families Our experience with these pitfalls is illustrated by presentation of a dozen of clinical histories of patients with unsuspected chromosomal aberrations (i e trisomy 21 with normal phenotype, Goldenhar syndrome associated with trisomy 18 or XYY caryotype, vanishing cystic hygroma with fetal Turner syndrome,), changing phenotypes of some well-known conditions (PW syndrome, campomelic dysplasia, Opitz G-syndrome) or paradoxical signs (gigantism in PW syndrome) The knowledge of these borderline states with respect to accepted clinical criteria may prove helpful in expanding the recognized phenotypes

9.068

Megathrombocytopenia (MTP) and deafness in a three-generation family : a new type IV collagen disorder ?

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A 20-y-old female requested genetic counselling for familial conductive deafness She had a progressive hearing impairment and asymptomatic MTP, as well as her mother and maternal grandmother (AO being 35y and 50y respectively) CBC revealed a MTP in the three female patients (platelets counts ranging from 8,000 to 45,000 per cubic millimeter) with a size up to 15 µ and inclusion bodies in leucocytes No renal involvement could be evidenced by urine concentration test, biology blood creatinine in clearance and analysis of urine sediment or analysis of the urine sediment Ultrastructural studies of platelets were normal except for their size On photonic microscope examination after May-Grunwald-Giemsa staining, small spherical inclusions were observed in approximatively 30% of neutrophils They were also present in eosinophils, but apparently not in basophils nor in monocytes By contrast to May-Hegglin inclusions, they were not spindle-shaped nor elliptic and were light blue instead of blue These were very similar to those described in Fechtner syndrome where deafness, MTP and nephritis are dominantly cosegregating (Blood 65 397, 1985) Since MTP is a rare feature of Alport syndrome we suggest the condition observed in our family might represent a further example of a type IV collagen defect

9.069

Clinical, cytogenetic and molecular studies of a child with partial duplication of chromosome 15

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The clinical, cytogenetic and molecular findings of a child having 46 chromosomes with a partial duplication of chromosome 15 are presented This contrasts with most reported patients with partial duplication of chromosome 15, who have a small extra acrocentric fragment The proband is the first child of unrelated healthy parents with normal karyotypes At birth he was small for gestational age and showed hypertelorism, low-set ears, anteverted nostrils, downturned wide mouth, broad philtrum, extra skinfold of the neck and club feet Cytogenetic studies of cultured lymphocytes (G-banding) revealed a bisatellited dicentric chromosome 15 with duplication of the proximal 15q, centromere and 15p Painting analysis confirmed the extra chromosome segment to be from chromosome 15 FISH studies with chromosome 15 centromeric and Prader Willi probes are pending Furthermore, molecular analysis with microsatellite markers from chromosome 15 will be performed in order to characterise more precisely the duplicated segment and the parental origin of the abnormal chromosome 15

9.070

The investigation of Gilbert Syndrome as a cause of hyperbilirubinaemia of the newborn.

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In man, the inherited hyperbilirubinaemias (Crigler-Najjar and Gilbert Syndromes) are caused by mutations in the gene encoding the UDP-glucuronosyltransferases (UGTs) Our laboratory recently demonstrated that Gilbert Syndrome (GS) is associated with homozygosity for a 2bp insertion in the TATA box of the UGT1*1 exon GS occurs in 10-13% of the population and is characterized by mild elevation of the unconjugated bilirubin level in the serum Heterozygotes (45-51%) are present in the normal population Neonates have reduced hepatic bilirubin glucuronidation capacity compared to adults for the first 3-4 postnatal months We are investigating whether the existence of GS further compromises the neonate's glucuronidation capacity precipitating the development of jaundice Preliminary results in prolonged jaundice (>14 days) neonates using a radioactive polymerase chain reaction methodology shows that this group contains more heterozygotes (56%) and less homozygotes with the mutation (2%) than are found in the general local population We are currently investigating neonates with early/severe (but not prolonged) jaundice and this data will also be presented It is not always possible to give concerned parents an explanation for their child's neonatal jaundice It is hoped that the outcome of this research will enable clinicians to allay some parental concerns

9.071

The molecular genetics of Familial Hypercholesterolaemia in the Eastern Scottish population.

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Familial Hypercholesterolaemia (FH) is an autosomal dominant disease characterised by gross elevations in the low- density- lipoprotein (LDL)- cholesterol, secondary to mutations within the gene encoding the LDL receptor protein FH is predominantly caused by heterozygous mutations within the LDL- receptor gene, or more rarely by homozygous or compound heterozygous mutations We are currently investigating the genetic basis of FH in the Eastern Scottish population with the view to providing presymptomatic screening in families affected by FH Genetic analysis was performed on 3 clinically diagnosed, unrelated FH patients from Tayside Polymerase Chain Reaction (PCR) amplification and Denaturing Gradient Gel Electrophoresis (DGGE) revealed they do not have a defect in the gene encoding apolipoprotein B-100 which can give rise to a form of hyperlipidaemia with similar clinical symptoms to FH PCR and direct sequencing confirmed that these patients do not have mutations in exons 3, 4, 6, 8 or 9 of the LDL receptor gene, all of which are known to be mutation hot spots A polymorphism has been identified in exon 10 Further work including Southern analysis of patient DNA will be presented Elucidation of the genetics of FH in Scotland will enable its clinical diagnosis to be confirmed in affected individuals and identify other family members at risk

9.072

Segmental neurofibromatosis.

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Approximately 130 cases with segmental neurofibromatosis have been reported, the majority of which are isolated cases They are presumed to be somatic mosaics for Nf1 mutations, with those cases with affected children representing gonosomal mosaics These hypotheses remain unproven at the molecular level At the Oxford Nf clinic 19 patients with segmental Nf have been seen in the past 5 years Six cases had unilateral cutaneous involvement, 7 cases had associated major Nf1 complications and 3 patients more than one body segment affected, these were all isolated cases One patient had unilateral cutaneous involvement and a daughter with full blown Nf1, and a father with cutaneous neurofibromas in several segments of the body had a daughter with a segmental pattern of café-au-lait spots and freckling Our findings suggest that segmental Nf is more frequent than suggested by the medical literature The approximate prevalence for our region is 1 in 400,000 Whilst most segmental patients are isolated cases they have a small risk of having children with full blown Nf1 The apparent vertical transmission of segmental Nf in one of our families is difficult to explain pathogenically It appears that the Nf1 gene for some reason is expressed normally and abnormally in different parts of the body This might be explained by position effect variegation Investigations of segmental Nf at the molecular level are in progress

9.073

Dominant myopia ? Report on a large Italian family.

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Genetic effects are known to play a role in the etiology of myopia The mode and impact of inheritance in myopia are still a matter of debate among those supporting the multifactorial mode of transmission According to McKusick (1994) high myopia can be inherited as an infantile severe autosomal recessive trait (MIM 255500), as an X-linked trait (MIM 310460 - Bornholm Eye disease, MIM 310600 - Norrie disease, MIM 311000 - Myopia-Ophthalmoplegia syndrome, MIM 310500 - Hemeralopia-Myopia, MIM 300600 - Aland Island Eye disease) or as autosomal dominant trait (MIM 1600700) We observed a large Italian family (fifty-one members) with 24 males and 8 females affected by high myopia through 4 consecutive generations Age at onset of visual impairment was between 1 and 5 years Neither dysmorphic features nor ocular and extraocular anomalies were noticed in all family members, intellectual abilities were normal in all individuals Ophthalmologic examination revealed a bilateral myopia ranging between - 8D and - 15D with a typical myopic fundus in all the affected members as well as regular astygmatismus Three patients suffered from horizontal nystagmus The dark-adapted ERG showed a reduced amplitude in almost all patients Myopia of severe degree, transmitted through several generations was reported in the past only in two families (Franceschetti A J Genet Hum 1953,2 283-284, Francois J Heredity in Ophthalmology, St Louis, Mosby Co, 1961) Our findings suggests that at least some high myopia can be inherited in an autosomal dominant trait

9.074

A new case of fibular aplasia and syn/ectrodactyly.

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We present a fetus with fibular aplasia, tibial hypoplasia and ectrodactyly diagnosed ultrasonographically at 21 weeks gestation A normal 46,XY karyotype had been documented by a previous amniocentesis performed because of advanced maternal age A therapeutic abortion was induced at 22 weeks The complete post-mortem examination showed bilateral aplasia of the fibulae and hypoplasia of the tibiae, oligodactyly of the left hand and ectrodactyly of the feet Both feet had rocker bottoms and only two toes with apparent syndactyly of the 4th and the 5th on the left No additional malformations were disclosed A similar pattern of skeletal malformations, in the absence of other major defects, had been previously observed in two other unrelated Italian families

9.075

Cytogenetic and clinical aspects of four trisomy 9p new cases.

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Trisomy 9p is a clinical well-documented syndrome, the most common abnormalities are brachycephaly, microcephaly, facial dysmorphisms and dermatoglyphic anomalies Regarding cytogenetic analysis the trisomy 9p derives mainly from parental balanced translocation, more rarely from a de novo translocation or partial duplication of 9p We describe four new cases of trisomy 9p derived from parental balanced translocation in three cases and from the presence of an additional marker chromosome in one case The karyotype was carried out on peripheral blood cultures both on the propositus and the parents Chromosome analysis was performed using QFQ, RBA, DA-DAPI and CBG banding techniques In the three cases a parental balanced translocation was identified, involving the chromosome 9 and chromosome 14 in two and chromosome 21 in one case In the fourth patient the trisomy 9p was an additional de novo chromosome, being the analysis of parental karyotypes normal A better comprehension of the cytogenetic rearrangments was achieved by means of fluorescence in situ hybridization (FISH) Clinical and cytogenetic aspects of these cases are presented in details

9.076

A de novo unbalanced t(4;10)(p15.2;p15.3) in a child with severe malformations and mental retardation.

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We report a 10 year old child who has referred to cytogenetic analysis due to a severe combination of malformation and developmental delay At the clinical examination he showed short stature ,obesity,dismorphic face,broad feet, broad hands with bilateral camptodactily and hypoplastic thumbs,cryptorchidism,severe delay of the psychomotor development and seizures The MNR evidentiated an arachnoid cyst in the temporal lobe and dysgenesis of the corpus callosum for the absence of the splenum of the corpus callosum High resolution QFQ,GTG and RBA banding analysis showed the presence of a 10p+ chromosome in all the metaphases studied (>50) The karyotype of the parents were normal FISH analysis by means of chromosome 10 library allowed to rule out either chromosome 10 duplication or translocation of chromosome 10 to other chromosomes In order to establish the origin of the extra material on chromosome 10p+, a search based on the overall clinical signs of the patient was made using the Possum,London Dysmorphology and Schinzel DB Among the candidate chromosomes indicated by the above procedure, chromosome 4 turned out to be involved in the rearrangements as shown by CISS with chromosome 4 library In addition loss of the 10p telomere in the rearrangement was inferred by the absence of an interstitial signal on the 10p+ chromosome by FISH Partial 10p monosomy should be considered together with partial 4p trisomy in the definition of the clinical phenotype

9.077

Genetic mapping of the human homologue (Hu-T) of the mouse T (Brachyury) gene and a search for allele association between Hu-T and spina bifida

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The protein product of the T gene is a transcription factor crucial in vertebrates for the formation of normal mesoderm T mutant Brachyury mice die in midgestation with severe defects in posterior mesodermal tissues Heterozygous mice are viable but have short or absent tails, fusions between gut and neural tube, and occasional malformations of the sacral vertebrae T has intrigued geneticists because of its association with the mouse t-complex This is a region of mouse chromosome 17 containing several genes including the MHC locus A naturally occurring genetic variant of the t-complex, the t-haplotype, is characterised by transmission ratio distortion, male sterility and recombination suppression Our mapping data presented here show that human T (Hu-T) maps to 6q27 between TCP1 and TCP10 and add to the evidence that in man the loci of the t-complex are split into two main locations on the short and long arms of chromosome 6 We have also re-investigated the idea that Hu-T might be involved in susceptibility to the neural tube defect (NTD) spina bifida Heritability for NTD has been estimated to be about 60%, but there is uncertainty about the mode of inheritance and penetrance, and

there is evidence for genetic heterogeneity Bearing in mind these problems we have used an allele association test which does not require prior knowledge of mode of inheritance and is independant of penetrance and family size

9.078

XX male - molecular diagnosis resources with y probes

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The XX male syndrome is caracterized by a male phenotype, short stature, gynecomastia, testicular atrophy and infertility It is know that a y sex determining region (Gene SRY) is located on the short arm of the Y cromossome and plays a role in the male sex development when differentiation takes place in gonadal ridges initiating testis determination The Authors report a case of a patient with normal genitalia referred for genetic evaluation after a testicular histology witch displayed seminiparous tubules normal in size and containing only Sertoli cells with no hyperplasia of Leydig cells PCR analysis of DNA extracted from blood lymphocytes was positive for SRY and for the pseudo autossomal boundary sequences located on the short arm of chromossome X were detected as well It is concluded that the presence of the ZYF sequence in the XX male could have carried the sex reversal For detection of molecular defects on the ZFY gene this casae has been subject for further investigation

9.079

Autosomal dominant, benign familial chorea, mild intellectual impairment and anticipation- a new syndrome?

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The female proband aged 45 had delayed development, starting to walk aged 5, learning difficulties, (no school qualifications) and a non progressive movement disorder since early childhood She was choreic in both upper and lower limbs CT scan of brain, EEG, CSF protein, serum copper and caeruloplasmin, and lysosomal enzyme assays were normal Her eldest son was reported to have similar restless movements since childhood He had walked at 3 5y and had been at a special school Aged 26, he is living independently and is in a manual job Her second son also had restless movements from the age of 5, had walked at around 2 5y and had attended normal school, although he required remedial teaching He is presently employed in a manual job Her youngest son is healthy with no movement problems or intellectual impairment Her second son's daughter, aged 1 was born at 37weeks with mild perinatal asphyxia She has developmental delay, most marked in gross motor abilities and abnormal posturing of the left arm The proband reported that her father, who had a University degree was also affected with a movement disorder PCR analysis for the Huntington's disease trinucleotide expansion revealed two normal alleles in the proband

9.080

CNS abnormalities in a family with a connexin 32 mutation and peripheral neuropathy

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X-linked Hereditary and Motor Neuropathy(HMSN) has been found to be due to mutations in the connexin 32 gene in the majority of families described In the families described to date, the clinical features of affected males is related to defects in the peripheral nervous system We would like to describe a three generation family in which affected males have clinical features consistent with X-linked HMSN but who have in addition involvement of the central nervous system In the two affected males in generation one of the family, the clinical features, in addition to those of a peripheral neuropathy, included severe tremor (lifelong) and marked generalised atrophy of the cerebral cortex and cerebellum as demonstrated by MRI scan In the affected, in generation three, reflexes in the lower limb were brisk and in addition to features of HMSN he had evidence of spasticity A mutation at codon 93 of connexin 32 segregates with these clinical features The mutation is a base substitution ATG>GTG which results in an amino acid change of a methionine to a valine in the second transmembrane domain of the connexin 32 protein

9.081

CYP2D6 genotyping in Russian population using a novel approach for the identification of CYP2D6A mutation.

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The frequency of 29A and 29B mutations in the CYP2D6 gene (debrisoquine-hydroxylase) has been analyzed in the Russian population For the detection of 29A mutation a new one step ARMS PCR approach has been developed Our purpose was also to compare CYP2D6 mutant allele distribution in aged and young individuals and study whether PM phenotype influences on longevity, since the CYP2D6 genotype was shown to be associated to certain cancer forms The 29B mutant allele typing was performed by means of Bst NI RFLP method (Daly et al 1991) For the 29A mutation identification genomic DNA was PCR amplified with three oligonucleotide primers The forward primer is designed for the specific amplification of the CYP2D6 gene sequence by mismatching two highly homologous CYP2D7 and CYP2D8 pseudogene sequences at its 3'-end nucleotide Two reverse primers are specific for the normal and the 29A mutant allele , each matching allele specific nucleotide at its 3'-end And one of them has an additional 10 nucleotides long stretch at its 5'-end that is not complementary to the basic CYP2D6 sequence, and thus allele specific PCR products differ in their lengths Among 344 Russian individuals analyzed 88 heterozygous and 11 homozygous for 29B mutation were found, 10 heterozygous and no homozygous for 29A were detected, one had both mutations in compound, and thus 3 5 % of Russian population was identified as poor metabolisers The frequency of the 29B mutation (0 17) in Russians appears to be significantly lower than those reported for Caucasoids and higher compared with Asia that can be explained by the mixed origin of the population inhabiting Russia Comparative analysis of the mutation frequencies among the aged and young individuals showed no age-related differences

9.082

VNTR haplotyping and mutation analysis of the phenylalanine hydroxylase gene in St.Petersburg phenylketonuria families.

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About 90% of mutant alleles of phenylalanine hydroxylase (PAH) gene were identified by means of SSCP and sequence technique in the group of 70 PKU patients Eleven different PKU mutations have been detected We found the following distribution of allele frequencies R408W-70,7%, R261Q-4,3%, P281L-4,3%, R252W-2,9%, IVS12-2,1%, R158Q-1,4%, R261X-0,7%, R243X-0,7%, E280K-0,7%, IVS10-0,7%, Frameshift 363 (-G)-1,4% We determined the association between mutations and VNTR All identified mutation associations were the same as mentioned in previous publications [Y Okano,90, R C Eisensmith,92, A Goltsov,92] except R261Q and R243X The R261Q mutation is in linkage disequilibrium not only with 8-copy VNTR allele as in Europe but also with 3-copy VNTR allele The mutation R243X is associated with allele containing 3 repeats whereas in previous investigations only the association with 8 repeats has been found The data obtained allow us to propose that both mutations were recurrent A new frameshift mutation 363(-G) was detected in haplotype with 8-copy VNTR The

combined use of the VNTR system and the mutation spectrum knowledge permits us to create the reliable system of prenatal PKU diagnostics in St Petersburg region

9.083

Angiotensin-I converting enzyme (ACE) polymorphism in patients with borderline hypertension in St.Petersburg.

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The genes encoding components of the renin-angiotensin system (RAS) present the attractive candidates for hypertension Angiotensin -I converting enzyme (ACE) is a key component within the RAS, where hydrolyzes angiotensin I to generate angiotensin II (vasoconstrictor) and the kallikrein-kinin system, where it inactivates bradykinin (vasodilatator) The recently detected ACE gene polymorphism results in three genotypes of II, ID and DD, the last one is associated with increased level of enzyme concentration in plasma We have studied the frequency of the ACE insertion/deletion (I/D) polymorphism in nonobsese (BMI , 27 kg/m2) young men 17-30 years of age Subjects were classified as normotensive (NT) N=79 with BP , 140/90 mm Hg or borderline hypertensive (BHT) N=120 with BP 140/90 mm Hg All subjects had clinically normal glucose tolerance Secondary hypertension was excluded by clinical and laboratory findings The genotype distributions in borderline hypertensive and normotensive subjects were in Hardy-Weinberg equilibrium DD=34, ID=65, II=21 and DD=26, ID=36, II=17, respectively The allele frequencies for BHT group were D=0,554 and I=0,446, for the NT group were D=0,557 and I=0,443 respectively No genotypic or allelic differences were observed between two groups

9.084

Study of the molecular genetic predisposition to heart disease in random population of schoolchildren in St.Petersburg.

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We examined whether the genetic variation of the apoB, apoCIII, apoE and ACE genes are involved in the determination of serum lipid level already in childhood For this purpose 403 schoolchildren (age range from 6 to 18 years old) from St Petersburg were taken into analysis Total serum cholesterol,LDL CH and triglyceride levels were determined by enzymatic methods (Labsystem,Finland) The apoB XbaI, apoCIII SstI and apoE genotypes were studied by the polymerase chain reaction with the following digestion by appropriate restrictases The ACE gene genotype was determined by PCR according to (Rigat et al ,Nucl Acid Res ,1992) All statistical analyses were made using the statistical software STATGRAPHICS Genotype distributions of the apoB, apoCIII, apoE and ACE genes in St Petersburg population are XIXI-25 2%, XIX2-58,7%, X2X2-15 1%, SISI-79 9%, SIS2-19 1%, S2S2-0 9%, E3/E3-60 3%, E2/E3-16,6%, E2/E2-0 7%, E4/E4-1 5%, E3/E4-17 4%, E2/E4-3 5%, II-25 1%, ID-48 1%, DD-26 8%, respectively The analyses of the relationship of the ApoB 100 XbaI, ApoCIII SstI, ApoE, ACE genotypes to serum lipid levels showed the statistically significant increase in the triglyceride level in girls compared to boys with the DD genotype of the ACE gene in group aged 6-9 years (P=0 02) We detected also the statistically higher triglyceride level in subjects with the S1S2 apoCIII genotype compared to those with the S1S1 apoCIII genotype in group aged 13-15 years (P=0 04) However, the association between the apoCIII genotypes and tryglyceride level was seen in girls only when the sexes were analyzed separately The serum cholesterol and the LDL cholesterol levels were significantly higher in subjects with the E3/E4 apoE genotype compared to subjects with the E3/E3 apoE genotype in group aged 16-18 years (P=0 02 and P=0 03, respectively)

9.085

Left ventricular mass among schoolchildren with different genotype of angiotensin-converting enzyme.

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Great attention is paid now to the possible positive linkage between insertion-deletion(ID) polymorphism of angiotensin-converting enzyme(ACE) gene polymorphism and heart diseases It was also indicated that the deletion polymorphism (DD) is associated with left ventricular hypertrophy, which is a risk factor for cardiovascular morbidity and mortality In this respect the purpose of present study was to investigate left ventricular mass (LVM) among 107 schoolboys with the DD, ID and II ACE genotypes We have taken 107 boys with age range from 5 to 17 years old (41 individuals with the DD genotype, 34 with the ID and 32 with the II genotype) for LVM measuring by means of echocardiography All individuals were healthy and without intensive training The genotype of ACE gene was determined by polymerize chain reaction All statistical analyses were made using the statistical soft STATGRAPHICS The comparisons of the following parameters between the ACE genotypes age, body mass index (BMI), systolic and diastolic blood pressures were conducted by the Kruskal-Wallis One-Way Analysis by Ranks Stepwise regression analyses were performed to identify variables for LVM and LVM/S, where S is the body surface area The analyses of the relationship of the ACE genotype to LVM and LVM/S were carried out by the analysis of covariance (ANOVA) with the age, BMI and systolic blood pressure as the covariates Among 107 boys were not any statistical differences in their age, systolic and diastolic pressures and BMI We have not detected any statistical significant differences in LVM between the II and ID, ID and DD subjects However, we have obtained the statistically significant decrease in LVM (P<0 03) and in LVM/S (P=0 03) in group with the DD genotype compared to those with the II genotype

9.086

Active screening for genetic pathology in the child population

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The study is focused on genetic characterization of the child population of the Orava region, where genetic isolates persisted as long as the middle of this century Active screening for genetic pathology over a period of 5 years involved examination of 1 058 children aged 0-14 years Genetically determined pathological conditions were diagnosed in 757 children, which represents 1 67 % of the child population of this region Chromosomal aberrations were established in 55 children (0 12 %), monogenic diseases in 193 children (0 43%), of these autosomal recessive conditions in 88 children (0 19 %), and multifactorially determined conditions in 478 children (1 06 %) Focal occurence of an inherited disease was not recorded Genetic load of the population studied was found to be comparable to that in panmictic populations The occurence of diseases inherited as autosomal recessive traitssuggests that the influence of genetic isolates does no long-er persist in Orava

9.087

Oculo-oto-radial Syndrome (IVIC Syndrome) in discordant identical Twins

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Arias et al (1980) described this autosomal dominant syndrome in 19 members of a Venezuelan family over six generations Features included radial defect of very varying severity , strabismus and mixed hearing loss LW, is one of a pair of twin girls delivered after a normal pregnancy She has bilateral radial aplasia and absent thumbs There is a mild scoliosis and severe sensorineural deafness General

development and eye movements were normal Karyotype 46, XX , Fanconi anaemia excluded by normal chromosome breakage studies Her twin sister is normal apart from strabismus Multi-allelic polymorphic markers showed the probability of monozygosity to be greater than 0 1 % Family history Father, his sister, their mother and maternal grandfather all have abnormally small thumbs with absent thenar eminence Other family members have hearing loss and strabismus The family probably has 7 affected members over four generations the majority being mildly affected We believe this family has the Oculo-oto-radial (IVIC) syndrome with very variable expression most interestingly shown in the MZ twins The variability in expression is therefore unlikely to be genetically controlled and the twinning process itself, or the very early intrauterine environment must provide the clues to explain this developmental dilemma

9.088

The example of application of the functional models of complex characteristics (statusmetry) for the analysis of the factors causing chromosome nondisjunction.

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For the construction of the models mathematical theory of states of complex natural objects was applied The methodical basis of this theory is statusmetrics, which applies the following methods computerised experiments methods of image identification, theory for the creation of models of states, methods of mathematical theory of experimentation, methods of ranging and selecting the most informative characteristics, methods of constructing scales of generalised characteristics for the complex analysis of the state of an object 35 parameters (parental and grandparental ages, obstetrical and gynaecological anamnesis, frequency of intercourses) were studied in 188 women delivered to newborns with Down syndrome (DS) confirmed cytogenetically and in 287 mothers of healthy newborns All women were interviewed in several days after delivery Sets of the most informative parameters were determinated and the significance of each factor was estimated quantitatively In young DS mothers (<30 yr) the most influencing factors are number of children prior to proband (0 6237), hormonal imbalance (0 3025), previous artificial abortion (0 2927), infrequent intercourses (0 2583) In aged mothers (30+ yr) the set of factors is quite different miscarriages and spontaneous abortions prior to proband (0 5158 and 0 3991), spontaneous abortions and other miscarriages after artificial abortions (0 2554 and 0 2083)

9.089

Low doses of ionizing radiation and chromosome anomalies in man.

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Chromosome anomalies are frequent hereditary pathology and have to be taken into account in estimation of genetic effects of hazardous factors including ionising radiation However, opinion is spread that no or few evidences of induction of chromosome nondisjunction by radiation exist Analysis of 26 published reports is presented The most pronounced differences in Down syndrome (DS) rate were revealed in the studies of populations living in areas with different background radiation Majority of the reports concerning effects of diagnostic radiation indicates possible connection between irradiation and chromosome aneuploidy Studies of the effects of the largest nuclear accidents are considered separately There are some reasons for escaping of examined results One of them - genetic differences between races and populations in predisposition to chromosome nondisjunction Analysis of the data on prevalence of DS over the world allows to accept such suggestion, revealing the lowest levels of DS in Mongolians, middle levels in majority of European populations, high levels in Bedouins, orthodox Jews and Latin Americans One of the proven effects of nuclear accidents is hormonal imbalance in children According to our recent data, hormonal imbalance and hypofunction may cause chromosome nondisjunction Hypothesis on the mechanisms of indirect effect of irradiation on chromosome segregation is suggested

9.090

Rare skeletal anomalies in Turner syndrome

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The clinical, cytogenetical, and roentgen study of 148 patients with Turner syndrome revealed both typical frequent and some rare bone and joint changes Congenital absence of left arm and phalangel hypoplasia and aplasia of right hand were in one case Deep acetabular cave is typical for Turner syndrome But 11 patients have, contrary, acetabular dysplasia, 10 of them with femoral head subluxation or luxation Besides, there were dislocations of radial head (2 cases), kneecap (2), ulnar head because of unlar shortening (1) In sum, the dislocations were found in 15 patients, 5 of them were operated Frequent features of this syndrome are exostoses of proximal tibial and fibular metaphyses But 3 patients had exostoses of other locations, multiple in 1 case Tibia vara was in 6 patients, one of them was discussed for surgical correction Total frequency of these rare dysplastic changes and frequency of luxations surpasses significantly the levels in general population Pathogenesis of these rare changes is different For some of them a combination of chromosomal and gene mutations and loss of an epistatic gene might be supposed

9.091

Congenital malformations in newborns in St. Petersburg

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Congenital malformations are the most serious infant pathology This group of diseases represents a significant part of stillbirths and early neonatal death and appears to be one of the reasons for disabilities in childhood In this connection a study of the epidemiology of congenital malformations in St Petersburg has been started In 1993 the rate of newborns with congenital malformations was 1 15% of the total number of newborns (33610 live births) The highest proportion of all cases comprises cardiac malformations (24 2%), the lesser - Down syndrome (12 1%) and anomalies of central nervous system (7 5%) Considerable increase in the population rate in comparison with 1992 was found for several pathologies gastroenteric atresia (from 0 28 to 0 68 per thousands), urogenital pathology (from 0 17 to 0 36 per thousands), limb reduction (from 0 2 to 0 53 per thousands) Differences in prevalence of congenital malformations between sexes exist 57% of malformed children were males The majority of malformed children /69%/ constitutes the firstborns, only 8 5% - the third and the fourth children in families On the basis of further analysis of numerous factors promoting the congenital malformations occurrence the measures for reduction of the congenital pathology in St Petersburg will be developed

9.092

Satellited Y-chromosomes and Y;acrocentric translocations: possible clinical implications

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Four cases of Yqs and six cases of Y,acrocentric translocations are reported Yqs were found in a boy with bilateral cryptorchism and in his father, in a boy with multiple stigmata, and in two males referred to cytogenetic examination because of habitual spontaneous abortions (SA) in their wives Translocations of heterochromatic region of Y-chromosome on acrocentrics were found in woman

delivered to child with multiple congenital malformations (Y,13), in father of child with Down syndrome (Y,14), in female with SA (Y,15), in husband of a woman with SA (Y,15), *in a criminal male* (Y,15), *in a male with 48, XXXY karyotype and in his* mother t(Y,15) Data from literature have been compiled Out of 27 cases of Yqs in 11 there were revealed numerical and structural chromosome aberrations in proband, and 2 cases of habitual SA Out of 76 cases of Y,autosome translocations 15 cases of chromosome aberrations, 4 cases of SA and 7 cases of abnormal sexual development In 4 cases the disturbances of sexual development were accounted by the absence of the second gonosome due to segregation of marker acrocentrics Thus, we consider the presence of either Yqs or t(Y,acrocentrics) to be a serious reason for referring the patient to prenatal diagnostics

9.093

Hormonal imbalance and Down syndrome occurrence

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It is well know that the risk of aneuploid conception increases with advancing maternal age Meanwhile, several age-independent factors favouring to chromosome nondisjunction are discussed Among them genetic predisposition resulting in reproductive failures preceding to aneuploid child and hormonal imbalance In St Petersburg the long-term multiparameter study on ethiology of aneuploidy is carried out, with the purpose to reveal the easily recognisable factors at-risk which could be controlled Nearly all mothers delivered of Down syndrome (DS) newborns in 1993-1995 have been interviewed in several days after delivery Mothers of healthy newborns delivered at the same time have been questioned as a control There were studied 188 mothers of DS children and 287 mothers of healthy children Pronounced differences were revealed between young women (<30 yr) by both irregular menstrual cycle (16 2% of DS mothers versus 6 0% of controls, P<0 006) and hormonal disorders (25 9% versus 8 8% P<0 01) In aged DS mothers (30+ yr) comparing to controls there was the higher rate of miscarriages after artificial abortions (37 4% versus 12 7%, P<0 009) which might be accounted by delayed recovering of hormonal status (hypo function) Additionally, in mothers of 35+ yr group the enhanced rate of miscarriages not connected with artificial abortions was revealed (37 8% versus 10 3%, P<0 007) There were no differences either in the rate of inflammatory gynaecological diseases, or in the frequency of fibromyoma of uterus

9.094

Application of home microcolumn HPLC for the diagnostics of the inherited metabolic diseases.

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The diagnostics of the inherited metabolic diseases requires the evaluation of various metabolic compounds amino acids (AA), their derivatives, carbohydrates, hormones etc This problem has been resolved with the home-made microcolumn liquid chromatographic device LC-1311 supplied with the spectrofluorimetric detection The fluoroplastic microcolumn (300 x 0,5 mm I D) is packed by Nucleosil-5-C_{18} The separation is fulfilled by gradient eluting Using the device we can identify 43 compounds in their DNS-derivatives forms The sample volume varies from 10 µl for AA to 500 µl for prostaglandins The analysis period ranges from 15 min for histamine to 60 min for AA These procedures have been used for analysing more than 400 body fluid samples from the children with mental retardation, psychoneurological disorders, defects of speech development Histidinemia, prolinemia, homocystinuria, hyperphenylalaninemia, distrubance of Tryptophan metabolism are revealed with high frequencies The observation of the children, confirming diagnosis, control of a treatment has been done in close co-operation with the Department of Medical Genetics of MAPE, Professor S Klueva head Using LC-1311 we will be able to study the population frequencies of the inherited metabolic diseases in St Petersburg and Province

9.095

On the development of Medical Genetic Service in Leningrad province..

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Medical Genetic Room (MGR) based on District Children's Hospital was organised in September 1992 according to the Order of Russian Ministry of Public Health on the development of Medical Genetic Service The MGR staff includes a geneticist, a cytogeneticist and a laboratory assistant They work in close co-operation with the Department of Medical Genetics of the MAPE, Research Centre of the MAPE etc Medical genetic service of Leningrad province includes 1 neonatal screening for phenylketonuria (PKU), congenital hypothyroidism (CH) and cystic fibrosis, 2 *selective screening for chromosomal and inherited metabolic diseases*, 3 second trimester prenatal screening for congenital defects (triple-test), 4 confirmation of diagnosis of hereditary disorders, 5 treatment and dispensary system for the patients with hereditary diseases, 6 genetic counselling, 7 organisation of the the Register of probands and their families Children population of Leningrad province is equal 318000, the number of annual deliveries - 12000 92,2-94,6% of the infants has been examined through neonatal screening PKU frequency 1 6600, CH frequency 1 4700 Owing to realisation of second trimester prenatal screening we have managed to prevent annually the delivery of 3 newborns with severe defects The Register includes 400 families of probands with congenital and hereditary diseases

9.096

Screening programmes as a routine practice of the Biochemical Laboratory of St Petersburg Centre of Medical Genetics (StPCMG)

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There are three directions in the routine work of the Biochmical Laboratory of StPCMG 1) Second trimester prenatal screening for congenital defects is based on the measurement of ÂFP and hCG in the maternal blood serum (enzymeimmunoassay, "Roche") Pregnant women with ÂFP value differing from normal data (after second test) both with low ÂFP and high hCG levels are composed a risk group referred to the Centre for Prenatal Diagnostics 2) Neonatal screening for phenylketonuria (PKU) and congenital hypothyroidism (CH) is done in dry blood spot collected from child heel on the 4-5 day of life PKU-screening is based on the assessment of Phe-Ninhydrine ("Fluoroscan"-IBM) The accepted cut-off value is equal 2 06 mg/dl CH-screening is based on the fluorimetric enzymeimmunoassay of hTSH ("Delfia"-IBM) The CH diagnosis is based on both elevated hTSH (above 20 U/ml) and decreased serum T_3, T_4 levels In 1991-1994 there were 183437 infants examined through neonatal screening programmes The PKU and CH rates coincide with European population values 3) Selective urinary screening is used as the first examination step when inherited metabolic disorders are suspected, further examination has been done under cooperation with different Laboratories and Research Institutes

Section continued

9.097
The search for genetic markers of predisposition to chronic bronchitis (CB).

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Chronic bronchitis (CB) is one of the wide-spread chronic non-specific pulmonary diseases Recent reports witness multifactorial nature and the role of genetic factors to the origin of CB That was why several genetic polymorphic systems (morphofunctional isoantigenic and biochemical) were investigated in 60 men with CB (aged 39-63) and compared with control data (1380 donors, the latter in brackets, %) The study of the $(GATT)_n$ polymorphism in intron 6a of the CFTR gene and Glutathione-S-transferase M1 polymorphism has been started Morphofunctional signs were polymorphic, bitter taste PTC-76(66) and dry cerumen-57(92) particularly, blood groups 0-32(32), A-32(41), B-27(20), AB-10(7), Rh(D)-89(83), M-19(35), N-24(13), MN-57(52), secretor status (+)-83(75), Hp_{1-1}-36(12), Hp_{2-2}-18(42), Hp_{1-2}-45(46), $\hat{A}_1$-proteinase inhibitor M_1M_1-51(63), M_1M_2-15(20), M_1M_3-15(12), M_2M_3-12(4), MZ-3(0,8), $(GATT)_6$/$(GATT)_6$-0(3,5), GSTM1-/GSTMI- - 29(39) On the basis of our data genetic structure of patients with CB has been revealed, which is essential for estimating the contribution of genetic factors to the origin of CB and for determination the complex of "small" genes of predisposition

9.098
Clinical genetics and music therapy: building a bridge

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The main problem for the children with hereditary disorders is the problem of adaptation, stimulation of their development and potential abilities In St Petersburg CMT music therapy (MT) has started as a method for rehabilitation and socialization of children with hereditary and congenital disorders These patients have behavioural and communicative delays, severe autism, mental retardation, hearing disorders, vision and speech impairment For these children music is of special meaning, being the only way they can "talk" MT is a creative active form of developmental, rehabilitative and psychotherapeutic treatment In clinical practice MT makes contact with patients unresponsive to traditional forms of verbal behavioural and pharmaceutics therapies Chidren with several diseases Down (3), Rett (1), Williams (1), Sinkler (1), Lesch-Nyhan (1), fra-X (3), phenylketonuria (3) are under our observation In the course of treatment of such patient the goals of MT sessions include eye-contact, attention span, on-task behaviour, awareness of self and other, motor planning, etc Sessions last 25-30 min, once a week Using various instruments we establish a music dialogue that allows handicapped children to express themselves Among the benefits of MT there are improvements in behaviour outside the sessions, improved interpersonal skills, greater confidence in everyday life

9.099
Chromosomal anomalies in patients with reproductive failures

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A chromosome analysis of lymphocytes was carried out in 387 patients 29 donors of sperm have the karyotype 46,XY, among 120 patients with oligozoospermia 1 man has 47,XXY, 4 - 45,XY,t(13,14), 1 - 45, XY,t(14,15), out of 76 patients with azoospermia 8 persons have 47,XXY, 3 - 46,XX,male, 1 - 45,XY,t(13,14); 1 - 46,XY,del(Y), among 40 men with astenozoospermia 1 has 47,XY,mar, 1 - 46,XY,t(1,22), 1 - 45,XY,t(14,21), one of 11 patients with teratozoospermia 46,XY,t(4,19), one of 18 fertile persons with normospermia one has 46,XY,t(14,Y), 11 infertile patients with normospermia have karyotype 46,XY Among 27 boys (aged under 18 yr) with anomalies of development 11 have normal karyotype, the rest have gonosomal abnormalities 2 - 47,XYY, 12 - 47,XXY, 1 - 46,XX/47,XXY (15%), 1 - 46,XX,male Six transsexual female>male patients have 46,XX, 2 male>female patients have 46, XY, astenozoospermia 23 women with infertile marriages have normal karyotype, among 24 girls and women with anomalies of development 7 have 46,XX, 5 girls were found to have 45,X, 3 - 45,X/46,XX, 7 - 46,XY An analysis of sex chromosome constitution was carried out in some cases for detailing of the diagnosis in situ hybridisation of X or/and Y-specific alphoid DNA probes in interphase, mitotic and meiotic nuclei of lymphocytes, spermatocytes, spermatids and spermatozoa (from semen)

9.101
Testing children to identify carriers of balanced chromosomal translocations: a retrospective, qualitative, psychosocial study

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The testing of children to identify carriers of genetic disiorders is controversial, but there is little evidence of the long-term effects on families of such testing This study set out to examine the consequences of identifying children as carriers of balanced chromosomal rearrangements by intervewing the tested individuals and their parents at least 10 years after the event Members of 10 nuclear families were interviewed, and the conversations were recorded, transcribed and analysed The analysis revealed that learning about carrier status causes a transient psychological disruption, which can be accompanied by feelings of discreditable stigmatisation Mothers who underwent prenatal testing experienced great emotional strain as a direct result of the procedure, which - for some - was not relieved by a favourable (unaffected) test result Many of the parents expressed dissatisfaction with the genetic counselling they had received in the past

9.102
Mutation detection in patients with X-linked agammaglobulinaemia (XLA) and detection of grandpaternal germline mosaicism

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X-linked agammaglobulinaemia (XLA) is an immunodeficiency characterised by absent B cells and hypogammaglobulinaemia It is caused by mutations in Bruton's tyrosine kinase (Btk) Around a third of cases are caused by new mutations The diagnosis of XLA can be difficult due to a range of symptoms which can overlap with other X-linked immunodeficiencies In sporadic cases the only way to confirm the diagnosis is by identification of a mutation in Btk We have been developing a mutiplex approach to screen all nineteen exons, including intron/exon boundaries, and the promoter of Btk in a minimum of reactions by single-stranded conformation polymorphism (SSCP) analysis The usefulness of mutation detection in XLA families will be illustrated by a large Spanish family in which the disease-causing mutation originated in the germline of the maternal grandfather of affected first cousins All the other daughters of this man were at risk of being carriers of XLA, despite having different mothers Identification of the disease-causing mutation in this family has, therefore, enabled carrier status to be resolved in the other daughters

9.103
Using mutation detection to improve the diagnostic service for ornithine transcarbamylase (OTC) deficiency.

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Ornithine transcarbamylase (OTC) deficiency is a severe X-linked disorder of the urea cycle The majority of affected males present with acute neonatal hyperammonaemia and death and females can often be affected There are several intragenic polymorphic markers which can be used for carrier and prenatal detection but many families remain uninformative Additionally, it is difficult to resolve carrier status in female relatives of isolated cases since there is a high new mutation rate in OTC deficiency We are currently screening all our patients for mutations in the OTC gene by a multiplex deletion screen combined with single strand conformational polymorphism analysis (SSCP) New primers have been designed to include the splice sites of all ten exons To date seven exons have been screened, SSCP shifts being found in 11 out of 27 patients and the putative mutations sequenced 5/27 patients showed deletions Once a mutation has been found it can be used to unequivocally determine carrier status in female relatives Early studies gave low mutation detection rates Following screening 66% of the OTC gene we have detected changes in 59% of our patients We conclude that multiplex deletion screening combined with SSCP analysis is a suitable method for detecting mutations in the OTC gene

9.104
Clinical phenotype in FRAXE

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FRAXE was recognised as a distinct fragile site distal to FRAXA in 1992 An expansion of a triplet repeat with aberrant methylation has been described in those with the fragile site, similar to the abnormalities in FMR-1 seen in fragile X syndrome Families with FRAXE have usually been ascertained because of mental handicap, and many had been included with fragile X syndrome before the molecular pathology was recognised The clinical phenotype in FRAXE is therefore not well defined Clinical features of 8 males (5 primary and 3 secondary cases) from 4 families with FRAXE are presented There were no consistent dysmorphic features, although one boy had bilateral microtia One boy had an isolated IgG subtype deficiency but no other serious medical complications were reported Growth was normal All males had a degree of intellectual handicap, formal IQ assessment on 5 showed functioning in the mildly handicapped range Psychological assessment did not demonstrate any consistent abnormalities in 5 males, although one had an additional diagnosis of autism There did not appear to be a behavioural phenotype Further studies are needed to clarify the phenotype in FRAXE FRAXE may be a cause of non specific mental handicap The poster will invite international collaboration to define the phenotype

9.105
Genetic modifications of expression of TRK genes family in human neuroblastoma

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There is increasing evidence that neurotrophins and their receptors play an important role in regulating development of both the central and the peripheral nervous systems Neurotrophins bind to the trk family of tyrosine kinase receptors and the low-affinity receptor adn this lead to the regulation of survival, growth and differentiation of developing neurons The trk family of neurotrophin receptors share approximately 85% homology in their tyrosine kinase domains and 50% homology in their extracellular domains Each member of the trk family is encoded by a single gene Our work was focused on identification of expression of three genes - TRK A, TRK B andTRK C in human neuroblastoma in which was/ was not found the deletion of 1p36 and amplification of MYCN protooncogene, which are very important prognostic markers for this solid tumor Together with last two mentioned genetic changes the expression of trk genes can also play an importante role in prognosis and therapy of human neuroblastomas Recently a new gene of trk family TRK E was described and it was suggested that this gene can be a non-neuronal receptor for nerve growth factor (NGF) We localised this gene on chromosme 6, precisely on 6p21 In our work we are also interested in expression of TRK E in human neuroblastoma and its potential involving in evolution of this type of human cancer

9.106
Myotonic dystrophy: ascertainment of patients in Northern Ireland.

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Myotonic dystrophy (DM) is the commonest inherited adult neuromuscular disease Our aim was to ascertain affected patients and families as completely as possible A genetic and epidemiological study in Northern Ireland (NI) resulted in the identification of 61 families *Methods* Multiple sources of ascertainment were used and included - (a)medical records in the Department of Medical Genetics, Belfast City Hospital since 1967 (b)findings of a survey of inherited muscle disease in NI, 1993-1995 (c)patients attending the NI Muscle Clinic, established in 1983 (d)list of affected patients known to the NI Muscular Dystrophy Group (1973) (e)a diagnostic index (1950-1960) maintained by the late Dr Louis Hurwitz, Consultant Neurologist *Results* Pedigrees were constructed for the 61 families A total of 188 individuals, including the index cases, were affected with DM, 21 were mildly affected, 151 had a classical presentation, and 16 had congenital DM All 61 families were offered genetic counselling, and CTG repeat analysis where appropriate The overall estimated prevalence rate of DM in NI is 114 4x10-6 *Conclusions* By using several methods, the ascertainment of DM in NI should be almost complete Given the nature of this condition, there will still be unidentified families in the population The NI prevalence rate is in keeping with rates reported for similar populations, and the uptake of counselling and molecular genetic analysis has allowed further delineation of 1/2 and 1/4 at risk groups

9.107
A large family with nonsyndromic craniosynostosis - clinical description and linkage analysis with candidate loci

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Craniosynostosis (CS) is defined as premature closure of one or more cranial sutures It is a manifestation of more than 60 syndromes, which are usually inherited as autosomal dominant traits Nonsyndromic unilateral (plagiocephaly) or bilateral (brachycephaly) coronal synostosis occur predominantly as sporadic traits, but families showing autosomal dominant inheritance with reduced penetrance have also been reported (Lajeunie, E et al 1995, Am J Med Genet 55 500-504) We present clinical data in respect of a family over 4 generations with nonsyndromic unilateral coronal synostosis Eight family members show plagiocephalus of variable expression, in six of them the synostosis is on the left side The deformity (plagiocephalus) of two sibs has been surgically corrected No family member with bilateral coronal synostosis was observed, and so far there is no evidence of reduced penetrance in this family We performed linkage analysis with the following loci corresponding to known CS syndromes FGFR1 (Pfeiffer syndrome), FGFR2 (e g Crouzon syndrome), MSX2 (CS type Boston), and the Seathre-Chotzen syndrome locus near D7S507 and D7S664 The nonsyndromic CS segregating in this family was excluded from these loci Analysis on further loci (GLI3, Greig syndrome, D4S115-D4S394, CS type Adelaide) is in progress

9.108
Genetic counselling of patients with Kennedy's disease using DNA analisys.

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Kennedy's disease or spino-bulbal muscular atrophy (SBMA) is an X-linked recesive disease Most patients show the early signs of disease when they are 40-50 years old The typical symptoms of disease are facial muscle weakness, tongue muscles atrophy, fasciculations affecting tongue, perioral and body muscles, peripheral tetraparesis which is more significant in the proximal parts of extremities, dysphagia, dysartria, finger tremor and cramps Affected males frequently exhibit gynecomastia, redused potency, testicular atrophy, redused fertility Kennedy's disease is caused by defective androgen receptor (AR) gene, which was mapped to Xq 12-21 The mutation is connected with expansion of the (CAG)n repeat in the first exon SBMA patients show roughly double the number of CAG repeats in contrast to an average repeat number We have studied 37 patients affected by Kennedy's disease 18 of them had affected relatives, the other 19 were classified as sporadic cases The age of onset ranged from 12 to 54 years The most often early symptoms of disease were fasciculations, gynecomastia, potency alterations, cramps, i e complaints that didn't make the patients appear to neuropathist or stayed unnoticed being ignored as the signs of future disease Gynecomastia was shown by 80% of our patients, lack of potency by 73%, increased estradiol values by 71%, decreased testosteron values by 28% Sperm was analized in 12 cases Only 1 result was normal, while 4 males showed aspermia Kennedy's disease may be diagnosed using DNA analisys methods which is especially important in diagnostically difficult and nontypical cases Besides, DNA analisys enables revealing of carrers among the patient's relatives These methodes enable carring out the DNA diagnostics in 15 families We identified 5 female carriers and revealed 2 boys with mutant gene, who were the sons of female carriers

9.109
DNA analisys in families with fascioscapulohumerial muscular dystrophy from Russia.

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Fascioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant neuromuscular disorder with a prevalence of 1 per 20000 The clinical symptoms of this disorder are progressive weakness and dystrohy of facial, shoulder girdle and proximal parts of extremities muscules The FSHD gene was mapped to 4q35-4q ter in 1990 The distance between this gene and the group of markers cen - D4S171 - F11 - D4S139 - FSHD - tel is 4 cM Recently the DNA markers of the distant part of 4q35 locus have been described cen - D4S139 - D4F35S1 - D4F104S1 - D4Z4 - tel The gene coding sequence has not been revealed, but it was shown that deletions of an integral number of D4Z4 tandem repeats 3 3 kb long are associated with FSHD Polymorphic markers inherited together with the FSHD gene may be used for presymptomatic and prenatal DNA diagnostics They may also be used in studies aimed to find out whether the disorder is genetically geterogeneous or not To solve this problem, the clinico-labaratory blanks for patients with FSHD were worked out taking into account the European expert group recomendations and the bank of DNA of affected persons and their relatives was collected In present the bank of FSHD DNA includes 65 samples 41 of affected patients from 33 families among which in 10 there were sporadic cases and in 6 there were more than 2 affected per family Microsatellite marker D4S171 typing was made for 20 FSHD families We studied D4F104S1 marker which shows at least two-allele polymorphism according to publications We measured the length of amplified fragments of this marker in denaturing and nondenaturing gels using the methods of heteroduplex analisys and SSCP In the groups of 10 affected and 18 unaffected persons it was shown that D4F104S1 did not exhibit any polymorphism All the studied individuals had two DNA fragments with different mobility in gel These two fragments are presumably the products of amplification of two different polymorphic loci

9.112
The incidence of limb reduction defects in the German Collaboration CVS Study.

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In the German Collaborative CVS Study a total of 13 observations of distal limb defects was reported after the 1st trimester CVS among 11660 liveborn children (= 0 11%) A uniform pattern of distal limb reduction emerges from 10 of the 13 cases (= 0 09%) ranging from transverse defects at the metacarpal/metatarsal level in its most severe form over defects or hypoplasias at different phalangeal levels to simple phalangeal hypoplasia of single or multiple digits The incidence figure of limb reduction defects emerging from the German CVS data exceeds the incidence in the general population of 0 02% as reported by the US National Institute of Child Health (1993) as well as that of 0 06% as obtained from the British Columbia Study (Froster-Iskenius and Baird 1989) It is in accordance with the CVS data of the Gruppo Italiano Embrio-Fetali (1993) Relating the limb reduction defects after CVS to the gestational week at biopsy there were a low incidence (0 03%) in the 11th week and later but increased incidences of 0 19% (10 weeks) to 0 26% (8 weeks and before) in cases of earlier biopsies It has meanwhile been accepted that CVS should be performed only after the 10th gestational week to avoid this possible risk of the procedure

9.113
Goltz syndrome with atypical manifestation in the newborn

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The Goltz syndrome (focal dermal hypoplasia) is a multisystem malformation syndrome charactarized by widespread dysplasia of meso- and ectodermal structures Mean features are focal areas of underdevelopement of the dermis as well as skeletal defects almost invariably associated with visceral abnormalities The syndrome is presumed to be transmitted in an X-linked dominant mode of inheritance attended with male hemizygote lethality However, several authors presented affected women just as a number of cases of affected men The survival of men is explained by different hypotheses as i e non-lethal autosomal new mutations or mosaic status We report on a newborn seriously afflicted female with symptoms compatible with Goltz syndrome and with additional atypical and severe mostly visceral malformations Beside other rare anomalies such as aortic arch anomaly, diaphragmatic hernia, spina bifida occulta and partial aplasia of corpus callosum we found a thoraco-gastroschisis which has not been reported before Possible pathogenetic mechanisms responsible for the phenotypic expression and variability in Goltz syndrome will be discussed

9.114
Functional analysis of the Huntington disease gene product

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Mutations in the huntingtin (htt) gene, i e expansion of a translated (CAG)n-repeat to 37-120 units, have been shown to cause Huntington's disease (HD) The htt-gene has no homologies with other genes Consequently, the functions of the normal and the aberrant gene product remain elusive Detailed studies of the

protein, its expression, its interactions with other proteins and its subcellular distribution in normal and in affected individuals should elucidate its function and the resulting specific cellular pathology We have raised a set of polyclonal antibodies against htt, using both synthetic peptides and fusion proteins On Western blots, affinity purified antibodies detect a band consistent with the size of huntingtin Two bands are resolved in HD-patients, slightly variable in size between patients Immunofluorescence and biochemical cell fractionation studies, showed both a cytoplasmic and in some cell types (e g neurons) a nuclear signal This is consistent with our first results reported in 1993, but in contrast to other studies which failed to show the nuclear signal Transient presence of htt in the nucleus would allow for a role in the regulation of other genes, in addition to functions in the cytoplasm To study this discrepancy further, we have sought to identify proteins interacting with various domains of htt using the yeast-two-hybrid system The results of the ongoing analysis will be presented

9.115
Prenatal diagnosis of dominant chondrodysplasia punctata

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A primigravida 36 old lady has been referred to the ultrasound Department of the University maternity hospital of Sofia because of suspicious fetal ventriculomegaly The brain abnormality has been confirmed and, in addition, femur asymmetry and chest abnormalities have been detected At this stage the family was referred to the genetic counseling unit for dysmorphological expertise The examination of the mother disclosed short stature, dry skin, sparse, dry hear and bilateral cataract The diagnosis of dominant CDP has been made and the parents decided to terminate the pregnancy On clinical, X-ray and pathological examination the male fetus showed moderate hydrocephaly, body asymmetry with shortening of the right femur and kyphoskoliosis, as well as multiple periarticular and perivertebral calcifications The biochemical studies are in progress, but most probably this is a dominant case of CDP, Conrady - Hunermann form with varying expression in the mother and son The presence of hydrocephaly is unusual and remains unexplained The experience with this family is a good example of the potential of US examination in what is called prenatal dysmorphology, which requires close cooperation between the obstetrician ultrasonographer and the clinical geneticists

9.116
Interstitial deletion del (2)(q31q33)

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Two interstitial deletions del (2)(q31 2q32 3) and del (2)(q31q33) were found in a 3 years old girl and 4 years old boy respectively, both with psychomotor and growth retardation, dysmorphic facial features, dental malocclusions and clinodactyly The authors compare these cytogenetic and clinical findings with those of 10 previously reported cases with del (2)(q31-33) There are many common features in all reported cases mental and growth retardation, microcephaly, eyes malformations (microphtalmia and/or ptosis and/or antimongoloid slant and/or hypertelorism), large or low set ears, beaked or prominent nose, cleft palate, micrognathia, clinodactyly and/or camptodactyly of the fingers, equinovarus, syndactyly of the

9.117
CT scan in dysmorphic syndromes

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1299 patients with various MCA/MR syndromes have been seen and managed over a period of 3 years/1992-1995/ In 266 of them/20 5%/ a CT scan was performed in an attempt to highlight the extend of phenotypic involvement According to the primary genetic defect,the patients could be grouped as follows chromosomal abnormalities - 33/12 4%/,single gene defects - 51/19 2%/,known syndromes - 37/13 9%/,environmentally determined conditions - 10/3 8/,unknown aetiology - 135/50 8%/ Seventy eight patient examined/29 3%/ proved to have CT scan changes due to a ventricular system,parenchymal or vascular abnormality Among them 7/9%/ had chromosomal defect,16/20 5%/-single gene disorder 12/15 4%/ - sporadic syndrome,5/6 4%/ - hypoxemic-ischemic encephalopathy and 38/48 7%/ - unknown dysmorphic syndrome In 14 patients/17 9%/ the brain anomaly was the only major malformation Having in mind the low proportion of metabolic disorders/1 9%/ in the examined cohort,we were unable to find any specific CT scan changes in different groups of MCA/MR syndromes The only fact to be stressed out is the relative predominance of ventricular system abnormalities in chromosomal and single gene disorders It is also noteworthy to emphasise the substantial discrepancy between the results of ultrasound and CT scan examination,the latter being considerably more precise and valuable

9.118
Clinical and biochemical controversies in NPC disease

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Nieman - Pick disease, type C is an autosomal recessive lipidosis, characterized by lisosomal accumulation of unesterified cholesterol Most patients manifest progressive neurologic deterioration in the late childhood ,although other phenotypes have been observed including neonatal liver disease, early infantile onset with hypotonia/delayed motor development and adult variants with psychosis and demential A 9 years old girl with splenomegaly, tremor, ataxia, normal intelligence and diagnosis of Gaucher disease had been referred to the section of clinical genetics for enzyme replacement therapy and prenatal diagnosis of the disease in the advancing second pregnancy of the mother The diagnosis was made by the referring doctor on the grounds of existing splenomegaly and decreased activity of beta-glucosidase in leukocytes and skin fibroblasts The detailed clinical analysis however, raised some doubts in the diagnosis because of the history of neonatal liver disease and presence of vertical supranuclear ophtalmoplegia The biochemical reexamination confirmed low level of beta-glucosidase in skin fibroblasts, but also demonstrated clearly lisosomal cholesterol accumulation after filipin staining both in the patient culture and amniotic fluid cells Thus, NPC disease has been proven in the proband and fetus and the parents decided to terminate the pregnancy

9.120
A survey of phenotypic features in juvenile polyposis

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Background/Aims Extracolonic phenotypic abnormalities are well documented in patients with familial adenomatous polyposis and Peutz-Jeghers syndrome Though described, extracolonic abnormalities have not been clearly defined in juvenile polyposis Our aim was to determine whether patients with juvenile polyposis consistently exhibit extracolonic phenotypic abnormalities and whether there was evidence of other known genetic syndromes in these patients Methods Twenty four patients with juvenile polyposis were examined clinically and those who consented to further investigations had X-rays of skull, chest and hands and if clinically indicated, an echocardiogram Results Extracolonic phenotypic abnormalities were present in 19 (79%) patients and included dermatological (14), skeletal (12), neurological (6), cardiopulmonary (4), gastrointestinal (3), genitourinary (4), and ocular (1) features Nine patients had abnormalities of clinical significance Five patients had features of a genetic syndrome in addition to juvenile polyposis which included Bannayan Riley Ruvalcaba syndrome (2), Gorlin's syndrome (2), and hereditary haemorrhagic telangiectasia (1) Conclusions Extracolonic phenotypic abnormalities were found in over three quarters of the

patients with juvenile polyposis and were of clinical importance in over one third One fifth of patients had features of a well defined genetic syndrome

9.121
Prenatal diagnosis of Turner syndrome :about 22 cases

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Between 1986 and 1995, in Rennes (Britanny) we have done 22 prenatal diagnoses of Turner syndrome The mean of maternal age was 28 7 (+ 10) and the mean of the term was 19 weeks (+ 11,3) (with 2 late diagnoses at 27 and 34 weeks, where the single ultra sonic sign was intra uterine growth retardation) The first ultra sonic sign was a kystic hygroma in 16 cases (72 %) , it was associated with intra uterine growth retardation in 4 cases (25 %) with oligoamnios in 5 cases (31 %) , a foetal ascitis was the first sign in 2 cases, an intra uterine growth retardation in 2 cases The other ultra sonic signs were hydrops ascitis (7 cases) pleural (4 cases) or pericardic extravasation (3 cases), oligoamnios (8 cases), intra uterine growth retardation (8 cases), renal dysplasia (1 case) The diagnosis was a discovery of the caryotype prescribed for maternal age in 2 cases , in one case the ultra sonic examination was prescribed for an increased hCG The caryotype was 45,X in 17 cases (77 %), a mosaic in 5 cases, got after an amniocentesis (19 cases), foetal blood sample (2 cases), CVS (1 case) The outcome of the pregnancies was an abortion in 17 cases (77 %), an intra uterine foetal death in 2 cases , the pregnancy proceeded on in 3 cases (13 %) these 3 pregnancies will be detailed

9.122
A Search for uniparental disomy in Sotos syndrome_

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Sotos syndrome was described in 1964 and since that time over 200 hundred cases have been reported In 1994, Cole and Hughes, reported the largest single series of 40 cases and defined the diagnostic criteria The criteria were - characteristic facial appearance and three of the following, height greater than the 97th centile for at least 1 year, head circumference greater than the 98th centile, developmental delay and bone age greater than the 90th centile In addition the authors noted that mendelian inheritance and structural anomalies were rare but behavioural abnormalities were very common This constellation of symptoms are similar to those seen in cases of murine uniparental disomy (UPD) reported by Catternach and Kirk (1984) and Fergusson-Smith et al (1991) and in human syndromes known to be caused by UPD, for example Prader-Willi syndrome, Angelman syndrome and Beckwith-Wiedemann syndrome (Clarke 1991) We therefore began a search for UPD in Sotos syndrome using VNTR southern blotting and PCR analysis Initial studies concentrated on candidate regions, identified because of de novo translocations, in particular 3p and 6p in light of the patient reported by Schrander-Stumpel et al Results to date have not confirmed UPD in these candidate regions In order to broaden our screen to include all the autosomes, bar chromosome 15, we used the PCR multiplex system developed by Genethon in Paris Using 112 highly polymorphic microsatellite repeats, a minimum of three per autosome screened, no Sotos affected individual, in a panel of 29 cases, displayed whole chromosome UPD We can conclude that if whole chromosome UPD contributes to Sotos syndrome its frequency is less than 12 8% (95% confidence interval) assuming 100% detection with this methodology

9.124
Familial cases in Angelman syndrome: clinical and biological studies.

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A series of 69 Angelman syndrome (AS) patients, from 62 independant families, has been studied in the Center of Neuropediatrics and in the Department of Medical Genetics of Marseille While familial cases are rarely described in data, in our series, we found 13 subjects from 6 independant families (sibs, cousins and grandcousins) Every patient has been evaluated by precise clinical, cytogenetic and molecular protocol - 38 subjects (55%) presented a classical deletion in 15q11-q13 region including 2 subjects with a de novo unbalanced translocation - 3 paternal isodisomies were found (4,3%) - 2 patients presented abnormal methylation status with probe PW71/Hind III+HpaII (2,9%) - 26 subjects (37,8%) in 19 families exhibited neither deletion, nor disomy or abnormal methylation All of the familial cases were classified in this group Comparing clinically the familial cases to the sporadic cases with the classical 15q11q13 deletion, we found a significant difference in the severity of the clinical features By molecular analyses, we demonstrated that the same maternal chromosome 15q11q12 markers were inherited by all the affected children in the same family In these families, the model of the transmission was autosomic dominant with imprinting And a genetic counselling became possible

9.125
Features of Kabuki syndrome in a patient with a X-Y translocation

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Several chromosomal localisations and candidate regions have been proposed for Kabuki syndrome, on the basis of chromosomal rearrangements occurring in patients with features of the syndrome Autosomal rearrangements concern chromosomes 4, 6, 15, 17 and 13 In an other hand, similarities between the phenotypes of Turner and Kabuki syndromes have been reported many times Three patients exhibited structural abnormalities of sex chromosomes rX (p11 2-q13), inv Y (p11 2-q11 23), rY (p11 2-q11 2) We report on the case of a girl who was referred at age 6 for short stature and facial dysmorphism She was born to healthy unrelated 22 year-old mother and father Pregnancy and delivery were normal Birth weight was 2500g, height 45 cm and HC 31 5 cm The clinical course was complicated by recurrent urinary infections A small VSD spontaneously resolved Psychomotor development was slightly delayed At age 6, she had growth retardation (-2SD) Under examination, the girl exhibited facial dysmorphism with large palpebral fissures, sligth eversion of the lower eyelid, strabismus, large and prominent ears The neck was short but there was no webbing The hands were small with short IVth and Vth metacarpals Cellioscopy demonstrated the presence of bilateral streaks-gonads Chromosomal analysis was abnormal with an X chromosome being replaced by a X-Y translocation (p11-p11) C-banding and in situ hybridization with probes specific of X and Y centromeres showed that both centromeres were present This abnormal chromosome was found in 80% of lymphocytes and 98% of fibroblasts The remaining cells had a 45X karyotype This girl exhibit a clinical picture compatible with both phenotypes of Turner and Kabuki syndromes This observation further supports the hypothesis of a candidate locus for Kabuki syndrome on the sex chromosomes

9.126

Haptoglobin genetic polimorphism: Association with essential hypertension and acute stroke

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Haptoglobin (Hp) phenotype Hp 1.1 was described associated with salt sensitivity and hypertension Hp 2 2 with peripheral cardiovascular risk in hypertension Our purpose was to look for an association between one of those phenotypes and cardiovascular risk of stroke. Polyacrilamide gel (PAGE) electrophoresis was the method used for the determination of Hp polymorphism We studied 160 normotensive individuals and compared them with 155 hypertensive patients We also studied a subgroup of 45 patients with acute stroke (>65 years old) and compared them with 44 normotensive individuals and 124 elderly hypertensive patients without stroke We found in general group a frequency of allele Hp1 in hypertensive patients and controls respectively of 0,40 and 0,36 We found a frequency of the different phenotypes in elderly hypertensives was 8,1% (n=10), 56,9 (n=73) and 33,0 (n=4l) and in controls 15,9% (n=7), 50% (n=22); 34% (n=15) respectively for Hp1 1, 2 1, 2 2 The patients with acute stroke had the frequency of these alleles respectively of 15,6% (n=7), 24% (n=24) and 31,1% (n=14) When compared these patients with those with hypertension of the elderly without stroke we observed a $c_1 2=5,74$ ($p<0,05$) This data suggests that elderly hypertensive patients with Hp1 1 or Hp2.1 are more stroke prone than individuals with Hp2 2 Sodium sensitivity of blood pressure can signify a greater risk of stroke in general hypertensive population and this polymorphism can help in the prognosis evaluation

9.127

A case of spondylometaphyseal dysplasia and deafness

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The spondylometaphyseal dysplasias are a group of condrodystrophies caracterised by irregularity of long bone metaphyses associated with platyspondylia We describe a 4 5-year old girl with short stature, bowed limbs and deafness Her parent are normal and consanguineous She had neurosensory type of deafness Bone X-rays revealed spondylometaphyseal dysplasia resembling the Kozlowski type This type includes platyspondylia, short squared iliac wings, flat and irregular acetabulum, irregular rachitic-like metaphyses, hypoplastic carpal bones Although deafness has not been previously reported in this type of metaphyseal dysplasia, her clinical picture suggests a new variant of autosomal recessive inherited spondylometaphyseal dysplasia with deafness Another possibility is that deafness and skelatal dysplasia are the separate inherited entities in this patient

9.128

Evidence for a critical region for ambiguous genitalia and imperforate anus within or adjacent to chromosome 13q34

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Ambiguous genitalia have been described with various chromosome aberrations including partial monosomy of human chromosome 13 We describe three unrelated individuals with very small distal deletions of chromosome 13q and strikingly similar phenotypes of shawl scrotum or penoscrotal transposition, penoscrotal hypospadias, a reduced perineum, anal atresia, and mental retardation Cytogenetics and FISH indicated deletions of chromosome 13q32 2qter, q32-q33q34, and q34qter, respectively. Euchromatin from other chromosomes was not involved in the chromosomal rearrangements Molecular findings will be presented elsewhere at the meeting Brown et al recently defined a "critical region for severe malformations" on chromosome 13q32 (AJHG 57 859-866) and proposed that the most distal deletions, involving 13q33q34, are not associated with gross malformations (AJMG 45 52-59) However, our findings and the literature indicate for the 13q33q34 deletion a high risk of about 40 % for minor anogenital malformations such as hypospadias, biseptate uterus, or reduced perineum, and a substantial risk in the range of 10 % for major malformations e g penoscrotal transposition, imperforate anus, or common cloaca The findings indicate the presence of gene(s) which regulate the development of the ano-genital structures within or adjacent to chromosomal band 13q34

9.129

A case of Lenz microphthalmia syndrome

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Lenz microphthalmiz syndrome was first described by Lenz in 1955 The cardinal features of the syndrome are microphthalmia or anophthalmos, narrow shoulders, other skeletal anomlies, dental and urogenital malformations Here we present a case of Lenz microphthalmia syndrome which shows its typical characteristics and, additionally, corpus callosum dysgenesis assoicated with dilatation of the lateral ventricules The patient, a 13 year-old male was refered to our hospital by a dental hospital for genetic counselling On physical examnination height, weight and head circumference were below the third centile He had brachio-microcephaly, preauricular tage, microphthalmia, teeeth missing , narrow shoulders, long and proximally placed thumbs, hypospadias, criptoirchidism and a normal IQ level Opthalmological examination showed microcornea, sclerocornea, absence of the pupil, no vision in the left eye with decreased vision (4/10) and a small pupil in the right eye in addition to his bilateral microphthalmia Cranial MRI revealed dilatation of the laeteral ventricules and corpus callosum dysgenesis

9.130

DOOR syndrome: another case without elevated excretion of 2-oxoglutarate.

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DOOR syndrome is a MCA-MR syndrome characterized by profound sensorineural deafness, onychodystrophy, hands and feet osteodystrophy, and mental retardation Important variability occurs in its neurological manifestations from severe seizures and profound mental retardation to milder developmental delay Patton and al (1987) observed in three severely retarded patients an elevated excretion of 2 oxoglutarate in urine and plasma A young Algerian girl born of non consanguineous parents was referred to us because of MCA-MR syndrome She presented with typical DOOR syndrome with moderate psychomotor retardation without seizures Metabolic investigations including, blood and urine aminoacids, ketone bodies, lactates and pyruvates, urinary organic acids (GC/MS), blood carnitine, did not reveal any abnormalities, especially 2 oxoglutarate excretion was normal The basis of the neurological variability is not known In the literature, the excretion of 2 oxoglutarate was elevated in four severely retarded patients and normal in two others with mild neurological symptoms This report support the hypothesis that oxoglutaric aciduria is not constant in the DOOR syndrome and seems to be related with severe phenotype

9.131

Schinzel-Giedion Syndrome: A case with absence of tears

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Schinzel Giedion Syndrome (SGS) is a rare MCA-MR condition It is characterized by a typical dysmorphy (coarse facies with midface retraction and bitemporal narrowing), redundant skin, hypertrichosis, urogenital anomalies (ie hydronephrosis), poor skull vault mineralisation, wide occipital synchondrosis, variable anomalies of long bones, and severe developmental delay with seizures The syndrome is usually lethal in the first 2 years of age We present another case of typical SGS in a boy born of non consanguineous parents The characteristic findings of SGS were associated with absence of tears There was neither family history of alacrimia, nor others eye anomalies which could explain it, nor dysautonomia Thus we consider this finding as a possible additional manifestation of SGS, although it has not been described in the previously reported cases

9.132

Cri du chat syndrome: the changing phenotype when patients get older

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The Cri du chat or 5p- syndrome is a well delineated clinical entity with an incidence in newborns of 1 in 50 000 In 85 % of the patients the deletion of the 5pter region has arisen de novo The remaining 10 - 15 % are familial cases were 90 % of them is the result of a parental translocation and 5 % is due to an inversion of chromosome 5 Although the size of the deleted fragment varies between patients, it appears that the critical segment to present the typical Cri du chat phenotype is caused by a deletion of the 5p15 2 region In situ hybridisation techniques and molecular studies may further improve our insights into the relationship between the extend of the deletion and its phenotype In infancy and young children the clinical picture is well known and includes a characteristic high shrill cry, psychomotor retardation, failure to thrive, microcephaly, and facial dysmorphism (round facies, hypertelorism, broad nasal bridge, downslanting palpebral fissures and micrognathia) With advancing age the clinical phenotype becomes less striking This is demonstrated in 7 adult patients, all institutionalized, varying in age between 17 and 45 years In the adult microcephaly is present and the face is rather coarse and long, with macrostomia and prognathia Premature greying of the hair was seen occasionally In some of them, especially when seen for the first time at adult age, initially Angelman's syndrome was considered, because of craniofacial features, atactic movements, severe retardation and no speech The far majority of our patients had behavioral problems with aggressive disturbances, selfmutilation and were severely retarded We are aware this is a selectionbias, because all patients were selected from studies in an institutionalized population The value of early diagnosis and early intervention programs, as stated before, suggests that at least a part of all patients with a 5pter deletion can stay ambulatory with social behavior

9.133

Readjusting the localization of Long QT Syndrome gene on chromosome 11p15.

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Long QT syndrome (LQT) is an autosomal dominant cardiac disease characterized by ventricular arrythmia that causes syncopes and sudden death Three loci have been mapped to 11p15 5 (LQT1), 7q35-36 (LQT2) and 3p21-24 (LQT3) The first locus for LQT has been identified on chromosome 11p15 5, closely linked to HRAS To refine its location, microsatellites were genotyped in eight French families and the following order was determined tel - HRAS - DRD4 - D11S922 - D11S4046 - IGF2 - INS - TH - D11S1318 - D11S1323 - D11S1338 - D11S909 - D11S1346 - cen By haplotype analysis, twelve crossing-over events were identified in affected and unaffected subjects Combined linkage and recombinant haplotype analyses of microsatellite markers establishes D11S1318 and D11S1323 as the flanking markers for the LQT1, which is 5 cM proximal to HRAS These results exclude not only the genes for IGF2, INS and TH, but also potassium channel genes KCNA4 and KCNC1 The gene for LQT1 is now confined in a precise interval of 7 cM, where we identified new highly polymorphic microsatellite markers It appears crucial to reexamine individuals in all the families linked to chromosome 11p15 5 for presymptomatic diagnosis of this potentially fatal, but treatable, disorder

9.134

Readjusting the localization of merosin (laminin a2-chain) deficient Congenital Muscular Dystrophy on chromosome 6q2 : application to prenatal diagnosis.

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Mutations in laminin a2-chain gene (LAMA2) are responsible for merosin-deficient congenital muscular dystrophy (CMD) cases These patients form a clinically homogenous group and represent about 50 % of the CMD cases LAMA2 gene has been localized on 6q22-23 and the disease locus has been previously mapped in a 16 cM interval in chromosome 6q2 by homozygosity mapping We established, by haplotyping 21 microsatellites markers in 18 consanguineous families, and by radiation hybrid mapping, that LAMA2 gene is more centromeric than previously thought between the flanking markers, D6S407 and D6S1705, distant of 3 cM In this interval the microsatellite D6S1620 is homozygous for all patients These three markers highly polymorphic and informative for most of the merosin-deficient CMD families can be used for prenatal diagnosis in consanguineous and in non-consanguineous families with one affected (CMD) child with complete merosin deficiency In addition, we identified several LAMA2 polymorphisms which are very useful to confirm the haplotype's transmission

9.135

Anticipation of age-at-onset in familial amyloid neuropathy-typel (FAP-I): could it be associated with a genetic modifier of a dynamic-mutation type?

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In early genetic studies of FAP-I, anticipation was dismissed as a statistical illusion (Becker et al, 1964) We have previously shown that there was a significant overall anticipation (average 6 years), moreover, sons of affected mothers showed the largest anticipation (7 9 years), whereas daughters of affected fathers had, on average, onset 0 79 years later than their fathers This study now includes 770 affected parent-offspring pairs registered at CEP We have addressed (1) the regression of anticipation on the parent's age-of-onset, (2) the problem of simultaneous onset in parent and offspring, and (3) the search for complementary pairs Anticipation did not show regression to the mean the value corresponding to an anticipation of zero (28 6 yrs) was well below the average age-of-onset in the affected parent (36 8 yrs) After excluding all pairs with simultaneous onset (±5 years), mean anticipation of 5 1 yrs Complementary pairs could not be found, i e, no early-onset case (<30 years) was found among parents of late-onset patients (≥50 years) Thus, anticipation seems to be due to more than ascertainment biases FAP is associated with a classic point mutation, but still presents anticipation,

sporadic cases and incomplete penetrance Thus, we speculate that a closely linked modifier, maybe an unstable DNA sequence, could be responsible for the large variability found between generations

9.136

The sudden infant death syndrome (SIDS) in Portugal: preliminary results of a retrospective study (1979-1994)

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The sudden infant death syndrome (SIDS) is the main cause of post-neonatal mortality in developed countries SIDS occurs between 1M-1Y, peaking 2-4M, usually an isolated finding, it may occur in sibs We observed (MIM 107640) 6 cases of SIDS (one autopsied) and 3 cases of apparent life-threatening events, related to central sleep apnea, in two generations of one family We now present preliminary results of a retrospective study of SIDS at two forensic institutes Our aims were to identify risk factors and study frequency in relatives Beckwith's definition (sudden death not clinically expected and not explained at autopsy) and a medico-legal register of SIDS were used to review clinical history and autopsy reports of 97 cases Our results indicate i) a general increase of SIDS cases, ii) higher frequency in males (65%), iii) 62% cases between age 1-4M, iv) peak incidence during winter (57% December through March) and weekends (43%) A *prospective study will identify new probands, with extended autopsies and death-scene investigation,* in order to identify main epidemiological risk factors and estimate the heritability of SIDS

9.137

Linkage of Human Non-Insulin Dependent Diabetes Mellitus to the phosphoenolpyruvate Carboxykinase gene Locus on chromosome 20

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Linkage analyses were performed between four candidate genes for insulin resistance, glycogene synthase (GSY), insulin receptor substrate-1 (IRS-1), apolipoprotein C-II (APOC-II) and the phosphoenolpyruvate carboxykinase (PCK1), and NIDDM in 55 large multigenerational NIDDM families No linkage was found (p>0 05) using affected sib pair analyses when GSY, IRS-1 and APOC-II were investigated However, an indication of linkage was found between NIDDM and the PCK1, in sib pairs with overt diabetes whatever the age at diagnosis considered (n=135, p=0 01), and was more significant among affected sib pairs with overt diabetes diagnosed at age <-45 years (n=24, p=0 001) These 55 families were pooled with 115 nuclear NIDDM families giving a total number of 170 NIDDM families The results remained significant in all diabetic sib pairs (n=282, p=0 02) as in those with overt diabetes diagnosed at age <- 45 years (n=53, p=0 0003) Evidence of linkage was also found between NIDDM and two markers (D20S100 and D20S196) located 5 2 and 14 4cM proximal to PCK1 respectively, in sib pairs with overt diabetes diagnosed at age <- 45 years (n=50, p=0 004 and n=51, p=0 006 respectively) our results suggest a contribution of a gene located in the PCK1 region on chromosome 20, to the development of diabetes in NIDDM patients with an age of onset <- 45 years As recently reported in transgenic mice, a genetically induced overexpression of PEPCK enzyme, may increase hepatic glucose production leading to severe fasting hyperglycaemia in humans

9.138

X-linked recessive aortic coarctation in 2 families.

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The authors present a 1 year old boy seen for genetic counseling of congenital aortic coarctation Aortic coarctation was diagnosed at one month of age with a 3/6 systolic flow murmur with absent femoral pulses discovered on routine physical and confirmed by ultrasonography The presence of arterial hypertension prompted surgical correction at 3 months of age The boy has a normal physical examination, growth and development without any dysmorphic features at the age of one year His mother has a normal cardiac examination and a normal cardiac ultrasonography Her father was noted to have mild aortic coarctation of the isthmus with subvalvular aortic stenosis on catheterisation His malformation was revealed by a heart murmur and chest pain at the age of 47 and had surgical correction The transmission of this aortic coarctation via an asymptomatic carrier mother is consistent with X-linked recessive transmission The boy's father is *without cardiac problems and there are no other children A second family was* seen with a boy who was operated for correction of aortic coarctation at 6 years of age and is well after 18 year follow-up without dysmorphism, malformation or mental delay He has borderline hypertension of 150 over 90 The mother who is well with normal cardiac examination has a twin sister with a 14 year old boy who was operated for aortic coarctation correction at the age of 7 years His sister has a normal cardiac examination These 2 families (4 boys with cardiac coarctation) seem to be compatible with the dysfunction of an X linked recessively transmitted gene causing the coarctation

9.139

Linkage of the human ob gene region to extreme obesity

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Obesity is one of the major risk factors for coronary heart disease, hypertension and type 2 diabetes (NIDDM) Although a strong genetic component in human obesity is well established, the underlying genetic mechanisms are not well understood This is in contrast to a number of well defined rodent models of obesity like the autosomal recessive fa/fa (fat), the db/db and ob/ob mice and the autosomal dominant Ay (Agouti) mouse Especially the ob mouse has been intensively studied Mutations in the ob gene, leading to a non-functional protein, result in extreme obesity in the mouse Functional studies of the ob protein (leptin) suggest, that it might be involved in food uptake regulation For these reasons this gene is a potential candidate also for human obesity Recently Friedman and colleagues cloned the human homolog of the ob gene and designated its localization to human chromosome 7q31 To study the role the human ob homologue may play in human obesity we carried out family linkage studies with markers around the designated gene locus As the ob mutations in the mouse result in extreme obesity a BMI of > 35 kg/m2 (morbid obesity) was taken as the threshold for linkage analyses Eight markers positioned between approximately 0 5 and 7 cM to each side of the gene locus were genotyped in 66 affected sib-pairs from 101 french families Additional family members were typed where available Two point linkage analyses between the markers and obesity showed significant evidence for linkage with three markers (D7S530, D7S514 and D7S680) situated within 2 cM of the gene locus (p= 0 006, 0 006 and 0 009 respectively) Two of these markers (D7S530 and D7S680) also showed a significant decrease of allele sharing when linkage analyses were carried out between discordant sib-pairs In conclusion we here report significant evidence for the involvement of the human ob gene region in extreme obesity

9.140

Mapping NIDDM Genes: Studies with 20 Candidate Genes in 600 French Sib-Pairs.

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We have recruited 174 families with at least 2 non insulin dependent diabetes mellitus (NIDDM) patients in the sibship, representing 760 individuals, 360 NIDDM sib-pairs (according to WHO criteria) and 600 affected sib-pairs (defined as a fasting plasma glucose value of >6 1 mmol/1 or glycemia 2 hours after oral glucose load of >7 8 mmol/l) As NIDDM is clinically and genetically heterogeneous we divided the families into more homogeneous sub-groups according to the Body-Mass Index (BMI) and the age of diagnosis We studied 20 candidate genes/regions Genes implicated in the regulatory pathways of insulin secretion include the genes coding for the Human G-coupled Inwardly Rectifying Potassium Channel (KCNJ3), the ATP-sensitive Potassium Channel expressed in beta-cells (KCNJ7), the glucagon (GCG), the regulatory protein of glucokinase (GCKR), the receptor for the glucagon-like peptide 1 (GLP1R), the genes encoding gene transactivators such as the LIM/homeodomain Islet-1 gene (ISL1) and the caudal-type-homeodomain 3 gene (CDX3), the gene for proprotein-convertase 2 (PC2), which encodes the insulin processing protease, the gene for the receptor B of the cholecystokinin (CCKBR) Genes encoding the enzymes of glucose metabolism in beta-cells comprise Hexokinase 1 gene (HK1) (ubiquitous), the gene encoding for the mitochondrial enzyme FAD-glycerophosphate dehydrogenase (m-GDH) and the gene for the isoform of the pyruvate kinase (PKM) which is expressed in beta-cells Genes that affect insulin action and glucose metabolism in target tissues are also candidates for contributing to the development of NIDDM They include genes coding for the hexokinase II (HK2), the fatty acid-binding protein 2 (FABP2), the hepatic forms of the phosphofructokinase (PFKL) and the pyruvate kinase (PKL), the type-1 protein phosphatase (PP1), the low density lipoprotein receptor (LDLR) and the protein ras-associated with diabetes (rad) which is preferentially expressed in muscle from diabetic patients In addition, we studied the HRC locus on chromosome 19q for which recent studies suggested an indication of linkage in Caucasian NIDDM families The results of parametric and non parametric analyses excluded 18 of the genes as major contributors in the pathogenesis of NIDDM We found an indication of linkage for two candidates genes, the CCKBR locus on chromosome 11p and the rad locus on chromosome 16q in the sub-group of NIDDM sib-pairs with an age at diagnosis below 45 years (p=0,0046 and p=0 002 respectively)

9.141

A large family with limb-mammary syndrome : clinical and molecular findings

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We report on a large family with at least 30 living patients in 4 generations with as major characteristics hand- and feet abnormalities ranging from small fifth fingers to split hand/split foot and mammary gland aplasia or hypoplasia Lacrimal duct atresia was commonly seen Cleft palate or bifid uvula was occasionally seen, as were nail and teeth abnormalities The expression was very variable The least affected was a male with only athelia Non-penetrance was not observed This syndrome is different from the ulnar-mammary syndrome, the EEC syndrome and related syndromes Molecular studies are going on So far the regions 12q23-24 1, 7q36, 7q21-22 have been excluded

9.142

Inherited duplication of the 17p11.2 region in a patient with Dejerine-Sottas syndrome

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Dejerine-Sottas syndrome (DSS) has been clinically distinguished from Charcot-Marie-Tooth disease type 1 (CMT1) by an early onset, markedly reduced nerve conduction velocities (NCV < 10-12 m/s) and apparent hypomyelinisation along with large onion bulbs in the peripheral nerve biopsy Meanwhile, point mutations in the PMP-22 gene on chromosome 17p and in the PO gene on chromosome 1 have been detected in patients with DSS Here we report a girl with DSS who had severe muscle weakness and wasting from the second year of life Nerve conduction velocity was 7 0-7 5 m/s, in the nerve biopsy, onion bulb formations were present around hypo- and demyelinated axons The parents appeared to be healthy and were closely related, which made autosomal recessive inheritance most likely Surprisingly, a duplication in 17p11 2 of the PMP-22 gene was detected not only in the affected girl but also in both parents The patient has inherited the maternal duplication Her 5 year-old sister, so far symptom free, showed the paternal duplication A neurological examination of the mother gave evidence of a mild neuropathy (NCV 13-23 m/s) The patient's father refused electrophysiological investigations until now, but his sister had classical CMT1 from youth (NCV 22-28 m/s), so that the diagnosis could be clearly established in this family Our results underline the marked clinical variability of CMT1 Although further studies are required to clarify the situation in the reported family, it seems that the same genetic mechanism might cause DSS and CMT1 Thus, electrophysiological and neuropathological findings are only of limited value to differentiate genetic entities

9.143

Pathological characterization of nerve biopsies from duplicated and non-duplicated CMT-1 patients.

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A duplication at 17p11 2 involving the PMP-22 gene is the most frequent mutation in the Hereditary Motor and Sensory Neuropathy type-1 (HMSN-1) Point mutations of the PMP-22, P-O (1q21-23), and connexin-32 (Xq13.1) may also cause similar de-remyelinating hypertrophic neuropathies. A detailed definition of the pathological phenotypes associated with different mutations may help to elucidate the pathogenetic mechanisms So far, a duplication analysis was done on 34 HMSN-1 patients belonging to 26 families, by MspI-Southern blots with the probe pVAW409R3a (D17S122) 17 out of 27 informative DNA samples were duplicated while 10 were not Nerve biopsies from 10 duplicated and 10 non-duplicated patients were performed, a morphometrical analysis of pathological findings was carried out and results were matched between two groups A similar pattern of myelin stain was also observed in all examined nerves, by using an antibody directed against 16-63 residues of PMP-22 Pathological overlap was present between duplicated and non-duplicated cases, indicating that different genetic lesions may lead to similar pathological phenotypes The financial support of Telethon-Italy (grant 750 to R N is acknowledged)

9.144

Chromosome aberrations in children with haemoblastosis

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Haematological malignancies represent about 50% of all neoplastic diseases with children The cytogenetic studies on leukemias are of great importance for the accurate diagnosing They are a significant criterion for the therapeutical

approach, the prognosis of the evolution and the final result of the treatment We announce our results of 43 patients with haematological malignancies 18 of them were with CML, 20 with AL and 5 with another haematological malignancies We found chromosomal anomalies in 65 1% More of them were consistent and specific karyotypic rearrangements, t(9,22), del(7q), t(14,22), dupl(1q) but another we found at first - t(3,7), del(2q), del(6q) and mar Structural changes of chromosomes, particularly, traslocations and duplications were especially frequent in ALL Patients with ALL and ANLL and t(9,22) had additional changes The cytogenetic findigs indicate that the initial cytogenetic change is an important factor in determining the nature of subsequent chromosomal abnormalities developing in the malignant clone

9.145
Acute leukemia in twins

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Five unisexual twins with acute leukemia are observet in children's oncohematological clinic For determination of their belonging are investigated blood group, Rh and HLA-antigens Only one pair turned out to be di-zigotic Acute lynphoblastic leukemia have predominated among the sick children The youngest twin pair was of interest, since the children got ill in the end of the first month with identical clinical and hematological symptoms of acute nonlymphocytic leukemia In our case 25% of monozygotic twins are concordant of leukemia, whereas dizygotic twins are discordant Investing leukemia in twins will help us to get more knowledge about the etiological and the pathogenetic moments in the process itself of leukemia

9.146
Chronic myelogenous leukemia with the Philadelphia Cromosome in a newborn

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The clinical and the hematological data are not sufficient to determine the type of a chronic myelogenous leukemia The chromosomal analysis gives the possibility to establish the specific chromosomal marker, determing the subtype of the leukemia, the treatment and the prognosis of the disease Hematological changes, characteristic of chronic myelogenous leukemia, but the presence of the Philadelphia chromosome has determined the treatment with Myleran and alfa-interferon

9.147
SYNGEN: an electronic manual for clinical diagnosis of malformation syndromes.

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Genetic and non-genetic malformation syndromes comprise a great number of variable and overlapping forms of inborn dysmorphies which are difficult for clinical identification and differentiation To facilitate the task a computerized manual has been elaborated which includes a list of above 1900 malformation syndromes, a dictionary for 1200 phenotypic traits related to respective syndromes, a set of figures for 200 syndromes, and a list of relevant references The manual enables to complete registration cards and case descriptions in unified format and to obtain a ranked list of candidate syndromes Completed trials in a number of genetic clinics have shown that the manual having convinient multiwindow interface is easily operated on IBM-compatible PCs and is of great help to medical practitioners It can also be used for teaching malformation syndromology A database on chromosomal dysmorphies (CHRODYS) including about 1300 aneusomies involving thirteen of the human chromosomes is helpful in clinical cytogenetic studies

9.148
Turner's phenotype in a male with ring Y chromosome

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A 23 years old male with many stigmata specific for Turner's syndrome is presented short stature (height - 1,43 cm, weight - 50 kg), triangular face, low implanted hair on the neck, short IVth metatarsal bone on the right side External sex organs are well developed with a male type of hair growth He has got regular ejaculations with azoospermia The hormonal tests revealed a normal testosterone and growth hormone levels Exaggerated response of gonadotrops to LHRH stimulation The cytogenetic analysis revealed karyotype 46, X, r(Y) in all examined cells from whole blood culture A DNA analysis has been started The ring Y has the loci 50f2/A, 50f2/B (mid short arm) and 50f2/D(short arm near centrome), but lacks the loci 50f2/C and 50f2/E (in different positions in the long arm euchromatin) Since the phenotype is male, it looks as if the entire short arm is present from SRY to the centromere One breakpoint would be in the Yp pseudoautosomal region and one in the long arm between the centromere and 50f2/E The results make good sense in the light of the clinical picture A more precise localisation of the Yq breakpoint is in progress

9.149
Growth and pubertal development in children with Down syndrome

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Growth retardation and gonadal insufficiency are well known features of Down syndrome In this longitudinal study, 44 home-reared children and adolescents with Down syndrome, aged 10-24 years, living in the county of Uppsala, were followed yearly The male patients had a mean final height above that reported previously and a close correlation between target and final heights was found The mean final height in the female patients was below that reported earlier Mean peak height velocities in males and females were 8 5 and 7 3 cm/year respectively, significantly lower than in healthy children The mean ages at peak height velocity were 12 3 and 10 8 years respectively, indicating early growth spurts. The serum FSH concentrations, the small testes and the negative correlation between luteinizing hormone and testicular volume in the males may indicate some primary gonadal insufficiency For the girls, mean menarcheal age corresponded closely to that of their mothers

9.150
DNA repair genes in GID (Genome Interactive Databases)

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One of the main objectives of the human genome project is to map, then to identify disease genes in order to prevent and eventually cure diseases Therefore, a database devoted to mapped genes must afford a twofold resource to the scientific community It has to make available, a pertinent information on the tools needed for the mapping process It must offer a documented information on mapped genes, besides the definition associated to an esoteric symbol The information must include data on the category and possibly subcategory of the gene, i e transcription factor with the helix-loop-helix motif, the family to which it belongs, its homology to genes in other species, its function and the diseases associated to its mutations To illustrate how these principles are implemented in GID, we shall present the current information on Nucleotide Excision Repair genes, first described as ERCCs in rodents They have been mapped on human chromosomes, then cloned. Their

homology to RAD yeasts has been demonstrated A link has been made between photosensibility, cancer and these defects Now, their function are better known and some correlation must be made between the function of a gene and the kind of the cognate diseases, i e xeroderma pigmentosa or Cockayne's syndrôme, in their different groups of complementation

9.153

Karyotype 46,XY t(7;9) in the child with Warburg syndrome

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In August 1995 we recognized Warburg syndrome in new-born child with karyotype 46,XY,t (7,9/) The same type of translocation was found also in his mother The child died at the age of one month with diagnosis lissencephaly. hydrocephaly, agenesis corpus callosum and septum pellucidum, cerebellar hypoplasia with agenesis of the cerebellar vermis, microphthalmia, congenital cataracts, cryptorchism and micropenis We suppose the chromosomal aberration in the child with Warburg syndrome can be helpful for gene mapping of this disorder

9.154

Two case with fetal hydantoin syndrome

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An association between anticonvulsants and congenital abnormalities was first realized by Meadow et al in 1968 Manson and Frederic showed teratogenic effects of hydantoin more clearly in thier epidemiological studies in 1973 Malformations due to hydantoin intake during pregnancy may be various, including digit and nail hypoplasia, growth retardation, typical appearance, rib anomolies, abnormal plamar creases, hirsutizum, low set hairs Ambiguous genitalia is rarely associated with this syndrome We present two siblings, aged 3 years and 3 months, with fetal hydantoin syndrome They were born to an epileptic mother who was given diphenylhydantoin and phenobarbital throughout her pregnancy The patients showed many characteristics of fetal hydantoin syndrome and ambiguous genitalia Clinical and laboratory examinations revealed that both of them normal female type of internal genital organs and female karotypes

9.155

Common allelic variants of vitamin-D-receptor gene and genetic predisposition to low PTH levels in ESRD haemodialysis patients.

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High levels of parathyroid hormone are normally reached in haemodialysis patients with end stage renal disease (ESRD) Only a low percentage of ESRD patients show reduced levels of PTH that predispose to adynamic bone disease Until now, identified risk factors only partialy explain the predisposition to these patients to being the high or low PTH level group Allelic association has been reported between vitamin-D-receptor (VDR) gene allelic variants and bone mineral density or serum osteocalcin levels According to this we have studied common allelic variants of the VDR gene in ESRD haemodialysis patients as a risk factor for PTH levels A number of 89 ESRD patients, with more than 1 year in haemodialysis, were genotyped for the Bsm I RFLP of the 3' end of the VDR gene DNA was extracted and a 191 bp fragment, surrounding the Bsm I polymorphic site, was amplified by PCR Genotypes were detected by Bsm I endonuclease digestion and SSCP analysis of this fragment The results obtained were

	VDR genotypes			VDR alleles	
	BB	Bb	bb	B	b
General Pop (n=82)	12	45	25	69	95
Pts high PTH (n=44)	8	22	14	38	50
Pts low PTH (n=45)	13	24	8	50	40

The allelic frequencies in general population were under Hardy-Weinberg equilibrium, showing values according to the literature for a Caucasian population These allelic frequencies were applied to estimate equilibrium in the two patients groups Differences were not significatively for the high PTH group, whereas low PTH group show significatively differences ($p<0,035$ $c^2=6,79$, df= 2) from equilibrium In this group the frequency of the B allele was significatively higher than in general population ($p<0,05$, $c^2=4,24$, df= 1) These results seem to suggest that the B allelic variant acts as a better modulator for the parathyroid suppression activity of the vitamin D This fact will be explained according to the described hypothesis that B allele produces more VDR mRNA levels than b allele These results allow us to design individualized treatments in patients from early stages of chronic renal failure, according its VDR genotype

9.156

Cytogenetic analysis of a neurofibromatosis type-1 case with bilateral optic nerve gliomas

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A six year old boy was admitted to eye department of Ataturk University Medical Faculty with visual loss of both eyes His eye examination showed no abnormality except bilateral optic nerves atrophy Intracranial calcifications and bilateral enlarged optic nerves were seen in his computed tomography Six cafe-au-lait spots were found on his trunk Chromosomal analysis and sister chromatid exchange (SCE) were performed and no numerical or structural anomalies was found 7 9+-1 3 SCE frequency as an average +-SD value was determined by counting metaphase plates An autosomal dominance inheritance was found by his pedigree analysis It might be an interesting case with its bilateral involvement of optic nerves

9.157

A case of Wilms' tumour associated with mosaicism for an interstitial deletion of 11p with no other features of WAGR complex

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A 14 months old girl was referred with a left sided abdominal mass which was confirmed as a Wilms' tumour (stage III) without unfavourable histological features Cytogenetic studies performed on the tumour biopsy showed 46,XX,del(11)(p1) karyotype Subsequent constitutional analysis showed a mosaic karyotype of 46,XX/46,XX,del(11)(p13) with the del(11p) cell line constituting approximately 10% of cells The detailed clinical examination following this result found no evidence of features of WAGR complex except the history of Wilms' tumour This contrasts with the two cases previously reported with del(11p) mosaicism, one of whom had bilateral tumours, these showed clinical features of WAGR, although the proportion of del(11p) cells in these cases was greater (_50%) than in our patient The patient underwent treatment according to the UKCCSG protocol for inoperable tumours and

is well 21 months later A recent ultrasound scan showed no evidence for recurrence of Wilms' tumour and a normal right kidney Patients with a constitutional del(11)(p13) cell line are at increased risk of bilateral tumours and of passing on this risk to their offspring, without necessarily showing clinical features of WAGR syndrome There is a case for studying all cases of Wilms' tumour for mosaicism

9.158
Associations between cognitive markers of predisposition to schizophrenia and personality traits.

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In 18 families of schizophrenics phenotypic and genetic correlations were calculated between personality measures (trait anxiety and 10 scales of MMPI) and 6 cognitive characteristics, that had been previously found to be genetically informative and helpful for identification of individuals at high risk for schizophrenia (Alfimova & Trubnikov, 1994) These cognitive markers were short-term memory capacity, mediated memory, selectivity, activity - the number of responses in series of tasks, verbal fluency, and concentration There were few phenotypic correlations between cognitive and personality traits At the genetic level short-term memory capacity was independent of personality, and hypochondrias and hysteria did not associate with the cognitive markers Correlations between the other cognitive parameters and personality measures were predominantly 0 2-0 7 The most stable and high genetic correlations were found between cognitive markers and the depression scale Associations of cognitive parameters with the schizophrenia scale are of special interest It was found that the schizophrenia scale had a significant phenotypic correlation with selectivity and high genetic correlations with selectivity and cocentration (0 45 and 0 52 respectively) Thus, the results pointed out to existing of common genes for certain cognitive and personality peculiarities in schizophrenia

9.159
The approaches to genetic systematics of clinical variants of disease.

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The classification problems of clinical variants of disease are usually stipuplated by 1) complexity phenotype, 2) limited opportunities to study etiology of disease revealing of the forms of diseas, 3) orientation in use of clinical data as main information of classification The set of the clinical forms of disease arranged according to causal factors of their determination appear to be natural system For diseases with herediatary predisposition genetic classification should determinate the division of clinical forms to the spectrum of genotypic homogeneous groups Because the existing classifications reflect saved experience of many researchers at first it seems to be possible to evaluate adequacy of such classifications of available genetic heterogeneity of disease with the help of the genetic correlation analysis The existence of the high genetic correlations between forms can be stipulated as real genetic homogeneity or consequence of an inadequate clinical grouping at which the allocated forms reflect secondary displays pathology and are genotypic heterogeneous In this connection search for criteria of division of the disease on such groups is necessary for the genetic similarity between them would be minimum The decision can be received with the use of combined application of multivariate statictic methods and genetic correlation analysis Because the existing classifications are based on some traits from the large set an increase of trait number relating to categories of genetic informative will allow to receive stable genetic reasonable systematics

9.160
To the search of psychoneurophysiological markers in the group of genetic risk of schizophrenia

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A complex multidiscipline study was carried out in 40 families where one of relatives suffered from schizophrenia Present abstract covers a part of the study, namely electrophysiological comparison of 15 first-degree relatives of schizophrenics (age 15-28 years) with 17 age- and sex-matched subjects without psychiatrically affected relatives Narrow-band EEG and EP in oddball paradigm showed the significant increase of spectral densities for frequencies of delta-theta band as well as significantly smaller P3 peak amplitude in the former group The findings so close to those found in established schizophrenics can play an important role in individual prognisis for risk-group subjects Next step will be to study neurophysiological and psychophysiological correlations between probands and their first-degree relatives

9.161
Influence of apolipoprotein E on clinical platters of familial amyotrophic lateral scerosis

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Amyptrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder involving both upper and lower motoneurons causing general paralysis A genetic component is demonstrated by familial studies and molecular studies, 5 to 10% ALS being herediatary transmitted as an autosomal dominant trait Both genetic and clinical heterogeneity are observed Geneitc heterogeneity has been recently demonstrated by the identification of mutations in the superoxide dismutase Zn/Cu (SOD 1) gene, in only 10 to 20% familial forms of ALS (FALS) Clinical heterogeneity is due to the presence ofr the absence of sensitive troubles, variations in site of onset, age at onset, mean duration Intrafamilial clinical heterogeneity is also a common feature in FALS This clinical heterogeneity might be due to the modifying effect of candidate genes in FALS different from SOD1 gene As a first step, we studied the apolipoprotein E gene in 14 pedigrees within at least 2 affected kindreds analysed, displaying or not SOD 1 gene mutation Results will be presented at the meeting

9.162
Percentage of GST 1M genotype in women with endometriosis in different Caucasian populations.

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Endometriosis (E) is a severe disease with unclear etiology and a frequency of about 10% in Caucasian population The role of the genetic polymorphism of Glutathione S-transferase M1 gene (GST M1) in the development of E was under investigation in the present study As shown before, a homozygous deletion of GST M1 gene affects the detoxication process of endogenous and exogenous compounds and is present in 39% of the general Caucasian population We report the results of indentification of GST M1 0/0 deletion in 85 women with E from Russian, Ukrainian an French populations In all cases the diagnosis of E was confirmed clinically and by histological analysis GST M1 0/0 deletion was studied by PCR performed directly from blood spots collected from patients The primers for the 4th and 7th exom of GST M1 were used for amplification The internal and external positive as well as external negative controls were included in each reaction The percentage of this deletion was 81% for Russian, 77% for Ukrainian and 83% for French population Our data suggest that endometriosis is more

frequent in individuals homozygous for GST M1 deletion This could correspond to a contribution of environmental toxins in the pathogenesisofthis disease

9.163
How to count genes in multifactorial disease: schizophrenia.

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Concordance for disease in MZ twins indicates incomplete penetrance, which can be quantified by binomial mathematics Since genetic segregation occurs before formation of DZ twins, comparison of DZ and MZ concordances allows estimation of the number of genes jointly involved in causation of polygenic phenotypes Frequency of disease in relatives of different degrees provides a check on this deduction The purpose of this investigation was to test the validity of the theory by application to familial schizophrenia Results indicate causation of schizophrenia by a single, partially dominant allele of mean penetrance around 0 33 Penetrance is increased if there are affected close relatives, the influence of an affected parent being three times that of a sib The theory of a single gene is confirmed by empiric risks in other relatives, concordance rates in twins being enhanced by shared prenatal trauma It is concluded that the number of genes jointly involved in polygenic conditions is estimable from twin concordance rates and that familial schizophrenia is probably caused by a single disease allele of incomplete penetrance, which is brought to expression by several factors including prenatal trauma and intra-familial stress This interpretation does not however exclude the possibility of genetic heterogeneity (Ref Annals of Human Genetics (1996), 60 1-19

9.164
A study of symptomatic haemoglobinopathies in Oman.

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A comprehensive national study of all cases of symptomatic sickle cell disease (SCD), β thalassaemia and G6PD deficiency in the Omani population was undertaken through hospital based records 1620 patients with symptomatic SCD were identified giving a calculated population frequency of 1 in 950 and heterozygote carrier rate of 1 in 15 Interesting local differences in population frequency were noted In the genetically and geographically isolated Dhofar region the indigenous mosquito is Anopheles coustani which does not support the full replication of the malaria parasite and in this region sickle cell disease is virtually absent In other areas there is a high frequency which may partly be due to consanguinity It has been suggested that the severity of sickle cell disease is clinically milder in the Arabian Gulf, but preliminary cross sectional data from Oman shows there is a significant morbidity and mortality associated with SCD There were 233 patients with symptomatic β thalassaemia which gives a population frequency of 1 in 6300 and a calculated heterozygote rate of 1 in 40 The majority of cases of G6PD deficiency were asymptomatic

9.165
Maternal serum screening for Down syndrome: a french national survey on practices in 1994.

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To measure the adoption of the maternal serum screening for Down syndrome by the physicians and the public, a national survey on the ongoing practices was organized A questionnaire was administered to 454 obstetricians and 727 general practitioners and to all the cytogenetics laboratories, as well as to all the labs performing serum screening for Down syndrome The survey showed that 11% of all pregnant women had serum screening in 1994 , with major differences between regions The test was prescribed by 12 3% of the GPs and 68 4% of the obstetricians The gestational age was determined by sonography for 93% of the pregnancies followed by obstetricians and 61% of the pregnancies followed by GPs Among the 32 labs, 9 used a simple test (hCG), 12 a double test, and 11 a triple test (hCG+AFP=uE3) Half of the labs were involved in a quality control network The cut-off risk level for amniocentesis was 1/250 in 13 labs and 1/100 in the others (This was the cut-off level chosen during the pilot study in 1991) The risk was calculated using software by only 19 labs This survey demonstrates a widespread diffusion of the test despite the fact that it is not reimbursed, but also that the practice is far from optimal It shows that guidelines have to be produced and diffused among the professionals involves in this type of screening

9.166
Personal control - a new approach to genetic counseling

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Many studies have tried to find variables for the evaluation of genetic counseling Outcome studies focused on learning and decision making variables The present study tested a new approach to genetic counseling based upon the concept of perceived personal control (PPC) This concept refers to the counselees' subjective perception of how much control they believe they have with regard to their genetic problem Questionnaires given to 161 counselees, showed a significant increase in PPC after counseling in comparison to baseline measured before counseling (t=-7 6 p<0 001) Analysis of variance and multiple regression analysis showed that the change in PPC was influenced by medical variables knowledge of recurrence risk of the specific problem (F=19 9, p<0 0001), the possibility of prenatal diagnosis (F=8 8, p<0 001), the availability of definitive diagnosis (F=8 3, p<0 001) and the type of problem - whether physical or mental one (F=6 2, p<0 01) Previous studies in patients suffering from malignant and chronic diseases revealed that increased PPC is a marker for better coping with the medical problem Increased PPC after genetic counseling may represent a similar process Awareness to the possible influence of the PPC on coping can guide the counselor while providing the information to the patients

9.167
Variables influencing the decision of "low risk" couples to undergo amniocentesis

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Amniocentesis is the most widespread prenatal diagnosis method The motivation of couples under the age of 35 with "low risk" for a chromosomal or genetic problem to undergo this procedure was not investigated The present study prospectively compared 133 "low risk" couples who decided to undergo amniocentesis, to a second group of 125 couples who preferred to avoid the procedure The later were recruited while coming to the biochemical screening test The couples in the first group were significantly older (women 31±3 versus 27±4, men 34±4 5 versus 30±4 5 p<0 0001), their knowledge about the test and about having a malformed child (women 3 1±1 2 versus 1 9±1 3, men 2 6±1 1 versus 1 5±1 3 in a scale of 0-5, p<0 0001), as well as their risk perceptions (p<0.001) was higher than the second group Nevertheless they perceived the procedure's risk as lower (p<0 001) Logistic regression analysis identified those parameters as independent significant predictors for decision to undergo amniocentesis We conclude that part of the "low risk" population base their decisions about prenatal evaluation on a rather incomplete knowledge, that leads them to nonrational risk perceptions Public education is needed in order to give the target population the ability to decide on a more rational ground

9.168
The new genetics - what the public wants to know.

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This study assessed what the public want to know about "the new genetics", and how effectively new genetics research findings are being communicated to families affected by genetic disease To do this, we analysed 309 enquiries made during a television phone-in about genetics, and 476 enquiries received by the Genetic Interest Group over a 22 month period There was little evidence from this data that people who are not directly affected by genetic research have much interest in the subject, or the social, ethical and moral issues it raises However, the results indicate that information about genetic diseases needs to be made more readily available to affected families In addition, despite claims that the public are ignorant of genetics, most of this specific group were already familiar with many of its terms and concepts

9.169
Molecular genetic evidence for the efficacy of early copper histidine treatment for Menkes disease

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Menkes disease (MD) is an X-linked lethal disorder characterized by widespread defect in intracellular copper transport. Progressive neurodegeneration and connective tissue abnormalities are the main clinical features Allthough mild forms of MD exist most patients present with the severe form, which is usually fatal by three years of age In MD the underlying genetic defect disturbs the functioning of copper requiring enzymes and treatment aims to provide copper to these enzymes Though parenterally administered copper in various forms does not produce substantial clinical improvement, copper-histidine appears to be efficient when administered at a very early age Two unrelated, prematurely born patients receiving this therapy responded favorably and they are now alive at ages 19 and 9, presenting a mild clinical course However, an unresolved question has been the severity of the disease in these patients Given the possibility of phenotypic variability, we have characterized their genetic defects using single strand conformation analysis (SSCA) following the characterization of exon-intron structure of the Menkes disease gene Both patients have mutations that should result in severly truncated proteins, confirming the efficacy of copper-histidine in severe lethal forms We suggest that if these patients benefit from copper-histidine, so may others with different kinds of mutations

9.170
Maternal serum marker levels in twin pregnancies: changes between the first and second trimesters.

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Alphafetoprotein (AFP) and free beta human chorionic gonadotrophin (FβhCG) levels have been investigated in 97 twin pregnancies from which two maternal serum samples were obtained, one in the first trimester between 7 and 14 weeks gestation and another in the second trimester between 15 and 20 weeks gestation AFP and FβhCG levels were significantly increased in twin pregnancies at both stages compared with singleton pregnancies but the effect was more marked in the second trimester Median AFP and FβhCG levels, expressed in multiples of the appropriate gestational median of singleton pregnancies (MOM), were 1 74 MOM and 1 59 MOM respectively in the first trimester and 2 25 MOM and 1 80 MOM in the second trimester There was a clear trend of increasing levels with advancing gestation The AFP level in twin pregnancies was 1 30 MOM at 7-9 weeks and 2 09 MOM at 13-14 weeks The corresponding levels for FβhCG were 1 55 MOM and 1 83 MOM Possible explanations for these observations include differences in the relative size of the placental interface between singleton and twin pregnancies in the first trimester compared with the second trimester and the predominantly maternal rather than fetal origin of AFP in maternal serum before 10 weeks gestation

9.171
The pathophysiology of Down's syndrome pregnancies.

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Prenatal screening for Down's syndrome is based on the analysis of feto-placental markers in maternal serum However the factors which give rise to the varying patterns of maternal serum marker concentration seen in Down's syndrome pregnancies are unknown Using biochemical and immunocytochemical methods, we have investigated endogenous levels of various markers in placental tissue (alphafetoprotein (AFP), intact and free beta human chorionic gonadotrophin (hCG), pregnancy-specific β1 glycoprotein (SP-1), placental alkaline phosphatase (PALP), gamma glutamyl transferase (GGT)), in fetal liver (AFP and GGT) and in fetal intestine (GGT) obtained from 50 Down's syndrome pregnancies after theraputic abortion in the second trimester and in the corresponding tissues from unaffected abortuses Results indicate that placental levels of placental products reflect maternal serum levels intact and free beta hCG levels are elevated in Down's syndrome pregnancies while PALP and SP-1 are little changed This suggests that membrane passage is not affected but that there may be altered synthesis of hCG AFP levels in fetal liver from Down's syndrome pregnancies were low reflecting maternal serum levels However, placental levels were strikingly elevated pointing to a possible transport defect specific to AFP GGT levels were high in placenta and liver from Down's syndrome pregnancies but low in fetal intestine

9.172
Changes in maternal serum marker levels in Down's syndrome pregnancies between the first and second trimesters.

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Previous studies have shown that changes in marker levels associated with Down's syndrome pregnancies vary between markers and often between first and second trimesters We have investigated the change in levels with gestation for alphafetoprotein (AFP), free beta human chorionic gonadotrophin (FβhCG) and pregnancy associated plasma protein A (PAPP-A) using a series of 26 Down's syndrome pregnancies in which two maternal serum samples were obtained from each patient, one in the first trimester (7-14 weeks gestation) and another in the second trimester (15-17 weeks) AFP levels were reduced to 0 93 MOM in the first trimester and to 0 74 MOM in the second trimester FβhCG levels were significantly elevated in the first trimester (overall median MOM 1 98) and further increased in the matched second trimester samples (3 32 MOM) PAPP-A levels however were significantly reduced only in the first trimester (overall 0 42 MOM) The change in PAPP-A levels became less marked with advancing gestation from 0 35 MOM at 7-10 weeks, 0 64 MOM at 11-12 weeks, 0 76 MOM at 13-14 weeks to 0 97 MOM at 15-17 weeks The trends in FβhCG and PAPP-A levels with gestation cause Down's syndrome detection rates to vary with gestation using this combination of markers in the first trimester

9.173

Allele status of the CS.7/Hha I polymorphism 5' of the CFTR gene may be associated with female survival in normal Czech individuals

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An unexplained genetic phenomenon occurs near the CFTR gene DNA polymorphisms 5' of the gene display linkage disequilibrium with cystic fibrosis (CF) alleles Although a large part of this disequilibrium is explained by the prevalence of a single common mutation (ΔF508), many of the less frequent CF mutations occur on the same chromosomal background as ΔF508 This disequilibrium is consistent in all studied European populations and peaks in a short region delineated by polymorphisms CS 7/Hha I and KM19/Pst I To determine whether this region contains biologically important sequences, the frequencies of 6 CF-linked polymorphisms were analyzed in 417 elderly individuals (>75 years) from a stable Czech population Allele frequencies of markers CS 7/Hhal and KM19/PstI differ significantly ($p<0.05$) between elderly female and elderly male subjects The frequencies of the 6 polymorphisms were then determined in 646 contemporary newborns and 345 young adults (19-45 years) drawn from the same population Interestingly, the frequency of CS 7/Hhal alleles differed significantly between newborn females and elderly women ($p<0.05$) Taken together, these data suggest that a polymorphism in the CS 7 region is associated with female survival

9.174

Report of a family with North Carolina Macular Dystrophy and deafness

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The macular dystrophies are a heterogenous group of conditions characterised by progressive degeneration of the macula In the majority of cases the disease is limited to the retina, with no involvement of other systems In distinction to the pigmentary retinopathies there are no reports of deafness segregating with macular dystrophy North Carolina Macular Dystrophy (NCMD) is a distinct progressive foveal dystrophy characterised by the retention of colour vision, variable visual loss and onset in the first two decades of life There is marked ophthalmoscopic variability ranging from a few small specks in the fovea to larger, single white colobomatous lesions in the macular area The condition is transmitted in an autosomal dominant fashion and the has been linked to chromosome 6q14-q16 2 We report a three generation family with the classic history and ophthalmoscopic appearances of NCMD in which the condition appears to be segregating with sensorineural deafness This is the first report of a family in which a macular dystrophy segregates with deafness Karyotype analysis of affected members has been normal We have initiated linkage studies to determine if the disease in this family is linked to the NCMD locus on chromosome 6q

9.175

Glutathione S-transferase T1 polymorphism in Estonians detected by ELISA method.

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A polymorphic glutathione S-transferase GSTT1 has been recognized recently (Peters et al , 1992) The enzyme conjugating methylhalogenes to glutathione is present in 20 80% of the human population Monohalogenated methanes - GSTT1 substrates are widely used in the industry The ethnic distribution of GSTT1 polymorphism has been investigated only in a few populations We have developed ELISA test for quantitative determination of GSTT1 from whole blood, and applied it for studying the polymorphism in Estonian population The ELISA test revealed the presence of three groups of individuals GSTT1 negative persons and the GSTT1 positive individuals expressing the protein in intermediate and high concentrations 673 Estonians were tested The percentages of individuals with GSTT1 -/-, -/+ and +/+ phenotypes were 17 7%, 43 15 and 33 2% respectively The gene frequencies for GSTT1 - and GSTT1 + alleles were found to be 0 423 and 0 577 There were no significant age and sex differences There was a slight tendency to have a lower frequency of GSTT1 negative phenotype in older people Some significant differences in the GSTT1 phenotype frequencies were found between different geographical regions in Estonia

9.176

Teaching of clinical genetics at second medical school of the Charles University Prague

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Since 1990 medical genetics has been taught at three levels of our six year curriculum In the Biology Course during the first two theoretical years, in the preclinical third year in Pathological Physiology Course and in the fifth year during the clinical part of the curriculum as a new obligatory one week course ending with exam The clinical course is aimed at solving of model situations in families with various genetic problems by small groups of students They have at their disposal various books, they can consult with assistants, see patients, speak with parents or family members and experience difficulties and approaches of genetic risks solving in genetic counselling Experience and problems of genetic teaching will be discussed

9.177

ACE genotype distribution in Russian healthy subjects: an initial study

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Renin-angiotensin system (RAS) plays an essential role in development of coronary heart diseases (CHD) Being multifactorial, CHD also has a genetic factor(s) in its aetiology Polymorphic markers were located inside/nearly two RAS candidate genes encoding for angiotensin I-converting enzyme (ACE) and angiotensin II type 1 receptor An association between particular alleles of the above polymorphic markers and CHD was recently established Thus, homozygosity for deletion in ACE gene (DD genotype) is strong risk factor of myocardial infarction in several studied Caucasian populations, whereas homozygosity for insertion (II genotype) is strongly protective Since allele/genotype frequencies for many polymorphic genes are influenced by racial/ethnic factors, and data on population allele/genotype frequencies are prerequisite for disease association study and relative risk estimation, the aim of this work is to determine I/D ACE genotype prevalences in Russian healthy subjects Our study enrolled 118 patients from traumatological

departments of Moscow hospitals (traumas were considered completely met inclusion criteria "random selection") I/D ACE polymorphism was determined by PCR technique The following distribution of ID/ACE genotypes was found DD – 50 8%, ID – 33 1% and II – 16 1% We cannot yet explain such great proportion of this CHD high risk genotype in Russian population Theses findings, however, do not differ significantly from published by others in various European populations More wide and extensive studies (including patient with CHD) are needed and they are under way

9.178
Fetal ultrasound abnormalities in 34 pregnancies: Correlation with fetal karyotype, autopsy findings, radiological findings and postnatal outcomes

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GATA Medical Faculty, Depts Of Medical Genetics[1], Obstetrics[2], Radiology[3] and Pathology[4] and GEN-MED Center For Genetic Diseases and Prenatal Diagnosis[5]

The study included 34 pregnancies who were diagnosed to have fetal anomalies during routine ultrasonographic examination between 14-37 weeks of gestation Cases were evaluated according to the ultrasonographic, cytogenetic, pathological and radiological findings as well as outcomes of gestation Prenatal cytogenetic study was conducted in 24 out of 32 cases In 8 cases fetal karyotype was studied either after termination of the pregnancy or at birth Three chromosomal abnormality was found out of the 32 cases studied cytogenetically two cases of +21 and one case of +18 Sonographical findings of trisomy 21 cases were "isolated hydrocephalus" for one and "cystic hygroma and hydrops fetalis" for the other For trisomy 18 case "polyhydramnios and growth retardation" were remarkable Among 31 cases with no chromosomal abnormality, five were grouped as presenting a monogenic syndrome, five as a multifactorial inheritance, two as amniotic fluid volume abnormality, one as placental abnormality and two as normal 16 cases presented congenital malformations of unknown etiology 22 pregnancies were terminated, six were born at term, 2 cases ended up with intrauterine death and four were still ongoing The study is unique with regard to the multidisciplinary approach for the evaluation of sonographically detected fetal abnormalities

9.179
Application of "APT TEST" in Prenatal diagnosis to evaluate the fetal origin of blood obtained by "chordocentesis": Results of 30 pregnancies

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This study aimed to set up practical lab-side approach to discriminate fetal from maternal blood in samples obtained by chordocentesis To determine the fetal origin of the blood, modified "APT Test", originally used to differantiate adult from fetal hemoglobin in bloody stools of newborns, was applied to 30 cases of prenatal diagnosis After receival of the blood samples , just before the routine set-up of cultures for cytogenetic purposes, 0 05 ml (1-2 drops of blood with a Pasteur pipette) of blood was added to 5 ml distilled water plus 1 0 ml of 0 25 N NaOH in a test tube and gently agitated for a few seconds For each sample, known adult blood was tested simultaneously as a control At the end of the 60 seconds the color change was noted for each sample tested A color change from pink to dark green-brown was accepted as adult hemoglobin reaction where as persistence of bright-pink, as fetal Among 30 cases studied, one had two separate samples withdrawn, making up the sum of samples studied, 31 Out of these 31 samples, there was only one discrepancy with regard to the gender of the fetus, the cytogenetic result and the result of the "APT Test" the test yielded a fetal reaction, whereas the karyotype was 46,XY (8 metaphases)/46,XX (3 metaphases) The fetus was a male Another remarkable case was the one with two samples Sample 1 yielded adult reaction and the karyotype was 46,XX whereas sample 2 ended as fetal blood , the karyotype was 47,XY,+21 The fetus was a male with Down syndrome Maternal contamination in sample 1 was obvious

9.180
Specificity of the first and second trimester prenatal diagnosis: pathological data

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Comparison of prenatal and pathological findings was performed in 790 second trimester and 41 first trimester fetuses Postabortion second trimester fetal autopsy changed or completed the prenatal diagnosis in 29 1% of cases, termination of pregnancy was found to be based on incorrect prognosis in 0 6% of cases Diagnostic discrepancies were more often (34 1%) in abortuses of 15 to 22 gestational wk than in the group of 22 to 28 wk foetuses (16 3%), the results of prenatal diagnosis were better in Minsk-city (24% of incomplete diagnoses) than in Minsk region (29 1%) Detection rate of the first trimester ultrasound in our series of 685 parallel sonographical and pathological examinations was 54 7%, in the group of 10 to 12 week fetuses the percentage of ultrasound recognition was higher (73 7%) The first trimester nuchal translucency was found to be a marker of various malformations and their complexes other than Down's syndrome cardiac malformations with normal karyotype, trisomy 18, de Lange syndrome, limb-body wall complex and some other skeletal deformities were revealed on postabortion pathological examination of such first trimester fetuses All the first trimester therapeutic terminations of pregnancy were based on correct prognosis

9.181
Abortion, a personal or familial consent

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The offer of prenatal diagnosis and pregnancy termination can have an unexpected impact on the social and phsychological welfare of the women, but it is also possible to have an effect on the relations within the family We present the case of a woman who had a prenatal diagnosis of Down syndrome She had to decide wether the pregnancy should be terminated or not The husband's sister had a 2 years old child which was diagnosed at birth as Down syndrome Only during the pregnancy the woman become aware that the syndrome had a genetic mechanism Both the husband's and the woman's families had a big influence on the last decision In the present social and economic situation the children are often raised by their grandparents, who can decide when a child should be born We present the psychosocial consequences of the family influenced decision of the couple In the absence of prenatal diagnostic programmes and the presence of very close familial relations, the problems which emerge from the decision of aborting - in case of a desired pregnancy - may be not only specific to a certain family Should then genetic counselling be extended to other members of the family?

9.182
The structured, presymptomatic disclosure of a diagnosis:experience in newborn screening for Duchenne muscular dystrophy.

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Newborn screening for Duchenne muscular dystrophy entails identifying healthy infant males as being at high (approximately 50%) risk of a serious genetic disorder Whether, or under what conditions, such a newborn screening programme results in benefits for the affected boys or their families remains uncertain and under evaluation We have developed a protocol for informing the high-risk families of the screening test results This entails close liasion with the primary health care team and paediatrician for each boy, and enables each family to decide what further

investigations will be carried out on their child Forty-one families with a boy investigated for suspected DMD have been interviewed, of which 25 had the possiblility of DMD disclosed according to the protocol Of these 41 families, 11 had a boy with a traditional, symptomatic diagnosis of DMD, 15 had a transient neonatal elevation of the serum creatine kinase and 15 had a diagnosis of DMD confirmed after newborn screening The families who had the process handled according to the protocol expressed much greater satisfaction than the other families 88% as opposed to 19% thought the process had been handled well or excellently The protocol will be described

9.183

Trisomy 10 mosaicism and maternal uniparental disomy in a liveborn infant with severe congenital malformations

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Trisomy 10 mosaicism in a liveborn has to our knowledge been described only once previously in a newborn with the trisomic cell line restricted to fibroblasts We describe a severely growth retarded, malformed newborn with trisomy 10 in 25/30 mitoses in skin fibroblasts, while the karyotype as obtained on cultured lymphocytes was 46,XY in 50/50 mitoses The child died 37 days old Biopsies taken at autopsy from achilles tendon, costal cartilage and pericardium confirmed trisomy 10 in 19/30, 30/30 and 26/30 mitoses respectively Karyotypes of lymphocytes from both parents were normal The major malformations of the child as confirmed at autopsy were microcephaly, microphtalmia, cleft and lip palate, thoracic scoliosis, anal atresia and malformations of heart and limbs Parental origin of the extra chromosome 10 was investigated using the following microsatellites D10S88, D10S89, D10S249, D10S220 and GLUDP2 The additional chromosome was of maternal origin and data consistent with a meiotic non-disjunction Analysis on DNA extracted from cultured lymphocytes showed maternal uniparental disomy for chromosome 10 Karyotyping skin fibroblasts is important, if chromosome aberration is strongly suspected, irrespective of a normal karyotype of lymphocytes

9.184

Mental retardation, incontinentia pigmenti-like lesions and 12p trisomy mosaicism

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A 21 month old, tall and obese girl was evaluated because of psychomotor retardation She exhibited various informative morphogenetic variants -not representing a variant familial developmental pattern- such as coarse round face with prominent forehead and cheeks, flat midface, hypertelorism, slight mongoloid slanting of long palpebral fissures, long upturned eyelashes, short nose with broad and flat nasal bridge, short and broad columella, long philtrum, slightly everted lower lip, geographic tongue, bifid uvula, broad thorax, soft hands with mild syndactyly III+IV and broad halluces in valgus position Fan-like hyper- and hypopigmentations following the Blaschko lines on the trunk were evident after very careful examination Karyotype from lymphocyte and fibroblast cultures revealed mosaicism with two cell lines, one normal and one with extra chromosomal material on the long arm of a chromosome 9 arisen de novo All of the remaining investigations gave normal results FISH showed that the extra material was from chromosome 12 The clinical picture with initially hypotonia, normal somatic development, big head, psychomotoric retardation and the presenting dysmorphic features fits well with trisomy 12p phenotype To our knowledge this is the second case to be described with 12p trisomy in mosaic state

9.185

PCR-based analysis of a family with HNPP: A loss of heterozygosity account for a large deletion of the PMP^{22} gene as a cause for HNPP in affected family members.

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Large insertions and deletions at the Peripheral Myelin Protein-22 (PMP-22) gene locus have been demonstrated to cause inherited motor and sensory neuropathies (Charcot-Marie-Tooth Disease, CMT, Hereditary Neuropathy with liability to Pressure Palsies, HNPP) The majority of HNPP cases were found to be associated with a large segment deletion on chromosome 17p11 2, harbouring the PMP-22 gene To characterise the underlying genetic defect in a family with HNPP using simple PCR-based techniques, we identified four dimorphic nucleotide positions in the 3'-UTR of the PMP-22 gene and identified several haplotypes in the general population The overall heterozygosity rate was 0 8 Analysing these intragenic markers in the HNPP-family, we observed absence of heterozygosity in all of the 16 affected individuals Haplotype analysis revealed a loss of heterozygosity (LOH) in three of the affected individuals Direct sequencing of the PMP-22 gene showed absence of mutations within the coding region A semiquantitative PCR-assay, in which a PMP-22 exon is co-amplified with a reporter-gene segment as a biallelic control outside chromosome 17, was established By using fluorescent-labelled primers, the co-amplified fragments were automatically detected and quantified As a result, in the affected individuals we found a reduction of the PMP-22 signal intensity to approximately half of the values seen in unaffected family members We conclude that a large deletion of the PMP-22 gene accounts for HNPP observed in our family Our PCR-based method is a powerful tool to analyse genetic defects underlying HNPP by circumventing time-consuming laboratory work

9.186

Hair shaft defect in Prader-Willi syndrome

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As one of our patients presented with pili torti and Prader-Willi syndrome (PWS) due to maternal uniparental disomy (UPD) we decided to investigate several children and adults with PWS for hair shaft anomaly Hair probes of 6 children were investigated All fulfilled the clinical criteria for PWS according to Vanja Holm All were found to be nondeleted by fluorescence in situ hybridization using probes D15S11 and GABRB3 (Oncor) five showed UPD by DNA-analysis using several polymorphic DNA markers spanning chromosome 15 and one showed abnormal methylation pattern at the SNRPN gene by Southern blot and biparental inherited chromosome 15 by DNA-analysis Hair shaft anomaly was found by light and electron microscopy in four patients two showed flattened hair shaft and rotation (pili torti), one flattened hair shaft without rotation, one flattened hair shaft with incomplete rotation A number of metabolic and genetic disorders are reflected in changes of the hair shaft morphology, i e Menkes disease Our findings suggest that hair shaft anomaly is a feature in at least some PWS cases and might reflect a metabolic disorder involved in PWS Hair shaft microscopy of nondeleted and deleted persons with PWS and controls and further investigations are in progress

9.187

Presymptomatic testing of individuals at 25% risk of Huntington's disease (HD) - analysis of 87 UK candidates during 1994.

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The UK Huntington's disease prediction consortium consists of 23 Clinical Genetics units who offer presymptomatic testing for HD For 1994 the consortium has reports of 87 enquiries from individuals at 25% a priori risk for HD On commencement of direct mutation testing 14 centres made some contact and 9 chose not to contact previous patients Of the 87 enquiries, 52 tests were performed, 30 withdrew and 5 tests are still pending Of the 30 withdrawals, 6 were after the 50% risk parent decided to have a test Of the 52 tests performed there were 31 females and 21 males, age range 19 to 69 years Seven individuals were mutation positive and 43 negative, one was equivocal In 31 of 52 tests the 50% risk parent was still alive 21 had been offered counselling and 9 had taken up the offer Of the 17 tests performed with parental knowledge 12 were in favour of their child having testing but did not want a test themselves A survey of current practice relating to 25% testing was undertaken All centres always discuss the possible effects of testing on the 50% parent and would recommend they be offered counselling 16 centres said they would still offer a 25% test even if the 50% parent actively does not want their offspring tested and 17 said that if the 25% individual refuses contact with the at risk parent they would, after extensive counselling, still perform a test This will be discussed further with reference to the current international guidelines on predictive testing

9.188

The role of free radicals in etiology and pathogenesis of trisomy 21

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Pathogenesis of trisomy 21 is still unknown. Moreover, neither of the genes mapped in region 21q22 is directly related to clinical manifestations The results of examination of 115 families with trisomy 21 gave the increased level of free radicals, enhanced lipoperoxidation, disbalancing of antioxidant system and oxidative phosphorilation in parents, mainly in mothers and in patients with trisomy 21 The increased level of free radicals may possibly be the key element in the rigin and manifestation of trisomy 21 The effect of CuZnSOD gene in trisomy 21with the initially increased level of superoxidic radicals promotes the formation of more active radicals and peroxides We compared the clinical characters of Downþs syndrome with the effects of low dose radiation damages including the descendants of irradiated animals The striking analogy was obtained The increased level of free radicals, different grade and duration of disbalancing in donors of extrachromosome explains the age-dependent frequency of trisomy 21, recurrence risk, connection to other aneuploidies, degenerative, autoimmune, oncological disorders, etc in some families and pedigrees The causes of increase of free radicals initiating nondisjunction of chromosomes and possible role of mtDNA mutations in pathogenesis and etiology of trisomy 21 are discussed

9.189

Maternal serum AFP and hCG in diagnostics of fetal chromosomal abnormalities

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Mass screening programme of the detection of maternal serum (MS) AFP and hCG between 15 and 20 weeksþ pregnancies has been provided in the Donetsk region for about 5 years Among all women referred to amniocentesis due to combined high risk for age and MS markers (cut-off greater or equal to1 270) chromosomal deseases were diagnosed in 5 7%, and among them trisomy 21 in 3 5% Average values of AFP and hCG in cases of trisomy 21 were 0,59 MoM and 2,72 MoM We believe that the informativity of AFP as a screening marker was higher in 15-17 weeksþ pregnancies than in 18-20 weeksþ terms Such dynamics was not observed in hCG that was increasing irrespective of the pregnancy term In a group of women with the combined risk higher than 1 270 in 7,5% the familial pericentric inversions, mainly of chromosome 9, were found Changes in MS AFP and hCG levels manifested themselves more profoundly (0,5 MoM and 4,5 MoM respectively) The reasons of false-positive and false-negative test-results as well as the connection of pericentric inversion with trisomy 21 are discussed The necessity to estimate the risk of trisomy 21 with due regard to the burdened anamnesis and pregnancy course is stressed

9.190

Study of X-linked ALD carriers: Neurological evaluation

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Adrenoleukodystrophy (ALD) is an X-linked recessive disorder which affects mainly the adrenal cortex, central nervous system white matter, and testis The principal biochemical change is the accumulation of Very Long Chain Fatty Acid (VLCFA), particularly hexacosanoic acid (C26 0) in the adrenal cortex , nervous system white matter, plasma, red blood cells, leukocytes and cultured amniocytes Carriers may present, in their forties, a mild form of the disease, similar to Adrenomyeloneuropathy, and progressing in decades being the symptoms often misinterpreted as Multiple Sclerosis A study was made of using carriers, belonging to several families of X-ALD, all presenting abnormal levels of VLCFA The study included clinical evaluation, electromyography, Somatosensitive, audditive and visual evoked potentials and MRI We present the results of a group of carriers with age between 18 to 67 years old submitted to this study The carriers over 40 present abnormalities in the neurological examination and in most evoked potentials in opposition to the electromyography

9.191

Clinical heterogeneity in dominantly inherited atrial septal defect

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Atrial septal defect (ASD) accounts for approximately 10% of all congenital heart defects with a birth incidence of 6 4 per 10 000 Although most cases are sporadic, families have been reported in which non-syndromic secundum ASD has been inherited as an autosomal dominant trait, in some families being associated with an atrio-ventricular (AV) conduction defect We have ascertained two three generation families in whom ASD is segregating and carried out detailed clinical and cardiological assessment including electrocardiography (ECG) The two families showed significant differences in presentation Four members of Family 1 had ASD, all of whom also have an AV conduction defect and one who has an AV conduction defect alone All obligate carriers have evidence of cardiac involvement In Family 2 four members have ASD, one having multiple septal defects but none have evidence of an AV conduction defect Two obligate carriers in Family 2 have no evidence of cardiac involvement Evidence from these and families reported previously suggest that families with dominantly inherited ASD may be divided into those with conduction defect which appear to be associated with a fully penetrant gene (type 1) and those who present with ASD alone in which non-penetrance seems to be a frequent observation (type 2) We plan to carry out linkage analysis on these and other families Clinical data suggest that more than one gene may be implicated in non-syndromic inherited ASD

9.192

Delineation of a new contiguous gene syndrome around the multiple exostoses gene on chromosome 11.

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Multiple exostoses (EXT) is a genetically heterogeneous skeletal disorder with one gene recently identified on chromosome 8 (EXT1) and two additional loci, EXT2 on chromosome 11p11-p12 and EXT3 on chromosome 19p, respectively Here, we present 8 patients from 4 families showing a similar clinical phenotype with most prominent findings EXT and enlarged parietal foramina (FPP) Additional symptoms in some but not all patients include craniofacial dysostosis and severe mental retardation All patients have cytogenetic and/or molecular deletions of chromosome 11p11-p13 The breakpoints of the deletions differ both on proximal and on distal side, resulting in deletions from 7 up to 22 cM between the centromere and D11S914 In all patients the deletions overspan the EXT2 candidate region, located between D11S1355 and D11S1361, confirming the presence of EXT2 on chromosome 11p Furthermore, the gene for FPP, previously also reported as an dominant isolated trait, probably resides in the smallest region of overlap The presence of additional clinical features such as craniofacial dysostosis and mental retardation suggests that other genes might be involved in this new syndrome We propose to refer to this syndrome as DEFECT 11 syndrome (Deletions on chromosome 11 with Enlarged Foramina, Exostoses, Craniofacial dysostosis and menTal retardation)

Genodermatoses

10.001
Chromosome breakage induced by bleomycin in lymphocytes from patients affected by lamellar ichthyosis and from their relatives.

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Chromosomal fragility, spontaneous or drug induced, was reported in some dermatological diseases To contribute to clarify the still unknown molecular causes of lamellar ichthyosis (LI), a rare inherited disease, we have examined five patients and their relatives Lymphocytes have been stimulated in vitro, to evaluate spontaneous chromosome breaks and sister chromatid exchanges Furthermore we have analysed breakage induced by aphidicolin (APC), an inhibitor of DNA polimerase alfa and delta, and by bleomycin (BLM), a radiomimetic antitumoral antibiotic For statistical evaluation, we have considered three different groups LI affected patients, their parents, and healthy controls Lymphocytes from patients and from their parents were significantly more sensitive to BLM, compared with those from controls Various cellular factors can interact with BLM, and any of them could be altered in hypersensitive subjects i e the mechanism of transport to transfer the drug into the cytoplasm, antioxidant enzymes and bleomycin hydrolase that inactivates BLM Thus further studies are necessary to clarify what could be altered in LI families Moreover it would be important to understand if there is any relation between the point mutations of tranglutaminase 1 found in some LI patients and our findings

10.002
A homozygous splice site mutation in the keratin 14 gene in recessive epidermolysis bullosa simplex

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Molecular analysis was carried out in a pedigree from the Northern part of The Netherlands with recessive epidermolysis bullosa simplex Several affected individuals, one of which with consanguineous parents, showed no expression of keratin 14 (K14) in the epidermis The patients presented with severe skin blistering with only mild mucous membrane involvement and improved slightly with age K14 was completely lacking in immunofluorescence microscopy of epidermal basal cells and in Western blotting of extracts of cultured keratinocytes of the patients The expression of keratin 5, the obligate co-polymer of K14, was only slightly reduced Linkage analysis using microsatellite markers around the K14 gene (KRT14) at chromosome 17q showed identical haplotypes at both alleles in all affected individuals This suggests a local founder effect for this recessive KRT14 mutation Sequence analysis of genomic DNA extracted from leucocytes revealed a homozygous mutation in the splice acceptor site of exon 2 This mutation will lead to skipping of exon 2 in at least a proportion of the KRT14 transcripts A premature stopcodon is generated almost at the beginning of exon 3, leading to a truncated K14 protein

10.003
The bullous pemphigoid antigen gene 2 (BPAG2) locus is homozygous in most generalized atrophic benign epidermolysis bullosa (GABEB) patients

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Generalized atrophic benign epidermolysis bullosa (GABEB) (McKusick #22665) is a rare autosomal recessive disorder and a clinical variant of junctional epidermolysis bullosa (JEB) BPAG2 has been revealed as candidate gene for GABEB, since the BPAG2 gene product, a 180-kD bullous pemphigoid antigen, is not detectable in skin of most GABEB patients by immunofluorescence and immunoblotting using monoclonal antibodies Recently, mutations in the BPAG2 gene have been identified in a BPAG2 negative GABEB patient by McGrath et al (Nat Genet 11 83-86 (1995)). We postulate that for a rare autosomal recessive genetic disorder such as GABEB, most patients from a restricted geographical origin might be "identical by descent" Mutations will thus be present in a homozygous state in most patients In patients intragenic markers for a candidate gene may therefore show a significant higher degree of homozygosity than in randomly selected unaffected individuals We developed an intragenic BPAG2 microsatellite marker and observed homozygosity of different alleles in six out of seven fully BP180 negative GABEB patients of different European descent Compared with a heterozygosity of 82 6% of this marker in randomly selected unaffected individuals, this homozygosity is most significantly increased. In the three of these seven GABEB patients originating from the Eastern part of The Netherlands five out of six BPAG2 alleles were identical, as well as the haplotypes for flanking markers

10.005
Comparative analysis of the tuberous sclerosis 2 (TSC2) gene in Human and Fugu

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Germ-line mutations of the TSC2 tumour suppressor gene have been identified in humans with tuberous sclerosis and in the Eker rat Tuberin, the human TSC2 gene product, has a small region of homology with rap1GAP and stimulates rap1 GTPase activity in vitro, suggesting that one of its cellular roles is to function as a GTPase activating protein (GAP) We have undertaken a comparative analysis of the TSC2 gene in human and the pufferfish, Fugu rubripes In addition to the GAP domain, three other regions of the proteins are highly conserved (peptide sequence similarity >80%) These regions are likely to represent further functional domains To facilitate analysis of mutations within these domains we have determined the genomic structure of the human TSC2 gene It comprises 41 exons, including exon 31 which was absent from the originally described spliceform of the human TSC2 transcript and was identified following exon prediction from Fugu genomic sequence These findings support the proposal of the Fugu genome as a tool for human gene analysis

10.006

The genetic basis for Keratosis Palmaris and Plantaris.

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Recently it was shown (Nature genetics, 1993, 5, 158-162) that the gene for nonepideermolytic keratosis palmaris et plantaris, an autosomal dominant skin disorder detected in an extended Uzbek's pedigree is located on chromosome 17q in close vicinity or directly in keratin type 1 gene cluster Cosegregation of the high molecular weight allele of Kt 10 insertion/ deletion polymorphic allele with this disorder was demonstrated We sequenced Kt10 and Kt9 genes from this locus and found point mutation in Kt9 gene in affected individuals with KPP The palmar and plantar specific pattern of expression of Kt9 and Kt9 mutations in epidermolytic variants of hyperkeratosis have been demonstrated by other authors for unrelated pedigrees from Europe Possible association of mutations and polymorphisms in cluster of keratin genes which may underlay epidermolitic and nonepidermolitic expression of hyperkeratosis forms is discussed

10.007

Recessive dystrophic epidermolysis bullosa localisata: identification of two splicing mutations in collagen type VII gene (COL7A1) in an Italian patient.

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Collagen type VII gene (COL7A1) has been demonstrated to be altered in several variants of dystrophic epidermolysis bullosa (DEB), either with recessive or dominant mode of inheritance We have identified two mutations in a patient affected by a localisata variant of recessive dystrophic epidermolysis bullosa (L-RDEB), which is characterized by the less severe phenotype of the syndrome These mutations are the first splicing mutations so far described for COL7A1 in DEB One mutation is a paternally inherited A-G transition at position -2 of the donor splicing site of intron 3, which results in three aberrant mRNAs depending on the skipping of exon 3, on the usage of a cryptic donor site inside exon 3, or on the maintenance of intron 3 The second mutation is a maternally inherited G-A transition at position -1 of the donor splicing site of intron 95, which causes the activation of a cryptic donor site 7 nucleotides upstream the normal site and results in a deleted mRNA All aberrant mRNAs contain a shift of the reading frame giving rise to premature termination codons (PTCs) We observed a reduced expression of COLVII mRNA and a reduced level of COLVII, at the dermal-epidermal junction of the patient, if compared with his parents and a control donor Allelic specific analysis of the transcripts has shown that the patient synthesizes an aliquot of normal transcript, deriving from the maternal allele These findings are in agreement with the mild clinical phenotype observed in the localisata form of RDEB shown by the patient

10.009

Further data supporting genetic linkage of Incontentia Pigmenti to Xq28

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Incontinentia Pigmenti (Bloch-Sulzberger syndrome) is a genodermatosis transmitted in an X-linked dominant fashion It is characterised by typical abnormalities of the skin and teeth as well as neurological deficits in at least 10% of patients. Genetic linkage of the disease locus to the sub chromosomal band Xq28 has been previously shown [1] but a second locus at Xp11 has been suggested We report the results of genetic linkage analysis, using Xq28 polymorphic markers, in 12 families with Incontinentia Pigmenti, including a total of 35 affected females These patients were from two generations in five of the families and from three successive generations in seven families No recombination events were identified and the lod scores obtained were DXS52 (Z=6 32), RGCP (Z=3 01) and F8C (Z=3 31) The cumulative lod scores are now DXS52 (Z=14 86) and F8C (Z=15 16) Together these data indicate that a single locus in Xq28 is responsible for Incontinentia Pigmenti Further genetic linkage analysis with polymorphic markers distal to F8C is currently being undertaken with a view to identifying any recombination events and narrowing down the position of the gene within the distal region of Xq28 [1] Smahi A et al (1994) The gene for the familial form of incontinentia pigmenti (IP2) maps to the distal part of Xq28 Hum Mol Genet 3(2) 273-278

Genetics of Hearing

11.001
Autozygosity mapping of known deafness loci in British consanguineous families which originated from the Mirpur region of Pakistan

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Congenital sensorineural hearing impairment affects approximately 1 in 1000 children and is of genetic origin in at least one third of these cases Over 75% of congenital non-syndromic hearing impairment is autosomal recessive and to date eight loci have been implicated in this form of hearing impairment The eight loci described (DFNB1-8) have been identified in a number of genetically isolated populations by autozygosity mapping Each genetically isolated population is expected to have a unique subset of genes involved in hearing impairment in that population However, a number of genes in the subset will also be involved in hearing impairment in other populations To estimate the relative frequencies of the known deafness loci in the British population originating from the Mirpur region of Pakistani, we have analysed 27 consanguineous families, from this population, segregating non-syndromal recessive deafness by autozygosity mapping Identification of homozygosity unique to the affected family members at microsatellite markers tightly linked to the known deafness loci has been used to implicate that locus as responsible for the hearing impairment 3 of the 27 families investigated showed evidence of linkage to DFNB1 at 13q12 and 1 family showed definate linkage to DFNB3 (17p11 2-12) Although 2 families showed homozygosity at DFNB2 (11q13 5), analysis of a second marker discounted the possibility of linkage in these families to this locus In addition no linkage has been demonstrated to the loci DFNB4 (7q31) and DFNB5 (14q12) in families from this population These results show that other loci are responsible for hearing impairment in the population

11.003
Positional cloning of the gene responsible for Branchio Oto Renal syndrome (BOR)

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BOR Syndrome (MIM 113650) is an autosomal dominant disorder characterised by branchial arch abnormalities, structural defects of the outer, middle or inner ear, responsible for hearing loss and renal anomalies of various types, ranging from mild hypoplasia to bilateral renal aplasia This embryonic disorder has an incidence of 1/40 000 and accounts for approximately 2% of profoundly deaf children The analysis of a de novo 8q12 2 - q21 2 deletion carried by a patient affected by BOR syndrome enabled us to localise the corresponding gene to this chromosome interval A YAC and a P1 contigs, spaning this chromosome interval, were constructed and were used to localise the breakpoint of an 8q rearrangement, dir ins(8)(q24 11 q13 3 q21 13), in another patient Two techniques are being employed concurrently in order to identify the gene responsible for BOR syndrome, 1) exon trapping using YAC DNA from the region subcloned into lambda phages, and 2) cDNA selection using DNA from P1s and inter-Alu fragments from various YACs which span or surround the translocation breakpoint The combined use of these techniques has resulted in the isolation of various potential coding sequences in the region cDNA library screening is now underway in order to identify candidate genes

11.004
The Hereditary Hearing Loss Homepage: on-line information on deafness research

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Genes responsible for non-syndromic hereditary hearing loss are being localized at an increasing rate, and more than 10 new loci were discovered in 1995 alone Obviously, a new way of offering timely information to the scientific community is needed Over the last few years an increasing number of researchers are making use of the internet for the distribution of information Recently, the World Wide Web technology has made this process user-friendly and more accessible In collaboration with researchers in the field and with Dr Ph McAlpine, chair of the HUGO nomenclature committee, we have set up a web-homepage summarizing genetic information on non-syndromic hearing loss The address for the Hereditary Hearing loss Homepage is http //alt-www uia ac be/u/dnalab/hhh html This site lists information on the currently known loci for non-syndromic deafness, including the official name, the chromosomal localization, the best microsatellite markers to perform linkage analysis, and references Information about the families linked, the candidate region, and the type of hearing loss can also be found for most loci Direct links to the Genome Database for marker information, and to Medline and OMIM for references have been included whenever possible We believe that the Hereditary Hearing Loss Homepage can become an important platform for the rapid distribution of up-to-date information between researchers involved in hereditary deafness

11.005
The DFNA5 non-syndromic hearing loss locus: refinement of the localization.

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We recently reported the localization of a gene for non-syndromic, progressive sensorineural hearing loss to chromosome 7p15 in an extended Dutch family The hearing loss starts between 5 and 15 years in the high frequencies With increasing age and progressive high tone loss, also the low frequencies become affected The inheritance pattern is autosomal dominant with complete penetrance, and the family contains more than 100 affected individuals The gene responsible for this dominant deafness (DFNA5) is located in a 15 cM interval using markers from the Généthon genetic map, between D7S493 and D7S632 The candidate region remains large due to the limited number of genetic markers available on the Généthon map in this region (Van Camp et al , 1995, Hum Mol Genet 4, 2159-2163) In order to refine the candidate region, 11 additional genetic markers from other sources and covering the complete 15 cM candidate region, were analyzed This enabled us to narrow down the critical region to less than 6 cM, between D7S1791 and GATA-P28070 However, it must be noted that the definition of the candidate region depends upon only one recombinant on both sides Progressive hearing loss also has an environmental cause in some cases, and therefore, the possibility that one of the key recombinants is a phenocopy can never completely be excluded A YAC-contig spanning the candidate region was constructed, which will be useful for the identification of the DFNA5 gene

11.007

Thyroid Peroxidase: evidence for disease gene exclusion in Pendred Syndrome.

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Vaughan Pendred, exactly 100 years ago described the association of congenital neurosensory deafness and goitre and subsequently abnormal discharge of iodide following perchlorate challenge was noted Although Pendred syndrome may cause up to 5% of all cases of inherited deafness, the molecular basis of the association between hearing loss and the thyroid organification defect remains unknown We have studied a highly informative variable number tandem repeat (VNTR), located 1 5Kb downstream of exon 10 of the thyroid peroxidase (TPO) gene, using PCR and southern blotting, to search for linkage in sibships affected by Pendred syndrome In seven of the nine sibships analysed we found obligatory recombination between TPO and Pendred syndrome In the further two sibships mutation of the TPO gene as the cause of Pendred syndrome could not be excluded These data suggest that defects at the TPO locus on chromosome 2 are not the major cause of Pendred syndrome, and a selective genome search for the location of the disease gene is underway

11.009

Non syndromic hearing loss: linkage study of turkish families

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Due to genetic heterogeneity of non-syndromic deafness, there has been limited success in the identification of genes for this disease The absence of a clear clinical differentiation among the forms of the disease has seriously complicated linkage analysis In addition, positive assortative mating in western society contributes to the difficulty in mapping analysis Consequently, the successful chromosomal localization of the gene(s) involved in non-syndromic deafness requires large families showing clear segregation of a single deafness locus within the pedigree 52 families from Turkey, exhibiting non-syndromic deafness with 2 or more affected individuals have been ascertained Simulation analysis has been performed to establish the informativeness of all the pedigrees with a known mode of inheritance Our analysis showed that 6 of the pedigrees ascertained can independently establish linkage We are currently testing three consanguineous autosomal recessive families for known hearing loss loci Those families which do not exhibit linkage to known loci will be tested for candidate genes, and for those families where linkage could not be established using the first two steps, a genome scan will be used to localize new disease loci

11.010

Mitochondrial mutation 1555G affecting the 12S rRNA gene is common in Caucasian families with non-syndromic deafness regardless of antibiotic treatment

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Mutation 1555G in the 12S rRNA gene in mitochondrial DNA has been associated with antibiotic-induced deafness in several oriental pedigrees and in an Arab-Israeli pedigree with deafness without antibiotic treatment We have analysed 6 Spanish families with maternally transmitted deafness and found that all affected members were homoplasmic for mutation 1555G All patients presented bilateral, progressive and sensorineural deafness as the only clinical symptom The age of onset of deafness was between 1 5 and 60 years of age (mean 7 5 years) Deafness was related with antibiotic treatment in 2 of the 6 families, but most of the affected patients of 4 pedigrees did not receive antibiotics Mutation 1555G was not detected in a sample of 200 normals We conclude that mutation 1555G is frequent in pedigrees with mitochondrial inheritance of deafness in Caucasians and is not always related with antibiotic treatment Although it has been suggested that in patients with mutation 1555G and no antibiotic treatment, mutations in a nuclear gene might contribute to the deafness phenotype, the absence of consanguineity and antibiotics in 4 of our families suggest that other genetic or non genetic factors should contribute to deafness in the presence of the 1555G mutation

11.012

Usher syndrome type IB: an unconventional myosin at the origin of sensorineural deaf-blindness.

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Usher syndrome, a clinically and genetically heterogeneous disease, associates congenital hearing loss with retinitis pigmentosa It is the leading cause of deaf-blindness in humans Usher type I (USH1) is the most severe form Of at least three genes responsible for this form, USH1B accounts for approximately 75% of USH1 patients and 50% of all Usher cases We have previously shown that theUSH1B gene codes for an unconventional myosin, myosin VIIA (Weil, et al , 1994, Nature, 374 60-61) We have now established the complete cDNA sequence which predicts a dimeric motor protein bound to the membrane via a sequence homologous to the band 4 1 superfamily We have also established the gene structure which comprises 48 coding exons In situ hybridisation analysis on human embryos has revealed a sequential expression of the myosin VIIA gene in the pigmentary epithelium and photoreceptor cells of the retina, as well as expression of the gene in the embryonic cochlear and vestibular sensory hair cells The pathophysiology of this syndrome will be discussed in regards to these results as well as to the cellular distribution of the protein as revealed by immunochemical labelling

11.013

Strategies to isolate cochlear-specific transcripts in a mouse cDNA library.

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Two strategies have been adopted in order to identify genes specifically expressed in the cochlea, that could be involved in cochlear development, maturation, and the hearing process, which could thereby be responsible for non-syndromic deafness First, we generated a cDNA library from 16-day old mouse cochlea (mature cochlea) A total of two million independent clones were obtained PCR amplification products from 40000 clones, randomly picked, were ordered on high density filters We used a combination of substractive hybridisation and differential screening with cDNA library from various tissues Quantitative analysis of hybridisation signals and storing of data can be performed using adapted software We also constructed a cochlea-enriched cDNA library from 2-day old mice (immature cochlea) by substractive protocols with liver and cartilage cDNA The differential expression of candidate clones has been checked on mRNA dot blots and confirmed on Northern blots Interesting clones were subsequently sequenced To further characterise the corresponding genes within cochlea, the cellular distribution of their expression is studied by in situ hybridisation, they are mapped to mouse and human chromosomes and tested as possible hereditary deafness loci in both species

11.015

Title: Genetic mapping of autosomal dominant non-syndromic deafness in a Norwegian family: Localisation of DFNA7 to 1(q21-q23).

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Non-syndromic hearing loss accounts for the majority of genetic hearing impairment Previous reports on linkage studies on families from various ethnic backgrounds have already shown considerable genetic heterogeneity In a large Norwegian family high tone progressive hearing loss had been segregating for at least 5 generations The linkage analysis in the present family includes 41 family members Firstly, all the known gene loci for autosomal dominant (DFNA 1 - 6) and all known loci for autosomal recessive non-syndromic hearing impairment (DFNB1 - DNFB5) were excluded As part of a systematic genome screen using micro satellite markers 13 markers were tested before linkage was detected with the markers D1S194 and D1S196 The highest LOD score was obtained with D1S194 with a maximum LOD score of 6 3 without recombinational events The genetic interval between the flanking markers were approximately 5cM Further efforts will concentrate on possible candidate genes based on the genetic location and function of these genes In two other large Norwegian families we excluded linkage to this gene location and can thus further confirm genetic heterogeneity even within the Norwegian families with autosomal dominant inherited non-syndromic hearing impairment

11.016

Evidence for a third locus for non-syndromic X-linked Deafness.

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Profound congenital deafness affects 1 in 1000 children and over half of these cases are genetic in origin X-linked deafness accounts for only a small proportion of all non-syndromic deafness but is both clinically and genetically heterogeneous Reardon presented the first evidence for genetic heterogeneity and Lalwani subsequently mapped a second locus to Xp21 in the region of the Dystrophin gene We present an analysis of a series of families with X-linked deafness which include - families with mutations in the transcription factor POU3F4 in Xq21, families with the same phenotype which map to this region but in whom no mutations have been found in the gene, families with a different phenotype which also map to Xq21, families who do not map to Xq21 or to the locus at Xp21 This is evidence for a third locus for non-syndromic X-linked deafness in a family with profound sensorineural hearing loss, normal vestibular function and a normal CT scan of the petrous temporal bone Recent mapping data will be presented

11.017

Clinical and audiological investigations of autosomal recessive non-syndromal hearing impairment (ARNSHI) families of Pakistani origin resident in United Kingdom

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Approximately 1 in 2000 persons are affected from congenital hearing impairment Half of these can be attributed to be genetic in origin Autosomal recessive non-syndromal hearing impairment (ARNSHI) is the most common form of inherited deafness It is genetically heterogenous with estimates of the number of genes responsible ranging from 5 common to 36 or more loci Linkage studies in four different populations in which consanguinity is common have so far demonstrated 8 loci, DFNB1 to 8, on chromosomes 13, 11, 17, 7, 14, 3, 9 and 2 Our linkage studies in 27 consanguineous families of Pakistani origin have shown one family to be definitely linked to the locus DFNB1 and it is likely that three further families are also linked to that locus In addition, four further families, two each, are likely to be linked to the loci DFNB2 and DFNB3 Detailed clinical examinations were conducted on the relevant members of these families to note any dysmorphic features and to rule out any known cause of deafness from history and medical records Audiological investigations were performed to quantify the degree and to determine the type of hearing loss This included pure tone air and bone conduction threshold audiometry and tympanometry and were performed in affected individuals, their siblings and parents in all families Automatic controlled frequency scanning method (Audioscan) testing was conducted with an intent to detect possible carrier status in members of the family in whom hearing impairment was not detectable using pure tone audiometry Oto-acoustic emissions were carried out in a sub-group of the families The audiological findings showed inter- and intra-familial variation which is likely to reflect to represent the loci and mutational heterogeneity of this form of inherited hearing impairment

11.018

Non syndromal autosomal recessive deafness: linkage analysis in a large Italian pedigree.

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Approximately 1 per 1000 infants has a hearing loss sufficiently severe to prevent unaided developement of spoken language,genetic autosomal recessive etiology accounting for about 40% To our knowledge 8 loci for autosomal recessive non syndromal deafness (DFNB1-8) have been mapped by linkage analysis Genetic heterogeneity makes these studies particularly difficult in developed countries because of small familial size We studied a three generation family with multiple consanguineity and five affected subjects from three distinct matings The family comes from a geografically isolated mountain valley ,the population discloses wide isonomy supporting the hypothesis of high imbreeding Clinical assessment excluded syndromal elements Linkage analysis was performed for all the known DFNB loci Preliminary results are consistent with the involvement of DFNB2, one observed crossing over in the haplotype studied suggests a more precise localization of the gene Acknowledgements This work was supported by Telethon (project E 127)

11.019

Identification and characterization of DFN3 associated microdeletions 500 kb proximal to the DFN3 gene POU3F4

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X-linked deafness with perilymphatic gusher during stapedectomy (DFN3) is the most frequent cause of X-linked hearing impairment Computerized tomography studies demonstrated specific structural defects in the temporal bone of DFN3 patients Through a positional candidate gene cloning approach, we recently identified a causal gene, POU3F4, which was deleted or mutated in 8 patients with classical DFN3 In one of the novel sporadic cases, a mosaicism of mutated (missense mutation) and wildtype POU3F4 sequences was observed in EBV-immortalized B-cells and in peripheral blood cells of the patient Among 12 other DFN3 patients, we and others had identified 4 microdeletions and a duplication/inversion which were located up to 400 kb proximal to the POU3F4 gene To investigate the remaining patients in more detail, we constructed a partial cosmid contig from a 1400 kb YAC located proximal to POU3F4 Employing cosmids from this contig, we identified 5 novel microdeletions, which are situated approximately 500 kb proximal to POU3F4 The combined molecular data support the idea that a sequence involved in transcriptional regulation of POU3F4 is present 500 kb proximal to the gene, but do not rule out the presence of another DFN3 gene

Diabetes

13.002
Diabetes' sweet mysteries: separating the syndromes

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Inherited diabetes syndromes offer rare opportunities to search for diabetes causing genes, but rely on accurate clinical descriptions Wolfram or DIDMOAD (Diabetes Insipidus (DI), Diabetes Mellitus (DM), Optic Atrophy (OA), and Deafness (D)), is a recessively inherited syndrome occasionally associated with thiamine-responsive anaemia We aimed to characterise the syndrome and identify any subgroups In a cross-sectional casefinding study of 54 patients, we showed that DM and OA present in the first decade, followed by DI and D in the second, renal tract abnormalities in the third and neurological manifestations including ataxia and myoclonus in the fourth We analysed 12 affected families for markers on chromosome 4p, and obtained a maximum lod score 3 02 at theta=0 05 for DRD5, confirming the assignment of a gene to this region In one family the single affected offspring of a consanguineous marriage had inherited two completely different haplotypes which spanned the entire region An unnaffected sibling beyond the age of risk shared identical haplotypes, suggesting that an additional locus must be involved A subgroup of 7 children from 3 families presented with profound sensorineural deafness in infancy, milder DM in childhood, variable OA, and thiamine-responsive anaemia Genotyping obtained negative lod scores across a range of thetas (0-0 5), typically -2 at theta=0 01 with DRD5, suggesting this subgroup is phenotypically and genotypically distinct

13.003
Search for the gene(s) responsible for Lipoatrophic Diabetes

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Lipoatrophic diabetes (LD) or Berardinelli-Seip syndrome (MIM 151660) is a rare disease, characterized by severe insulin resistance, hypertriglyceridemia and generalized lipoatrophy A high incidence of parental consanguinity and/or family antecedents with diabetes are reported To locate the responsible gene(s), we have used complementary strategies, both of them based on the assumption that in consanguineous families, a child affected with a rare recessive disease, is homozygous by descent in the region spanning the disease locus Using the functional approach, we have excluded in some consanguineous families the implication of several candidate genes involved in the mechanism of insulin action or in lipid metabolism In the positional cloning approach, homozygosity mapping was used to identify the disease gene(s) in 20 patients either born to consanguineous unions or coming from geographic clusters Analysis of 250 AFM microsatellites distributed on each chromosome did not disclose consistent homozygosity However, a 50 to 70% homozygocity rate characterized a small number of potentially interesting loci Linkage analysis are currently performed in patient families using several markers from these loci. The identification of any genetic marker(s) will help to understand the physiopathology of LD and the insulin resistance which is commonly observed in other forms of diabetes

13.004
Unequal risk of IDDM for HLA-DQB1*0302 in haplotypes with different DRB1*04 subtypes

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Described so far, HLA-DQB1 alleles show the strongest association with insulin-dependent diabetes mellitus (IDDM) However, the contribution of other loci to IDDM risk, both within and outside MHC, is evident We have studied the role of the other MHC II class gene - DRB1, which is also known to be involved in the IDDM association, either due to the linkage disequilibrium with DQB1, or its own role Like in most of other parts of Northern Europe, among Northern-West Russian population the particular haplotype DRB1*04 - DQ B1*0302 is found to be the main marker of IDDM. DRB1 is a highly polymorphic gene, and using molecular genetic methods, serological typed DR4 has been divided into 19 subtypes The study of DRB1*04 subtypes in DQB1*0302 positive haplotypes could thus be informative A total of 160 IDDM patients and 164 control subjects from St -Petersburg in Russia were studied for HLA-DQB1 gene polymorphism Using PCR-TRF (time resolved fluorometry) method, which allowed the determination of DQB1 alleles 0201, 0302, 0301 and 0602/03 we confirmed in this population the association of 0302 and 0201 with IDDM risk as well as the protective effect of 0301 and 0602/03 DRB1*04 subtypes among DQ B1*0302 positive subjects (125 IDDM patients and 33 controls) were studied using PCR amplification of DQB1 gene segment followed by the hybridization with allele-specific oligonucleotides We found a slightly increased frequency of DR B1*0401 among patients (51% and 39% in patients and controls, respectively) and decreased frequency of DR B1*0404 (20% and 33%) DRB1*0403 was absent among patients, but also present in only 3 3% of controls The result suggests that the risk of IDDM is not equally associated with particular DRB1*04 subtypes in spite of presence of DQB1*0302 The finding support the hypothesis that either DRB1 or a closely related gene has its own participation in the IDDM pathogenesis or both DQB1 and DRB1 genes show the secondary association with IDDM due to the linkage disequilibrium with a still uncharacterized primary gene

13.006
DNA polymorphisms of human glucagon-like peptide-1 receptor gene (GLPR) in Russian healthy subjects and NIDDM patients.

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Non-insulin-dependent diabetes mellitus (NIDDM) is genetically heterogeneous disorder Glucagon-like peptide-1 (GLP-1) affects on the glucose-dependent insulin secretion in islets via its receptor Defects in insulin secretion are a common feature of NIDDM and as such the GLP-1 receptor is a candidate to the development of NIDDM. To evaluate the contribution of this receptor gene to NIDDM, we have studied the polymorphism of (TG)n tandem repeat located nearby GLPR gene in control (n=126) and NIDDM (n=110) patient groups in a Russian population A total of 10 alleles ranging from 138 to 156 bp in length were identified with heterozygosity of 0 85 Frequencies of two alleles (138 and 146 bp) were shown to be increased in NIDDM patients in comparison with control subjects (0 12 versus 0 04 in case of 138 bp allele and 0 31/0 20 for 146 bp allele) A significant increasing of the frequencies of the "138/146 bp" genotypes were observed in a group of NIDDM patient (7 2%, RR 2 6, p=0 004) in comparison with a group of control individuals (3 3%) The data suggest that GLPR gene might contribute to the development of NIDDM and could be used as a genetic marker for this disorder

13.007

Comparison of prevalences of IDDM genetic risk markers (HLA-DQA1/B1 genes) in juvenile and adult-onset diabetic patients

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To assess a contribution of insulin dependent diabetes mellitus (IDDM) genetic risk marker to clinical heterogeneity of adult-onset diabetes and its relevance to development of insulin dependency at different ages, we compared frequencies of HLA-DQA1/B1 gene alleles in 121 healthy control subjects and two groups of patients Group 1 and 2 consisted of 113 juvenile IDDM and 99 adult-onset diabetic patients, respectively Analysis of HLA-DQA1/B1 gene allele combination were performed to subdivide IDDM patients into several groups according to the number of susceptible (S) and protective (P) alleles The frequencies of four susceptible alleles (4S) were 52% in group 1 and only 26% in group 2 But opposite relation were found in case of 3S/1P 39% in group 1 vs 61% in group 2 Combination 2S/2P was found with frequencies 7% in group 1 and 13% in group 2 The frequencies of "most diabetogenic" alleles (DQA1 *0301, *0501 and DQB1 *0201, *0302) were significantly higher in group 1 in comparison with group 2 and, respectively, the frequencies of another "mild diabetogenic" alleles were found higher in group 2 We conclude that main differences in distribution of susceptible alleles indicate significant genetic difference between studied groups of patients

13.008

The Dunnigan-Kobberling Syndrome - an autosomal dominant form Partial Lipodystrophy

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We describe a further family with more than ten individuals spread over three generations, affected by the Dunnigan-Kobberling syndrome This condition was first described in 1974 but the mode of inheritance was unclear, X-linked inheritance with lethality in the male being inferred We now present evidence for an autosomal dominant form with at least one example of male to male transmission of the condition The phenotype is broadly characterised by lipodystrophy of the limbs and trunk with sparing of the face and neck and associated with insulin resistance and hyperlipidaemia, leading to the term "lipodystrophic diabetes" To further define the phenotype detailed auxology and MR imaging and biochemical estimations of glucose, insulin and non-esterified fatty acid levels during a standard oral glucose tolerance test will be described The molecular defect underlying this disorder is unknown but is likely to be pivotal in energy homeostasis. Putative candidate genes, including GLUT 4 and the adipocyte-specific adrenergic receptor are under investigation

Molecular dysmorphology

14.001
ACTH receptor mutation in a girl with familial glucocorticoid deficiency

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Familial glucocorticoid deficiency (FGD) has long been recognised as a clinical entity, but molecular studies have so far been performed in only a few individuals We report a girl with clinical and biochemical features of FGD The segregation of highly polymorphic microsatellite markers on chromosome 18 indicated that she was likely to be homozygous for the region containing the adrenocorticotropin (ACTH) receptor gene, and restriction enzyme analysis showed that the patient was homozygous for the R146H mutation of this gene without the need for direct gene sequencing Our patient is the third child with the R146H mutation, and the second child with this mutation to be born to consanguineous Pakistani parents Interestingly, she has tall stature, a clinical finding described in several children who have previously been shown to have mutations of the ACTH receptor gene Although the precise cause of the increase in height is unknown, we suggest that mutation analysis of the ACTH receptor gene be considered in children with clinical features of FGD

14.002
ATR-X phenotype without HbH inclusions and with a point mutation in the XNP gene

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X-linked α-thalassemia/mental retardation (ATR-X) syndrome is characterized by severe mental retardation, typical facial dysmorphism, urogenital abnormalities, and a mild type of hemoglobin (Hb) H disease Genital abnormalities can be missing The hematological expression (HbH inclusions in red blood cells) can be extremely mild The ATR-X syndrome results from mutations in the XNP gene (Gecz et al , 1994 , Gibbons et al , 1995) encoding a putative DNA helicase We report a family with severe mental handicap and characteristic facies including telecanthus, epicanthic folds, mid-face hypoplasia, flat nasal bridge, small triangular nose with anteverted nostrils and with columella not extending below the nasal alae, carp-shaped mouth, abnormal ears and incisors There was no genital abnormalities. HbH inclusions screening was negative up to three time Analysis of globin chain synthesis was not contributive RT-PCR of XNP on lymphocytes did not show any abnormal product Sequence of the coding region using a set of primers and compared to the reference sequence found in one patient a point mutation at codon 852, causing the non-conservative replacement of a proline by a serine This mutation occurs in the 3' part of the gene in between the putative helicase domains It is very likely to be the cause of the disorder as the conformation of the protein must be affected by this change, and as it has not been found in RT-PCR products originating from different individuals and tissues The XNP gene is a candidate for playing an important role in the central nervous system development In the mouse developing brain, XNP is widely expressed only in territories when neuronal proliferation and differenciation still occur Mutations in XNP lead to severe mental impairment in addition to constant facial features The phenotype of the ATR-X syndrome is highly variable including Juberg-Marsidi syndrome (Saugier-Veber et al , 1995) and potentially Smith-Finneman-Myers syndrome Thus, mutation in the XNP gene must be considered in patients with characteristic facial dysmorphism even without evidence of α-thalassemia

14.004
Refined mapping of Nance-Horan syndrome by linkage analysis.

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Nance-Horan syndrome (NHS) or cataract-dental syndrome is a rare X-linked clinical disorder characterized by severe congenital cataract with microcornea or microphthalmia, distinctive dental findings and evocative facial features In 1990 two teams separately published a linkage analysis in NHS Stambolian, in a study of three families, showed that the NHS gene is located in a large region extending from DXS84 in Xp21 1 to DXS143 in p22 31 and is linked to locus DXS41 (Zmax=3 44 for q=0 00) Zhu, in one large family, reported a weak linkage to locus DXS85 (Zmax=1 662 for q=0 16) and a region of localization from Xp22 11 to Xp22 32 More recently, in one family, Bergen reported a maximum Lod Score of 3 23 and recombinants with locus DXS451 reducing the region of localization on the centromeric side These combined data placed the NHS gene in a still large (of approximately 20-25 cM) region from DXS451 (Xp22 13) to DXS143 (Xp22 31) We present the results of a linkage study performed in several large families using more than 25 STR polymorphic markers from the Xp21 1-p22 32 region We observed a closer recombination both on the proximal side and on the distal side, allowing us to define a narrower region of localization, and obtained a significantly higher maximum Lod Score

14.005
Further demonstration of phenotypic heterogeneity in chromosome 22q112 deletion

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The DiGeorge syndrome, the velocardiofacial syndrome and the conotruncal anomaly face syndrome combine characteristic though variable facial dysmorphic features, congenital cardiac defects of the outflow tract, cleft palate and parathyroid and thymic hypoplasia or aplasia The acronym CATCH 22 has been used for this associated phenotype Developmental abnormalities of other organs can be present We describe 14 French infants with the velocardiofacial-DiGeorge syndrome, 5 boys and 9 girls, aged one month to twelve years Eight of these 14 patients had a congenital heart disease including 4 tetralogy of Fallot, 12 had variable facial dysmorhic features, 5 had thymus aplasia or hypoplasia, all but one had overt or submucous cleft palate and three were mentally retarded One patient had a laryngeal cleft Partial monosomy of chromosome 22q112 was demonstrated in all of these 14 patients Nine of them had a de novo deletion None of the four mothers who had the same deletion had cardiac, thymic or parathyroid defects associated with the condition although dysmorphie features were present These cases demonstrate the phenotypic heterogeneity of the 22q112 deletion This heterogeneity makes genetic counseling in this condition more difficult

14.007

Transcriptional mapping in the DiGeorge syndrome critcial region - new genes and a new SRO.

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The DiGeorge syndrome critical region (DGCR) has previously been described as an approximately 500kb region of 22q11 This region contained sequences disrupted by a balanced translocation breakpoint (ADU) and it was generally accepted that this breakpoint disrupted a major gene haploinsufficient in DGS To test this hypothesis we cloned the breakpoint and isolated a gene, DGCR5, which spans the ADU breakpoint It is distinct from the open reading frame DGCR3 described previously, and for which no cDNA can be identified DGCR5 is alternatively spliced, expressed during human and murine embryogenesis, but does not appear to code for any protein These are features shared by XIST, H19 and IPW, which may have a role in the cis-regulation of gene transcription No mutations of DGCR5 in non-deletion DGS cases have been discovered. recent refinement of the SRO placing the ADU breakpoint 100kb outside the DGCR is consistent with the translocation exerting a position effect, perhaps through disruption of a control element encoding DGCR5 New genes within the refined SRO have been isolated and include a gene of unknown function, related to a sequence found in C elegans Embryo expression and mutational analysis are underway

14.008

Are detectable 22q deletions less frequent in familial Di-George syndrome (DGS) compared to sporadic cases?

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In 1965 Di-George reported 4 cases of absent thymus and parathyroid glands In 1967 the name Di-George Syndrome was proposed The phenotype expanded during the 1970's to include cardiac and facial anomalies In 1978 Shprintzen et al described a branchial arch syndrome with clefting and learning difficulties This autosomal dominant (AD) syndrome was called the velo-cardio-facial syndrome (VCFS) Further reports confirmed both DGS and VFCS are AD, due to a 22q deletion and are part of the same spectrum In addition 22q deletions are now known to result in some AD cases of congenital heart disease These deletions are frequently not visible without In-Situ-Hybridisation (ISH) studies, the latter reported to detect 90% of cases We report 4 families with 2 or more affected individuals with DGS in two generations In at least 1 individual in each family there were 4 or more of the following systems involved, cardiac disease, hypocalcaemia, immune impairment, palatal anomalies, characteristic facies and learning difficulties ISH and further studies failed to identify a deletion in these four families We suggest 2 possible explanations The first is that familial cases of DGS have subtler molecular anomalies despite the absence of a milder phenotype, alternatively, that non 22q aetiologies are commoner in AD "DGS phenotypes" than sporadic DGS

14.009

De novo mutation of SRY gene in non sex-reversed patient

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The SRY gene located near pseudoautosomal region of the Y chromosome directs testicular differentiation Molecular genetic analysis has revealed point mutations, microdeletions and deletion of SRY gene in 46, XY females The role of mutations in SRY gene in other cases of sexual differentiation disorders is not so clear The SRY gene has been examined in two 46,XY patients The first case - boy with bilateral cryptorchism and hypogenitalism (micropenis, hypoplastic scrotum) After stimulation by human chorionic gonadotropin, the testes were palpated in the inguinal canals The second case - patient with ambiguous genitalia (bilateral cryptorchism, perineal hypospadias, vaginal pouch without uterus) The SRY-locus has been analysed with PCR, Southern blots and sequencing In the first case the mutation outside HMG-box (T136-C) resulting in Cys^{46}-Arg substitution has been revealed The SRY locus sequence of his father as well as of the second patient could not be distinguished from normal males The contribution of detected mutation to the phenotype abnormalities is discussed

14.010

Fibroblast growth factor receptor 2 mutations associated with Crouzon syndrome and non-classical phenotypes of craniosynostosis

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We have demonstrated mutations in FGFR2 in 49% (27/55) of cases with classical Crouzon syndrome, principally in the third immunoglobulin-like domain (Ig-III) of the extracellular region of the receptor Here we present cases which extend both the types of mutations found in Crouzon Syndrome and the range of phenotypes associated with FGFR2 mutations Screening by SSCP analysis in a cohort of 84 patients with Crouzon, Pfeiffer or atypical craniosynostosis has identified mutations in other areas of FGFR2 A mutation in the first immunoglobulin-like domain (Ig-I) in a Crouzon patient was determined by direct sequencing This revealed a missense mutation predicting an amino acid substitution tyr105cys Mutations in the Ig-III domain and the juxtamembrane region of FGFR2 have been observed in several patients with non-classical forms of craniosynostosis In some examples atypical cases have mutations within exon IIIc which either create or destroy a cysteine and are identical to amino acid changes which have previously been associated with classical Pfeiffer and/or Crouzon syndrome.

14.011

Evidence of genetic heterogeneity for Crouzon syndrome: A single mutation, ala391glu, in the transmembrane region of FGFR3 causes Crouzon syndrome with acanthosis nigricans

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Mutations in the fibroblast growth factor receptor 2 (FGFR2) gene have previously been identified in Crouzon syndrome, an autosomal dominant condition involving premature fusion of the cranial sutures Several different missense and other mutations have been identified in Crouzon syndrome patients, clustering around the third immunoglobulin-like domain We report here the identification of a mutation in the transmembrane region of FGFR3, common to three patients with classical Crouzon syndrome and acanthosis nigricans, a dermatological condition associated with thickening and abnormal pigmentation of the skin The mutation within the FGFR3 transcript was determined by direct sequencing as a specific gcg to gag transversion, resulting in an amino acid substitution ala391glu within the transmembrane region We also report the identification of a mutation in a single patient in the transmembrane region of FGFR2 Direct sequencing has revealed a missense mutation resulting in a substitution of a conserved amino acid gly384arg This was seen in a patent with a non-classical phenotype of cranyosynostosis, in whom there were no associated limb malformations This is the first evidence of genetic heterogeneity in Crouzon syndrome

14.012

Cytogenetic and molecular characterization of a critical region for sex differentiation on chromosome 13.

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We describe the cytogenetic and molecular findings in two patients with ambiguous genitalia Ambiguous genitalia are found in patients with chromosomal aberrations of the chromosomes 9, 10, 11, 13, 18, X, Y and in syndromes localized to chromosomes 1 and 7 The karyotypes of our patients are 46,XY,psu dic t(13;22)(13pterÄ>13q32 2 22p11 2Á>22qter) and 46,XY, r(13)(p12q34)/45,XY,-13, respectively According to the cytogenetic breakpoints we determined the haplotypes of polymorphic markers (D13S265, D13S159, D13S274, D13S173, D13S285 and D13S293) around the deleted region D13S159 is located within 13q32 The molecular breakpoints are located between D13S265 and D13S274 in patient 1 and D13S274 and D13S285 in patient 2, respectively In both cases the paternal allele is deleted Fluorescence in situ hybridization using the probes D13Z8 and D13S327 showed the absence of the terminal 180 kbp in our patients We conclude that the critical region for differentiation of the genitalia is located distal to D13S274 and 13q33 At present more polymorphic markers are studied in order to narrow the span the breakpoint is located in Less severe forms of genital malformations have been described for similar deletions as in our patients This variation might be due to mutations in corresponding genes on the other chromosome, imprinting or factors of other origin

4.018

A knowledge based model for the Fibroblast Growth Factor Receptor 2 (IIIc)

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The fibroblast growth factor (FGF) family currently consists of nine homolgous polypeptides which are involved in many stages of cell growth and development They are known to interact with high affinity to a family of four tyrosine kinase receptors to promote dimerisation of the receptor. Mutations in these receptors have been shown to be present in patients with human skeletal disorders such as Crouzon syndrome (FGFR 2 and 3), Pfeiffer syndrome (FGFR 1 and 2), Apert syndrome (FGFR2), achondroplasia, thanatophoric dysplasia and hypochondroplasia (FGFR3) Mutations have been observed in the extracellular domain, the transmembrane region and tyrosine kinase domains. The extracellular domains of the receptors have been predicted to belong to the I-set immunoglobulin family of proteins (Hapraz and Chothia, 1994) We present a model of the receptors based upon members of the I-set This model explains the action of many of the observed genetic mutations in human syndromes involving craniosynostosis and growth Hapraz,Y and Chothia C J Mol Biol (1994) 238 528-539

14.019

Localization of the gene for Möbius syndrome in a Dutch family on chromosome 3p.

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Möbius syndrome (MIM no 157900) is defined as a congenital pareses or paralysis of the seventh cranial nerve with or without involvement of other cranial nerves Furthermore, the syndrome is regularly accompanied by orofacial malformations and malformations of the extremities We performed linkage analysis in a large Dutch family with Mobius syndrome partly described by van der Wiel (Acta Genet Statist Med 7 348, 1957) Linkage was detected with the CA-repeat marker D3S1292 which showed the highest lod score of 5 76 at = 0 0 With markers flanking D3S1292, the locus for Mobius syndrome could be assigned to the interval D3S1589-1576, which spans approximately 10 cM on 3q21-25 2 The candidate gene CRBP1, located in this region, was excluded In a second family with Mobius syndrome, already described by Fortanier and Speyer (Genetica 17 471-486,1935) the locus on the long arm of chromosome 3 was excluded indicating genetic heterogeneity of the syndrome Reversed genetics is the only way to solve the unsettled discussion about the genesis of this diseae for which nuclear agenesis, failed axonal outgrowth or degeneration, or failure of axonal guidance by a primary muscle defect have been proposed

Dementias and cell death

15.001

Relationship of apolipoprotein E and amyloid precursor protein genotype to cognitive decline and dementia in Down syndrome.

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Adults with Down syndrome develop the characteristic neuropathology of Alzheimer disease and many demonstrate, with increasing age, a progressive impairment in cognitive function and the development of dementia This study reports the relationship between the longitudinal neuropsychological findings in 41 unrelated, Caucasian adults over the age of 30 years (48 1 years ± 1 1 SEM), with free trisomy 21 and their ApoE and Amyloid precursor protein genotype status using molecular genetic analysis at apolipoprotein E (APOE) and chromosome 21 loci, within, and flanking the amyloid precursor protein (APP) gene Two control groups of 37 euploid individuals and the other of 79 Caucasian children with free trisomy 21 were also studied Regression analysis of subject age and cognitive function show that age accounts for a significant proportion of psychometric test performance variability (F=9 5, p=0 004) Overall scores of intellectual deterioration and age are positively correlated (r=0 43, p=0 007) However, allele and genotype distributions at APOE and APP (Ivsl) with flanking markers D21S8 and D21S111, demonstrate no association with cognitive decline in this adult sample with Down syndrome These findings have implications for models of Alzheimer disease pathogenesis in the general population

15.003

Is the $tRNA^{Gln}$ 4336 mitochondrial DNA mutation a risk factor for Alzheimer's disease?

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One genetic risk factor that has been identified for Alzheimer's disease (AD) is the apolipoprotein E4 allele In addition, two recent reports have suggested that a mitochondrial DNA mutation within the $tRNA^{Gln}$ gene, located at position 4336, may be a risk factor for AD, as it was found in 10/246 (4 1%) cases with autopsy-confirmed AD We have determined the distribution of apolipoprotein E alleles, as well as the frequency of the mitochondrial mutation, within a community-based population of 65 demented individuals and age- and sex-matched controls and in an additional 44 non-demented group from the same population all aged over 70 In agreement with previous studies, we have found a significant association between the apolipoprotein E4 allele and demented cases However, the mitochondrial mutation was detected in 5/109 (4 6%) control individuals (aged 74, 81, 84, 86 and 91 years) but was not detected in any of the 65 demented individuals that were tested Since the age of onsot of dementia in AD cases with this mutation was between 60 and 76 years (mean 68) and because we find the mutation at a similar frequency in our non-demented controls as in previously-reported AD cases, our data challenge its pathogenic roles

15.004

Mutation analysis of the presenilin genes in families with early-onset Alzheimer disease.

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Several missense mutations have been identified in the presenilin (PS) genes causing familial early-onset Alzheimer disease (EOAD) The PS gene family comprises 2 genes encoding integral transmembrane proteins, i e PS-1 on chromosome 14q24 3 (AD3) and PS-2 on chromosome 1q31-q42 (AD4) Mutation analyses showed that 39 EOAD families carried a mutation in PS-1, while only 2 families had a mutation in PS-2 Most mutations are detected in the second putative transmembrane domain (TM-II) and the hydrophilic loop between the putative TM-VI and TM-VII The importance of these domains in the normal function and the pathological function of these genes is not yet understood To obtain a more accurate estimation of the frequencies of mutations in both PS genes and to identify functional domains, more extensive mutation analyses in additional EOAD families are needed Therefore we performed a genetic analysis in 6 EOAD families Linkage analysis using chromosome 14 and chromosome 1 markers indicated that in 1 EOAD family the disease was linked to the AD3 region Sequence analysis of RT-PCR products of PS-1 revealed a novel missense mutation in individuals of this family In the 5 remaining EOAD families linkage to the AD3 or AD4 locus could not be excluded Mutation analyses of the PS genes is currently performed in patients of these EOAD families

15.005

Search for APP gene mutation in DNA of Russian patients with dementia of the Alzheimer type

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Missense mutation in codon 717 of the amyloid precursor protein (APP) gene that codes for the precursor to the b-protein found in the amyloid deposits of Alzheimer disease was shown to be segregated with this disease in some kindred Further to our report on having searched for this mutation in a number of familial and "sporadic" cases with dementia of the Alzheimer type in Russian population we have investigated DNA isolated from the tissues of several patients with Alzheimer disease and dementia APP gene expression appears to vary in different tissues the highest levels of the amyloid deposits are detected in brain in liver, kidney and spleen amyloid protein has not been found In present work 48 DNA samples isolated from lungs, liver, muscles, adrenal gland, heart, kidney, pancreatic gland, spleen, thyroid gland and different parts of brain such as frontotemporal and parietal lobes, frontal pole cortex, hippocamp, cerebellum, subcortex and nucleus were tested for APP mutation None of them were positive These data support our previous results that APP gene mutation may be the cause of the disease rather rarely

EUCROMIC: European Collaborative Research on Mosaicism in CVS

Spoken presentations

Chorionic villi in prenatal diagnosis : The UK collaborative study update.

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Following the highly successful prospective data collection on diagnostic chorionic villus samples undertaken by the ACC working party on prenatal diagnosis for the years 1987-1989, a further retrospective period of 4 years has been added to the data (1990-1993) thus giving a cumulative experience of 7 consecutive years data A total of 28 laboratories provided data for this study with the majority supplying data for all the years covered From an initial collection of 7,500 diagnostic cases we now report the findings on well over 16,000 cases Data collected included gestational age, reason for referral, karyotype (direct and/or long term culture) cell ratio (for cases of mosaicism), karyotype of any prenatal follow-up, pregnancy outcome, and karyotype outcome This data allows us to determine the predictive value of the various mosaic and non-mosaic chromosome abnormalities encountered The success rates remained high (>98%) and the cumulative data adds support to the conclusions gained from the initial survey, although now the new data includes the finding of false positive diagnoses not encountered in the initial data

Karyotype discrepancies between CVS and foetus. Our data.

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Since 1986, our Unit offers cytogenetic prenatal diagnosis on chorionic villi samples From a total of 2,554 samples, carried out between 1986-1994, the foetal karyotype was achieved by the semidirect method in 2,516 cases (98 5 %) We found 31 discrepancies (1 23 %), 18 of which were mosaicism in CVS and 13 were "false positive". The chromosomes more frequently involved in this type of anomalies, in our series, were 9, 7, X, 18, 20 and 22, which were present more than 3 times On the other hand, chromosomes 1, 5, 6, 11, 12, 17, and 19 were never involved, except in two cases when a tetraploid set was found In 3 samples more than two chromosomes were involved in the mosaicism Structural chromosome abnormalities were detected in four cases (three were mosaics) The study of the outcome of these pregnancies, together with other data like maternal age or gestational age, can be good tools to help in the future to prenatal counselling

Cytogenetic experience on 4214 CVS.

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We performed 4214 CV analyses for foetal karyotype in the first trimester of pregnancy Both cultures and direct preparations were set up if the amount of CV sample was sufficient (82%of the cases), on the contrary only direct preparations were analysed We identified 164 abnormal karyotypes (3 9%),of which 26 were balanced and 158 unbalanced Among this last group 76 (1 8%)were true mosaicisms,confined mosaicisms and discrepancies In these cases a karyotype control was required on amniocentesis (or foetal blood) before considering the cytogenetic diagnosis as conclusive In only one case, a discrepancy between direct preparation (45,X) and culture analysis (46,t(x,x)) was not confirmed on the AF metaphases (46,XX) and resulted true on blood cultures after birth

Selective use of long-term cultures for the detection of false-positive cytogenetic findings with the (semi-) direct method at CVS

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In 1-2 % of prenatal chromosome studies with CVS, a discrepancy is found between the karyotype observed using a (semi-)direct method and the chromosomal constitution of the fetus To enhance the reliability of the CVS diagnosis, the combined use of a direct preparation and a long-term culture (LTC) has been recommended The data from our experience with the parallel use of LTCs and amniocentesis or other additional follow-up studies in combination with the (semi-)direct method are presented The results from 32 patients, with both mosaic and non-mosaic chromosomal abnormalities initially observed in the (semi-)direct preparation, clearly show that in the majority of cases (n=29), the fetal karyotype can be correctly predicted by the long-term culture In view of the possibility to preserve intact chorionic villi for up to 7 days before establishing the LTC (Hansson et al , in press), a policy is suggested in which the LTC is selectively used to detect false-positive findings in the (semi-) direct preparations Hansson, K , Schuring-Blom, G H and Leschot N J (1995) Prenat Diagn in press

CVS preparation techniques and ambiguous cytogenetic findings 1986-1994 in EUCROMIC (BMH1-CT93-1673)

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Type of preparation technique was reported to EUCROMIC for 85,197 karyotyped CVS 1986-1994 65 4% were karyotyped after direct preparation/short term incubation only, 6 3% after long term culture only and 28 3% after both Mosaicism or non-mosaic discrepancy was encountered in 1320 CVS (1 5%) The proportion of CVS with ambiguous cytogenetic results was 686/55,698 (1 2%) after direct preperation only, 56/5,408 (1 0%) after long term culture only and 578/24,091 (2 4%) after both methods The number of non-mosaic fetoplacental discrepancies out of the total number of ambiguous cases for each group was 153/686 (22%), 6/56 (11%) and 17/578 (3%) respectively, this difference is partly explained by cases where one preparation showed abnormal, non-mosaic karyotype, while the other revealed a mosaic or normal karyotype In CVS karyotyped after both preparations, the direct preparation was more often abnormal in centers using both methods infrequently (<15%), than in centers using both methods frequently (≥ 85%) (153/158 = 97% vs 149/240 = 62%). Most likely this reflects that in some CVS analyzed after both methods, culture was analyzed due to chromosome aberration on direct preparation, in others routinely Preparation techniques in relation to mosaic vs non-mosaic findings, false positive/negative diagnoses and chromosomes involved will be further discussed

Comparison of cytogenetic analysis of chorionic villi and amniotic fluid cells

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The cytogenetic results and the outcome of pregnancies in 2766 chorionic villus samplings and 1190 amniocenteses (AC) were evaluated from a fifteen-year period (1980-95) at the Haynal Imre Medical University of Budapest Transcervical CVS (TC-CVS) was performed between 9-12 weeks of gestation and transabdominal CVS (TA-CVS) at 13 6 weeks in average, amniocentesis was done usually between 15-19 weeks Mosaicism was noted in 0 76% of CVS cases and in 0 25% of AC cases The possibility of uniparental disomy occurred in three cases, and tests gave

negative results Despite of the higher rate of mosaicism in CVS, and due to the fact that the procedure-related fetal loss rate was lower in the TA-CVS group than in the TC-CVS or AC patients in our centre the TA- CVS is the preferable procedure The reasons of this attitude considering the local possibilities is discussed

Progress of the EUCROMIC ancillary studies on clinical follow-up and UPD research

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The European collaborative study on prenatal diagnosis (EUCROMIC) has collected cytogenetic and clinical information on CVS since 1986, the registry contains data on 89,347 CVS through 1994 Of these, 1382 (1 5%) involved mosaicism or other types of feto-placental discrepancies In 1993, EUCROMIC became a European Union concerted action (no BMH1-CT93-1673) Two ancillary studies, supported also by the Swiss government (OFES 93 0337-2057) are coordinated in Geneva In the first, clinical follow-up of children born after CVS mosaicism, pregnancy outcome is known for some 700 cases reported through 1993, birth weight (BW) is available for about 500 Long-term follow-up has been obtained on about 150 children thusfar Analysis of the birthweight data indicated a statistially significant difference between the distributions and regression slopes in the mosaic/discrepant study group as compared to control populations Our current task is to compare birthweights of children born following pregancies in which CPM for various autosomal trisomies was observed The second ancillary study is the search for uniparental disomy (UPD) in fetuses or children whose gestations were ascertained through CVS mosaic/ambiguous results To date, 90 UPD tests have been performed Fifteen cases of maternal UPD, for chromosomes 9,14,15,16 and 22, have been detected The UPD-positive cases were characterized by a higher proportion of CVS done because of intrauterine growth retardation and/or abnormal ultrasound findings and a greater percentage of abnormal cells (over 75%) on CVS

Investigations on the distribution of different cell lines in the placenta after prenatally diagnosed mosaicism

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About 1-2% of cytogenetic analyses after CVS lead to pathologic results which are not confirmed in the fetus Most of these cases show confined placental mosaicism (CPM) Even if the amount of the aberrant cell line in the placenta is high it still can not be concluded that the fetus carries the same aberration The stage of the mutational event and a cell selection against an abberant cell line (proven for different autosome aneuploidies) are reponsible for the distribution of the cell lines The aim of our investigations was to analyse the amount and distribution of mosiac karyotypes in 6 defined areas of the placenta These were from two opposite peripheral, intermediate and central regions We present 20 cases of prenatally detected mosaicism where additional tissues were analysed pre- and postnatally The aberrations comprised numerical and structural changes of autosomes (n=10) and gonosome (n=7), two tetraploidy and one triploidy mosaicisms In 17 cases placenta biopsies were available after delivery 10 of those placentae were studied more in detail by analysing 6 defined areas using FISH on metaphase and interphase nuclei The investigations showed that there are great differences in the amount of the different cell lines in the single placenta There is no obvious region which shows preferentially a higher or lower amount of a pathologic karyotype

Prenatal diagnosis of maternal heterodisomy for chromosome 15 following trisomic zygote rescue

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We present a case of maternal heterodisomy for chromosome 15 in the third pregnancy of a 39 year old patient who was referred for prenatal diagnosis because of a previous DS child, and also increased maternal age. A CVS sample taken at 11 weeks gestation showed a 47,XX,+15 karyotype (30 cells) on direct preparation and 46,XX/47,XX,+15(45 cells 25 cells) on cultured preparations An amniotic fluid sample taken at 15 weeks showed a 46,XX karyotype in 229 cells examined Molecular analysis on DNA extracted from cultured amniocytes gave informative results on two probes PMS620 and D15S113 Both loci showed maternal heterodisomy This was interpreted as being indicative of Prader Willi Syndrome and the pregnancy was terminated at 20 weeks Analysis of fetal tissue confirmed maternal heterodisomy for chromosome 15 This case therefore represents further evidence for trisomic zygote rescue, resulting in this instance in uniparental disomy for chromosome 15

Fetoplacental discrepancy involving trisomy 16

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Trisomy 16, the most frequent trisomy in human conceptions, was considered to be invariably lethal as it was found, initially, only in spontaneous abortions In recent years, this trisomy was reported also in ongoing pregnancies and in newborns in a mosaic status The increasing number of CVS's performed in several centers in the world has allowed to detect cases of trisomy 16 confined to the placenta with none or few manifestations in the fetuses or in the newborns that, when present, were thought to be due to uniparental disomy (UPD) We report a severely malformed fetus with a non-mosaic trisomy 16 confined to the cytotrophoblast UPD was discarded in the fetus by molecular studies Our hypothesis is that the trisomy 16, of prezygotic origin, disturbed the development of the embryonic mesoderm during the critical stage of blastogenesis This case alerts on that trisomy 16 confined to the placenta can have graver consequences on the fetus than was thought before We recommed that a more cautious counselling must be given to the parents in cases of trisomy 16 in the placenta

EUCROMIC registration of prenatal diagnosis 1986-1994

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90,981 chorionic villus samples were received for chromosome analysis at the 79 laboratories that contributed data to EUCROMIC during the period 1986-1994 Karyotypes were obtained in 89,347 (98 2%) of the samples CVS mosaicism was found in 1201 (1 3%) of the CVS, non-mosaic fetoplacental discrepancy in 181 (0 2 %) The trends in the use of CVS and changes in mosaicism, discrepancy and maternal cell contamination are calculated from the numbers registered for centers reporting all years since 1986 The data for 1993 and 1994 concerning prenatal diagnosis in general provides information on changes in the use of early and late CVS, early and late amniocentesis and cordocentesis The correlation between technique for prenatal diagnosis and single cell line non-ambiguous chromosome aberration, mosaicism, non-mosaic discrepancy, maternal cell contamination and failure rate is presented

Clinical evaluation of first trimester amnifiltration

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Chorionic villi sampling allows an early prenatal diagnosis but is hampered by a high frequency of discrepant cytogenetic findings in extraembryonic/embryonic tissues A major concern with early amniocentesis is the possible effect on fetal development of the removal of a relatively large proportion of amniotic fluid (AF) Hence, an amnifiltration technique with consecutive refunding of the AF into the sac is now introduced to early routine diagnostics In a pilot study among 100 pregnant women undertaking legal abortion in the 7th to 12th week a mean of 2 4 ml, 2 8 ml, 4 4 ml, 13 7 ml 15 8 ml, 20 7 ml AF, respectively, was obtained, till tenting occured (K et al PD 12 595ff,1992) Facit Filtration technique should allow AC as early as the 10th week (i e 15 ml AF as in midtrimester AC) In a 2nd study an appropriate filter system was evaluated 20 ml AF were aspirated in each of 80 mid-trimester routine-diagnostic biopsies 5 ml of the AF were filtered and the trapped cells were cultured according to the same regimen as the non-filtered cells As measured by minimum "culture time" a mixed cellulose filter membrane (pore size 5 µm) proofed to be the most efficient (K et al PD 13 247ff,1993) After a 0-series (35 women before termination at 8-14 weeks) the amnifiltration technique is carried out in a clinical trial We have now performed 42 diagnostic AC at completed weeks 11 (n=4), 12 (n=26), and 13 (n=12) allowing a definite cytogenetic diagnosis in all cases (mean culture time 13 3 ± 1 5 days, control 13 1 ± 2 5, mean number of colonies 16,6 ± 9 6, control 15 5 ± 5 4) No abortion within 2 weeks after AC came to our attention Conclusions AC performed in the 11/12th week includes all advantages of CVS (early result allowing for less invasive termination procedure) but avoids its disadvantages (lack of AF-AFP screening) This study is supported by a DFG grant

Coelocentesis - a new method for early prenatal diagnosis.

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The extra-embryonic coelom surrounds the amniotic cavity and the fetus during the first 10 weeks of gestation Puncture and aspiration of liquid from this cavity is possible under transvaginal ultrasound guidance between the 6th to 10th week of gestation The technique is called coelocentesis (Jurkovic,D Et al , Lancet 1993, 341 1623-24) Coelocentesis was done in 60 women undergoing legal termination of their pregnancies for psycological reasons In 30 samples attempts to culture the coelomic cells were made With one culture system 9 of 10 samples were successfully cultured and standard cytogenetic analysis were made The cytogenetic results always agreed with those obtained by analysing the corresponding chorionic villi samples Successful culture of coelomic cells has not been described before and with further investigation this could be a future method for very early prenatal diagnosis

Evaluation of rapid FISH method using gamma globin mRNA with X, Y, 18, and 13/21 DNA probes for the isolation and cytogenetic analysis of fetal nucleated red blood cells.

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The detection of fetal cells in circulating maternal blood using non-invasive methods for prenatal diagnosis represent a new field of application which is under development Three types of fetal cells are identified and can be isloated in maternal blood (trophoblasts, lymphocytes, and erythrocytes) Fetal eythrocytes or nucleated red blood cells (NRBC) from maternal blood could be used as material for non invasive prenatal diagnosis To study this, we have evaluated the use of a rapid flurescence in situ hybridization (FISH) system, Right ™ , using probes specific to the mRNA of gamma hemoglobin simutaneously with DNA probes for X, Y, 18 and 13/21 on a large number of NRBC Fifty four 5 ml samples of fresh newborn cord blood were analyzed The fetal NRBCs were enriched using Ficoll-Hypaque density gradiant centrifugation and deposited on slides Hybridzation was carried out for 15 min at 85%C The percentage of fetal NRBC ranged from 44% to 100% This evaluation demonstrated that this method using FISH in combination with mRNA gamma hemoglobin and DNA probes for chromosomes X, Y, 18, 13/21 could accuratley and rapidly identify fetal NRBC and enable analyse of chromsome copy number

Posters

Analysis of oocyte meiotic chromosome pairing by SC immunocytology, FISH and TEM

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We have used a monoclonal antibody against the meiotic proteinaceous pairing structure the Synaptonemal Complex (SC), in combination with FISH to study the pairing behaviour in oocytes of structural chromosome aberrations ascertained by prenatal diagnosis We present the results of a sequential LM and transmission electron microscopy (TEM) investigation of a Case diagnosed by FISH of amniocytes (using the whole chromosome 18 probe) to have a karyotype 46,XX,-18,+der(18) (t18,?)(p13,?) Complex pairing configurations were seen, which we interpret to be the result of subterminal homologous pairing initiation of the unbalanced 18p translocation There was evidence both of lack of synapsis of 18p and triple pairing of chromosome 18 In addition there was an indication of an interchromosomal effect with the appearance of univalence of chromosomes not involved in the rearrangement To our knowledge the meiotic oocyte pairing configurations association with structural heterozygosity has only been previously investigated in one case (Speed, RM , 1986, Hum Genet 72,256) The new combination of SC immunocytology with FISH and TEM allows a more precise identification of the pairing abnormalities and should in the future further our insight into the process involved in normal meiotic chromosome pairing and synapsis, which still remains an enigma

Trisomy 10 mosaicism and maternal uniparental disomy in a liveborn infant with severe congenital malformations

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Trisomy 10 mosaicism in a liveborn has to our knowledge been described only once previously in a newborn with the trisomic cell line restricted to fibroblasts We describe a severely growth retarded, malformed newborn with trisomy 10 in 25/30 mitoses in skin fibroblasts, while the karyotype as obtained on cultured lymphocytes was 46,XY in 50/50 mitoses The child died 37 days old Biopsies taken at autopsy from achilles tendon, costal cartilage and pericardium confirmed trisomy 10 in 19/30, 30/30 and 26/30 mitoses respectively Karyotypes of lymphocytes from both parents were normal The major malformations of the child as confirmed at autopsy were microcephaly, microphtalmia, cleft and lip palate, thoracic scoliosis, anal atresia and malformations of heart and limbs Parental origin of the extra chromosome 10 was investigated using the following microsatellites D10S88, D10S89, D10S249, D10S220 and GLUDP2 The additional chromosome was of maternal origin and data consistent with a meiotic non-disjunction Analysis on DNA extracted from cultured lymphocytes showed maternal uniparental disomy for chromosome 10 Karyotyping skin fibroblasts is important, if chromosome aberration is strongly suspected, irrespective of a normal karyotype of lymphocytes

Specificity of the first and second trimester prenatal diagnosis: pathological data

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Comparison of prenatal and pathological findings was performed in 790 second trimester and 41 first trimester fetuses Postabortion second trimester fetal autopsy changed or completed the prenatal diagnosis in 29 1% of cases, termination of pregnancy was found to be based on incorrect prognosis in 0 6% of cases Diagnostic discrepancies were more often (34 1%) in abortuses of 15 to 22 gestational wk than in the group of 22 to 28 wk foetuses (16 3%), the results of prenatal diagnosis were better in Minsk-city (24% of incomplete diagnoses) than in Minsk region (29 1%) Detection rate of the first trimester ultrasound in our series of 685 parallel sonographical and pathological examinations was 54 7%, in the group of 10 to 12 week fetuses the percentage of ultrasound recognition was higher (73 7%) The first trimester nuchal translucency was found to be a marker of various malformations and their complexes other than Down's syndrome cardiac malformations with normal karyotype, trisomy 18, de Lange syndrome, limb-body wall complex and some other skeletal deformities were revealed on postabortion pathological examination of such first trimester fetuses All the first trimester therapeutic terminations of pregnancy were based on correct prognosis

Simple and rapid prenatal DNA diagnosis of Down syndrome

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In 1991 a quantitative PCR based technique to determine gene dosage for sex chromosome aneuploidies was reported (Lubin et al , 1991, Mol Cell Probes 5, 307-312) This strategy was later modified to determine Trisomy 21 using quantitative automated analysis of small tandem repeats (STR) markers (Mansfield ES, 1993, Hum Mol Genet 2, 43-45) STR markers are a class of hypervariable repeats found throughout the genome, consisting of repeat elements of 3-6 bases in length Our aim was to apply and evaluate this procedure as a diagnostic test for Down syndrome to provide a rapid inexpensive alternative to present prenatal diagnosis by cytogenetics We have used the ABI scanner with DNA extracted from surplus amniotic fluid (range 0 5-5ml) and the tetranucleotide repeat polymorphism at the D21S11 locus (where the PIC value is 0 82) in 1,000 Cases, ascertained for routine prenatal Down cytogenetic screening A total of 15 Cases of Trisomy 21 were identified by the DNA analysis and confirmed by cytogenetics No Case of fetal Down syndrome was missed by this alternative technology when using adequate amniotic fluid samples, not heavily contaminated by maternal blood Heterozygous control samples showed 2 peaks of equal intensity, which is easily distinguishable from heterozygous Down syndrome samples, where there are either 3 peaks of equal intensity or 2 peaks with a dosage ratio of 2 1 Our results indicate that prenatal diagnosis of Trisomy 21 Down syndrome by DNA technology may *per se* provide an adequate alternative to traditional cytogenetics The potential benefits include (1) the rapid diagnosis with results readily available within 48 hours, and (2) the cost benefit with one molecular scientist estimated to be able to process 1,500-2,000 Cases per year

Neonatal outcome of 1035 CVS and 2076 amniocenteses with normal karyotype.

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We started to study the outcomes after prenatal diagnosis and up to now we have collected data about 3111 CVS and amniocenteses, representing the 80% of the sample of the Prenatal Centre of 5th Obstetric Clinic, University of Milan The incidence of unbalanced chromosome anomalies was 4 0% after CVS and 2 14% after amniocentesis, while the incidence of balanced chromosome anomalies was 0 78% for CVS and 0 74% for amniocenteses In the present study the stillbirths, the therapeutic abortions, the spontaneous abortions and newborns with abnormal karyotypes have been excluded Neonatal outcome including gestational age and weight at birth of the newborn with normal karyotype of 1035 CVS and of 2076 amniocentesis, compared to the Italian population of newborn in 1985 and 1991, is presented This work was supported by C N R PF"Prevention and Control Disease Factors" subproject 7 Grant 41 115 19572

Discrepances between the karyotype in chorionic villus sampling and the fetal karyotype.

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Approximately 1% of the pregnancies studied by chorionic villus sampling (CVS) show confined placental mosaicism (CPM) We have studied 1798 CVS Thirteen of them (0 72%) showed discrepances respect to the fetal karyotype The cause for prenatal diagnosis study in these cases was maternal age except two cases who had parental translocations All CVS were performed by trasncervical way before the 13th week of pregnancie and by transabdominal way after the 14th week In one case, the first study was performed on amniotic fluid (AF) and the discrepance was found in CVS trying to confirm the karyotype All studies were performed by standard cytogenetic procedures Three of them were followed up by Fluorescence In situ Hibridization and in one case by DNA analysis The chromosomes involved in CPM were of C and D group, 18, 21, X and marker chromosomes In CVS, all but one case were mosaics The remainder case was tetraploid in all cells We confirmed these karyotypes in AF, fetal fibroblasts or peripheral blood cultures Two cases were confirmed in a second CVS

Cytogenetic experience on 8820 amniocenteses.

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Since 1983 we have analysed 8820 AF cultures for foetal karyotyping We identified 265 total chromosome anomalies 170 (1 9%) omogeneous unbalanced anomalies, 49 (0 55%) balanced (including de novo rearrangements) anomalies, 46 (0 52%) mosaics (II and III level) For the last group a check on foetal blood cultures and/or second amniocentesis was carried out In cases where a single unbalanced colony was detected, only a genetic counselling and an echographic control of foetal morphology in selected centres was suggested We observed also a change regarding the indication to prenatal diagnosis In the last years the malformed foetus observed at US examination appears as group of analyses, dense of chromosome abnormalities, which let us to indicate the correct prognosis of pregnancy and a better genetic counselling for future pregnancies More recently, the use of biochemical maternal serum screening for Down S risk introduces a new indication In the present experience, 200 amniocenteses were performed because of a positive test and a 2,5% of chromosome anomalies detected . This work was partially supported by C N R PF "Prevention and Control Disease Factors" subproject 7 Grant 41 115 19572