Identification of a new nt480delG mutation in Multiple Endocrine Neoplasia Type 1

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The gene for Multiple Endocrine Neoplasia Type 1 (MEN1) was identified in 1997. It is presumed to be a tumour suppressor gene but the function of the protein product, menin, remains unknown. The gene has 10 exons; mutations are widely dispersed throughout the gene and there is no common mutation. We undertook SSCP analysis of exons 2, 3 and 7 (because these contain ~70% of previously reported mutations) from 9 independent families with MEN1 attending an endocrine clinic. In one family, band shifts were noted in exons 2 and 3. DNA sequencing identified the change in exon 2 to be (G at nt480), and that in exon 3 was a R171Q polymorphism. The mutation was confirmed in other family members by restriction enzyme digests of PCR products from genomic DNA. This particular mutation has not been reported previously. The polymorphism co-segregated with the mutation in this family. As a result of identifying this mutation, screening tests can be targeted appropriately in this family. SSCP analysis is being undertaken for the remaining exons in the other 8 index cases.

Determination of SMNt and SMNc copy number in SMA families

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Determination of copy number of the survival motor neurone gene SMNt and the centromeric pseudogene (SMNc) is becoming increasingly important as our understanding of the mutational mechanisms of spinal muscular atrophy (SMA) develops. Elucidation of SMNt and SMNc copy number is important in three groups of individuals: Firstly, patients homozygously deleted for SMNt (approximately 95% of affected cases.) Recent publications have outlined a model in which Type I patients differ from Type II and III patients in their mutational mechanisms. Determination of SMNc copy number in type I, II and III patients should increase our understanding of these mechanisms. Secondly, non-homozygously SMNt deleted patients. Affected patients in this group are likely to be heterozygously deleted. Confirmation of heterozygous deletions would identify patients for further point mutation analysis, and subsequent confirmation of diagnosis. Lastly, parents of affected individuals. Detection of carriers of heterozygous deletions of the SMNt gene will aid future management of SMA families. We have developed an assay for the determination of SMNt and SMNc copy number by fluorescent DNA dosage analysis and have tested 100 patients in total from the three categories described.

New PCR-Based Test for Fragile X Mutation Detection

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Fragile X mutation detection by DNA analysis enables the accurate diagnosis of normal, premutation and mutation states of the fragile X syndrome. Analysis relies on the ability to detect the expansion of a CGG repeat motif in the FRAXA locus. This is currently carried out primarily by Southern blot analysis however this procedure is time consuming, labour intensive and requires relatively large amounts of DNA. The use of PCR offers a solution to these problems but the amplification of the CGG motif under normal conditions is problematic. We present a novel PCR-based test that is able to quickly and accurately amplify and size normal alleles and expansions of up to 800 repeats. We have designed this test to work on extractions of whole blood simplifying sample preparation. The test also includes an internal amplification control that allows the estimation of gene copy number for the identification of homozygotes and so-called “male heterozygotes”. The resulting PCR products are run on an ABI PRISM 310, 373 or 377 using the GeneScan fragment analysis software. The data is automatically interpreted by downstream software. This test therefore provides the accurate diagnosis of DNA analysis with the sensitivity of PCR but in a greatly simplified and automated manner.

Variation of site-specific methylation patterns in the factor VIII (F8C) gene in human sperm DNA

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The pattern and efficiency of cytosine methylation in vertebrate genomes appears to be influenced by a number of different factors which exert their influence upon methylation patterns in a complex combinatorial fashion. An integrated analytical approach was therefore adopted so as to assess the relative importance of these factors. The methylation status of 12 CpG sites in three exons (8, 14 and 23) of the human factor VIII (F8C) gene was examined by bisulphite genomic sequencing of human sperm DNA from 14 individuals (8 European Caucasians and 6 Asians). Different CpG sites were found to vary in their methylation status both within and between individuals. Strand differences in methylation status were also detected at certain sites, a finding which could reflect hemi-methylation. No evidence for systematic deviations in methylation status were found, however, between the two ethnic groups. Only a limited correlation was observed between the level of methylation of specific CpG sites in sperm DNA and their mutability measured in terms of their frequency of detection as a cause of haemophilia A. This is probably due to the pattern of methylation observed in mature spermatocytes not being representative of that of the germline.
14.12  
APEX nuclease mutations and motor neurone disease  
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Apurinic/apyrimidinic endonuclease (APEX nuclease) on chromosome 14q11.2-12 is a multifunctional DNA repair enzyme with an important role in the protection of cells from oxidative stress and in the regulation of the DNA-binding of several nuclear factors. We have screened genomic DNA from 120 sporadic, 24 familial MND patients and 64 anonymous control samples for mutations in the APEX nuclease gene. SSCP and heteroduplex analysis identified six different potential mutations in the samples investigated. Subsequent sequencing identified two rare polymorphisms in the 5 untranslated region, one rare polymorphism in the 3 untranslated region and one common 2-allele polymorphism (D148E). D148E variants were analysed for differences in allele frequency between sporadic MND patients and controls (p=0.029). A conserved amino acid base change in codon 315 occurred in three sporadic MND patients and a 4-base pair deletion (TCCA) was identified in one sporadic MND patient. The exonic deletion created a premature stop codon 174 amino acids from the expected end of the gene. This preliminary data indicates that the APEX nuclease gene may contribute to the aetiology of MND.

14.13  
Nearest-neighbour Effects upon Germline Nucleotide Substitution Rates in Human Genes  
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The spectrum of single base-pair substitutions logged in the Human Gene Mutation Database (7271 different lesions in 547 different genes) was analysed for nearest-neighbour effects upon relative mutation rates. To this end, a novel estimation procedure was devised, correcting for the confounding effects of differential codon usage and considering different clinical observation likelihoods for different amino acid replacements. Over and above the well-established hypermutability of CpG dinucleotides, only a subtle and locally confined influence of the surrounding DNA sequence upon relative single base-pair substitution rates was observed which extended no further than 2 bp from the substitution site. A disparity between the two DNA strands was evidenced by the fact that, when substitution rates were estimated conditionally upon the 5' and 3' flanking nucleotides, a significant rate difference emerged for 10 of 96 possible pairs of complementary substitutional events. Mutational bias favouring substitutions towards flanking bases, reminiscent of misalignment mutagenesis, was apparent and exhibited both directionality and reading frame sensitivity. A moderate but significant correlation was noted between the relative mutability and thermodynamic stability of DNA triplets, suggesting either hindrance of the DNA replication process in regions of high stability or the transient stabilization of misalignment intermediates favouring nucleotide misincorporation.

14.14  
Mutation analysis of the TSC1 gene reveals unequal expression of both alleles.  
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Tuberous Sclerosis (TS) can be caused by mutations in either TSC1 or TSC2. Virtually all disease-causing mutations in TSC1 are stop codons or frameshifts predicted to truncate the protein product. Mutation screening has produced two important findings: 1. So far mutations in TSC1 have correlated precisely with clinical diagnosis. The only well-documented case of non-penetrance in TS is now solved. Here we report a remarkable case where an affected grandchild carries a sporadic TSC2 mutation (R751X) in a family already carrying a TSC1 (L250X) germline mutation. This finding is important for genetic counselling. 2. Seven TSC1 mutations were analysed on cDNA using RNA extracted from lymphoid cell lines. Here semi-quantitative RTPCR and phosphoinosine analysis showed that same of the seven patients analysed showed reduced levels of the mutant transcript as compared to normal transcript. Ratios of mutant to normal transcript varied from 0.06:1 to 0.36:1. Equal intensities of both alleles were observed at the genomic DNA level. By using this technique on polymorphisms within the TSC1 transcript, it may be possible to distinguish patients in which TSC1 is mutated as compared to TSC2.

14.15  
A common RET polymorphism results in failure to detect mutations in MEN 2 patients  
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Mutations at cys 634 in exon 11 of the RET proto-oncogene account for approximately 87% and 50% of ME N 2 and FMTC cases respectively. Mutations at this codon are detected by amplification using published primers and digestion with four restriction enzymes. We describe a MEN 2 family with a known cys 634 mutation which did not delete due to a common polymorphism underlying the antisense primer. In the index case, both the polymorphism and mutation were on the same allele, resulting in preferential amplification of the normal allele and failure to detect the mutation. In contrast, the mutation was identified in a positive control homozgyous for the polymorphism. Of the MEN 2 samples received in the laboratory, 23% were heterozygous for the polymorphism. We are in the process of repeating the cys 634 screen on these samples. It is possible that varying dete clon rates reported for cys 634 mutations may have been due to this polymorphism. This study illustrates that in order to prevent mutations being missed, primers for diagnostic tests must be investigated for underlying polymorphisms.
14.16
A Fluorescein-based approach to identify mutations in the
Fanconi Anaeemia Group A gene
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Fanconi anemia (FA) is a genetically heterogeneous autosomal recessive
disorder associated with chromosome fragility, bone-marrow failure, congenital
abnormalities and cancer. The group A gene (FAA), mutations in which
account for 60-65% of FA cases, has an open reading frame of 4.3kb, dis-
tributed among 43 exons. The heterogeneity of the FAA mutational spec-
trum and high frequency of intragenic deletions makes mutation detection a
challenge. We have designed a strategy for FAA mutation screening using a
combination of two fluorescently based PCR techniques. Quantitative fluo-
rescent multiplex PCR assays were developed to detect deletion heterozy-
gotes. These two assays simultaneously amplify 5 and 4 exons respectively
from the FAA gene and 2 exons from the FAC gene as external controls.
Point mutations are detected by fluorescent chemical cleavage of mismatch
in 6 overlapping RT-PCR fragments. Fluorescent sequencing of cleavage
products is then carried out to confirm any mutations. Preliminary results
indicate that a combination of these two methods will lead to a higher detec-
tion rate than previous studies. Use of this strategy will allow rapid classifica-
tion of patients as FAA or non-A and knowledge of the causative mutation
will permit rapid prenatal diagnosis.

14.19
Characterisation of a second hit in a plexiform neurofibroma
from a neurofibromatosis type I (NF1) patient
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Neurofibromatosis type I (NF1) is an autosomal dominant disorder affecting
1 in 3,500 individuals. The NF1 gene is located on chromosome 17q 11.2
and spans 350kb of genomic DNA. A hallmark of the disease is the occur-
rence of benign dermal and plexiform neurofibromas. Genetic and biochemi-
cal data support the hypothesis that NF1 acts as a tumour suppressor
gene. Analysis of a number of NF1-specific tumours has shown inactivation
of both NF1 alleles during tumour development consistent with Knudson's
two hit hypothesis. Identification of germline and somatic mutations in a
dermal neurofibroma from an NF1 patient has been recently reported. In this
study, we report our analysis of a plexiform neurofibroma from an NF1
patient, in which mutations in both NF1 alleles have been characterised. The
constitutional mutation involved a splice site change in obligate GT doublet
(288+1 G-A), and the somatic change, a nonsense mutation (R816X).
On screening 40 NF1 blood-tumour pairs for loss of heterozygosity (LOH) in
tumour samples, using a panel of 16 intragenic and extragenic NF1 mark-
ers, only 3 tumours demonstrated LOH. These results suggest other mech-
anism could be involved in NF1 tumourgenesis and are currently being
investigated.

14.18
Application of the protein truncation test (PTT) to mutation
analysis in the neurofibromatosis type I (NF1) gene
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The neurofibromatosis type I (NF1) gene is located at 17q 11.2 and con-
tains 60 exons. Mutation analysis in the NF1 gene has been hampered by
the size of the gene, the high rate of new mutations, a lack of mutational
clustering and the presence of homologous loci. A mutation detection
method based on RNA offers rapid screening of large multi-exonic genes.
In this study the complete NF1 cDNA was screened in five reactions using
PTT. Analysing RNA for mutations can produce false negative results if the
mutant mRNA is unstable. The extent of this allelic exclusion was examined
by analysing RNA from 15 NF1 patients with known truncating mutations.
PTT detected the mutant allele in 11 samples. One of the remaining four
samples was informative for the exon 5 coding polymorphism and exhibited
allelic exclusion. PTT was applied to a further 41 NF1 patients with unknown
mutations. Aberrant bands were identified in 20 samples and 11 of these
changes have been fully characterised (495del4, 1127del3, del 1466-1537,
1541delAG, 2537insTG, 3456deIACTC, R1278X, R1362X, ins4047-4217,
6364+2G, 7458delC). PTT is proving to be a valuable and rapid method for
NF1 mutation analysis, and in this study had a detection rate of 50%.

14.20
932delA - a novel LDLR mutation causing FH in N. Ireland
shows evidence of common ancestral origin.
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Familial Hypercholesterolaemia (FH) is a co-dominant single gene disorder
affecting 1 in 500 individuals and caused by mutations in the LDLR gene at
ch19p13.2. DGGE and fluorescent sequencing has detected mutations in 51
of 60 families who clinically and genetically meet the criteria for definite FH.
A novel mutation in exon 6, 932delA, was detected in 5 unrelated families.
Haplotype analysis using 2 intragenic RFLPs and 2 closely linked
microsatellites was performed on the families comprising 41(932delA +ve)
and 38 (932delA -ve) individuals. The mutation co-segregated with a com-
mon haplotype, except for one crossover event in an affected individual,
suggesting a founder mutation in the N. Irish population. The lipid profiles of
family members >16 yrs were analysed and the average total cholesterol for
the 932delA +ve group was 10.08 (SD1.87) and for the 932delA -ve group
4.92 (SD0.99). Two of 20(932delA +ve) individuals tested had T-chol levels
below the diagnostic criteria for FH and 3 of 18(932delA -ve) individuals had
equivocal levels. Mutation screening enables presymptomatic detection of
at risk individuals, particularly children, allowing improved disease manage-
ment and clarification of disease status in individuals with equivocal lipid
profiles. Haplotype analysis has applications for definite FH families with no
detectable mutation and for confirming linkage to the LDLR locus in families
with ambiguous clinical symptoms.
14.22
Vohwinkel's keratodermia: a novel loricrin mutation
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We report a kindred with autosomal transmission of Vohwinkel's keratodermia. Eight affected members had a diffuse honeycomb-type palmoplantar keratodermia, mild pseudo-aunium and an associated generalised ichthyosis most prominent over the extensor aspects of the limbs. Microsatellite marker analysis showed no evidence of recombination with D1S1664, D1S305 and D1S196, supporting linkage of the disorder to the epidermal differentiation complex on chromosome 1q21. This cluster includes the genes for loricin, involucrin, filaggrin, small proline-rich proteins and calicyn. Subsequent direct sequencing of the loricin gene identified a heterozygous mutation with insertion of a thymine (T) residue at codon 209 introducing a frameshift. Only one mutation has been described previously in the loricin gene, in this subtype of Vohwinkel's keratodermia. This is a guanine (G) insertion producing a frameshift after codon 231. The novel mutation we report is likely to have similar functional effects, with impairment of transglutaminase-mediated cross-linking of the cornified envelope.

14.23
Mutation analysis of the GPC3 gene in Simpson-Golabi-Behmel Syndrome (SGBS)
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Simpson-Golabi-Behmel Syndrome (SGBS) is an X-linked disorder with prenatal and postnatal overgrowth as its main characteristic feature. Affected individuals usually have distinctive facial features and delayed motor milestones, but are of normal intelligence. Abnormalities of other organs may also be present and some affected individuals develop embryonal tumours. The gene responsible for SGBS is Glypican 3 (GPC3), a heparin sulphate proteoglycan. GPC3 is thought to interact with IGF2 and may play an important role in growth control in embryonic mesodermal tissues. The GPC3 gene comprises 2,130bp arranged in eight exons which span approximately 500kb of genomic DNA. We have tested DNAs from a large series of 96 patients with classical SGBS or several features consistent with SGBS, initially for large-scale deletions (by PCR) and currently for smaller mutations (by SSOP and bidirectional dideoxy fingerprinting). To date, we have identified 7 patients with deletions (5 reported previously) and 10 patients with small-scale mutations which are being characterised. This supports our preliminary conclusion that large-scale deletions would be responsible for only a minority of SGBS cases and should provide sufficient information to assess genotype-phenotype correlations.
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14.24
Genomic CTG/CAG repeat size is significantly enlarged and inversely correlated with age-at-onset in Type II diabetics
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PURPOSE: To perform a preliminary examination of the genomic CAG/CTG repeat size in diabetics compared to controls. METHODS: a sample was drawn from East Yorkshire consisting of fifty diabetics and 28 healthy controls. All are Caucasians with no significant difference in age or gender between patients and controls. The age-at-onset was obtained for 70% of the patients. The repeat expansion detection method (RED) was applied to the genomic DNA of subjects at least twice. SUMMARY: The maximum size of genomic CAG/CTG repeats is elevated in significantly more subjects in the diabetic group than in the control group (p<0.01). The frequency of the largest CAG>11 repeats in the type II diabetics (16%; 4/25) is markedly higher than that estimated using ten reports of its frequency in control Caucasians (1.8%; 18/977). Moreover, three of these four patients with type II diabetes and CAG>119 repeats presented with an early age-at-onset of ~36 years (p<0.05). So we tested the correlation of the larger CAG repeat subset (>40 triplets) with age-at-onset of type II diabetes. The genomic CAG repeat size is inversely correlated with age-at-onset (Spearman rank correlation coefficient=-0.81, p=0.002). None of the corresponding associations were found for the type I diabetic group. To try and identify the locus of these genomic repeats, the CTG size of SEF-2 on chromosome 18 was examined. The size of this repeat did not correspond to that detected by the RED... [Abstract truncated at 1500 characters]

14.25
Cystic fibrosis mutations and variants associated with male infertility
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Male infertility as a result of congenital absence of the vas deferens (CVA) is a recognised complication of cystic fibrosis (CF). However, a recent paper reported that CF mutations are also present at higher than expected frequency in infertile men without CAVD. We studied 244 males without CAVD who presented with azoospermia or oligospermia (<20 million sperm/ml) at a Dublin infertility clinic. Eight males with an abnormal karyotype and 4 with Yq microdeletions were excluded. We analysed lymphocyte DNA for 8 cystic fibrosis mutations: DeltaF508, R117H, Delta507, G542X, G551D, R560T/K, R352Q, and 621+1G→T. Eighteen mutation carriers were identified in the 232 males tested (7.8%), significantly higher than the CF carrier frequency in the Irish population (5%, p=0.039). Sixteen men carried the DF508 mutation, one carried G551D, and one carried the 621+1G→T mutation. All of these mutations would be expected to result in a marked reduction of the amount of CFTR expressed. We also tested for the '5T' variant in intron 8 of the CFTR gene, as it is known to be associated with reduced CFTR expression. We found 35 of our 232 men (15.1%) to have the 5T variant (expected 5%, p<0.001). The excess of 5T alleles was highly significant in men with 1-5 million sperm/ml, and in men with 5-20 million sperm/ml, but less significant in men with very low sperm counts or azoospermia. Only one CF mutation carrier and two 5T variant carriers had azoospermia, indicat... [Abstract truncated at 1500 characters]
14.26
Molecular analysis of exon 15 of the APC gene in 17 Northern Irish families with Familial Adenomatous Polyposis: 9 germline mutations identified by restriction digest, PTT and sequencing.
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Germline mutations of the adenomatous polyposis coli (APC) gene are responsible for familial adenomatous polyposis (FAP) an autosomal dominant predisposition to colorectal cancer. We have investigated a series of families in Northern Ireland in order to identify and characterize the specific APC mutations. Restriction enzyme digest was used for the common deletion mutations followed by PTT analysis of exon 15 from genomic DNA. This identified 9 variants from 17 families (53%). Subsequent sequencing of these mutations identified 7 frameshift mutations, 4 consisted of a 5 base deletion (del ACAAA at codon 1061) producing a termination at codon 1063 and 2 detected for (del AAAGA ) at codon 1309 producing a termination at codon 1313. A single base deletion (2746 delG) resulted in a termination at codon 955. Two nonsense mutations (CAG>TAG), Q1191X and (C>T) Q1529X were identified. The present study indicates that the combined use of RE digest, an optimized PTT and subsequent sequencing offers an effective strategy for direct mutation detection and presymptomatic diagnosis in FAP.

14.27
Rapid Throughput Mutation Analysis of the Hereditary Non-polyposis Colorectal Cancer Genes hMLH1 and hMSH2
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Hereditary non-polyposis colorectal cancer (HNPPC) is an autosomal dominant disorder characterised by early onset cancer of the bowel. Mutations in at least four mismatch repair genes have been detected in HNPPC patients. Two of these genes, hMLH1 and hMSH2, account for approximately two thirds of affected families, and co exist of 19 and 16 exons respectively. In order to undertake a mutation screen of all the exons in both genes, 35 PCR reactions at 9 different cycling conditions would normally be required per patient sample when using the published primer sets. To create a more efficient test, we have devised a 96 well microtitre plate rapid through hptt screen, using multichannel pipette transfer and intron-derived primers, designed to work at the same optimum isd PCR cycle conditions. Mutations are detected by visualising SSCP's and heteroduplexes in the same tracks on 12% acrylamide gels run in Sanyo cold cabinets overnight and then silver stained. Products which are found to have a band shift are sequenced using the ABI 310 with dRhodamine chemistry. We have found this new high throughput screen to be an invaluable development in order to accommodate the increasing demands of multigene cancer diagnosis in the molecular diagnostic setting.

14.28
Rapid Mutation Analysis of Charcot-Marie-Tooth Type 1
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Charcot-Marie-Tooth (CMT) disease describes a clinically and genetically heterogeneous group of hereditary peripheral neuropathies. In our laboratory we screen all CMT patients for a 1.5 Mb duplication encompassing the PMP22 gene at 17p11.2 which is associated with over 70% of CMT cases. Patients negative for the duplication are then selected on the basis of family history for subsequent screening of the Cx32 (chromosome Xq13) and P0 (chromosome 1q23) genes by a combination of SSCP/heteroduplex analysis and sequencing analysis. Eight of 70 unrelated patients (11%) without a PMP22 duplication were found to have a mutation in exon 2 of Cx32. These included 3 previously unreported sequence changes: an S93G mutation in codon 11, a 1 bp deletion of nucleotide 186 in codon 42 producing an early stop at codon 63 and an A487Q mutation in codon 142. A limited screen of P0 exon 2 was carried out in 50 patients with no previously detected mutation; this has yielded 3 further mutations including one previously unreported mutation in P0, a V94F substitution in codon 32. These results demonstrate the benefits of a sequential screening strategy for CMT analysis.

14.30
Mutational analysis in the mucopolysaccharidosis disorders
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The mucopolysaccharidoses (MPS) are lysosomal storage disorders resulting from defects in the catabolism of glycosaminoglycans. Affected individuals can be diagnosed by enzyme analysis, whereas for carrier testing, it is less reliable and DNA analysis is required for confirmation. MPS I is an autosomal recessive disorder due to a deficiency of the enzyme alpha-L-iduronidase. There are 3 clinical subtypes of the disease ranging from the severe Hunter syndrome to the milder Scheie syndrome, with an intermediate Hunter/Scheie form. Eighty-nine patients, who had been diagnosed clinically and enzymatically as MPS I, have been screened for 8 common mutations W402X, Q70X, P533R, A75T, 474-2a>g, R89Q, 678-7g>a and L218P, by PCR amplification of the IDUA gene and restriction enzyme digestion. Hunter disease, MPS II, is an X-linked recessive disease resulting from a deficiency of iduronate-2-sulfatase. Mutational analysis has been carried out in 38 patients by SSCP analysis and sequencing. Mutational and linkage analysis for MPS I and MPS II is currently being transferred to the Clinical Molecular Genetics laboratory at Great Ormond Street Hospital, with the prospect of including Sanfilippo disease (MPS III). Other lysosomal storage disorders such as Gaucher disease, Fabry disease, metachromatic leukodystrophy (MLD), and arylsulphatase A pseudodeficiency are also being included.
14.31
Novel mutations in the 3' region of the Polycystic Kidney Disease 1 (PKD1) gene suggest important protein domains
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It is estimated that ~85% of ADPKD is due to PKD1. Mutation detection in the PKD1 gene is difficult because more than two thirds of the gene is reiterated several times on chromosome 16. In this study, mutation screening in 90 ADPKD1 patients was carried out on some of the exons in the single copy area (37, 38, 39, 44, 45) using genomic PCR and SSCP. One family was found to have a 15 bp intrame deletion in exon 39 in two affected members [Arg-Leu-Arg-Gln-Val (3747-3751)]. Three different mutations were observed in exon 44 in a few other cases. There were two transitions [12341A->G (4044V), 12252T->C (F4014S)] and one G insertion at position 12290. 12341A->G has already been reported as a pathogenic mutation but in our study it is clearly a normal variant. The second and third mutations in exon 44 are reported here for the first time. The latter produces a frameshift that starts with a substitution of Glycine for Valine and is predicted to produce a novel, 127 amino acid polypeptide. Three affected members of another family were found to have a novel 3 bp intrame deletion in exon 45 (12601-12603). This results in deletion of a Leucine residue (L413del). One of the novel mutations described here in exon 39 deletes the same five amino acids as a previously published mutation but exhibits a different nucleotide deletion indicating that this particular repeat-rich DNA sequence is a deletion hotspot. All these novel mutations suggest functionally important... [Abstract truncated at 1500 characters]

14.32
Mutation analysis in the duplicated part of the polycystic kidney disease 1 (PKD1) gene
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Mutation screening in the PKD1 gene has mainly been confined to exons 35-46 in the single copy 39 end of the gene owing to the presence of several highly homologous copies of the 59 end. These have made PKD1 specific PCR amplification difficult. Techniques that have allowed limited definition of mutations in exons 22-34 of the duplicated region include anchored RT-PCR and long range PCR (LR-PCR) using one primer located in the single copy region, and the protein truncation test. Exons 1-21 which represent nearly 60% of the coding region remai n to be screened. We have developed a LR-PCR strategy that allows specific amplification of PKD1 sequences from genomic DNA from a unique region identified in intron 1. Products ranging in size from 5-17 kb have been gene rated that allow mutation detection by direct sequence analysis of exons 2-21. The specificity of the intron 1 primer for the PKD1 gene was tested using radiation hybrids. LR-PCR products were sequenced directly with ABI Big DYE terminators. In a preliminary trial of the technique in 85 patient samples all amplified exons could be sequenced directly. As has been previously reported silent polymorphisms are common in this gene compared to pat hological mutations which include substitutions and insertions. Interestingly large deletions that can be resolved on conventional agarose gels were rare with only one deletion of 2.9 kb being found. This deletion extended from within intron 1 into exon 5. This technique and the ability to specifically amplify exons 22-34 in an addi... [Abstract truncated at 1500 characters]
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Mutation screening strategy for craniofacial disorders
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The Supra Regional Craniofacial Genetics Service at Great Ormond Street Hospital was established in 1997 to provide genetic analysis to support the four UK craniofacial referral centres. In the first 6 months 76 samples were received with the following suggested diagnoses: 10 Apert, 14 Crouzon, 6 Pfeiffer, 6 Saethre-Chotzen and 40 non-specific craniosynostosis/no details. The patient referral criteria are briefly reviewed clinically and the majority are tested for the FGFR3 P250R mutation first. 15.5% of those tested were positive (6 non-specific craniosynostosis/no details, 2 Crouzon, 1 Saethre-Chotzen). Pfeiffer samples were also tested for the FGFR1 P252R mutation (1 positive). Negative samples and all Crouzon samples are screened for mutations in FGFR2 exons 5 and 7 (by SSCP). Cases which are still negative are screened for FGFR2 Exon 8. Apert samples are tested for the common FGFR2 P253R and S252W mutations (6, 1, 3 patients had S252W, P253R or neither, respectively). Two prenatal samples (1 Apert, 1 Crouzon) have been tested and reports issued. The clinical details of any negative samples following the above procedure are reassessed to determine if further analysis is appropriate or whether a negative report should be issued.

14.34
Type VII Collagen (COL7A1) Mutation Screening in Severe Recessive Dystrophic Epidermolysis Bullosa Patients using the Protein Truncation Test and Fluorescent Chemical Cleavage of Mismatch
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Severe recessive dystrophic epidermolysis bullosa is an autosomally inherited disease characterised by fragility and blistering of the skin. The disease is caused by mutations in the structural protein type VII collagen, which, with a few exceptions, cause protein truncations on both alleles. COL7A1 is a large multi-exonic gene with a known mutation spectrum and is expected to be an ideal candidate for an RNA based protein truncation test. A nested RT-PCR PTT protocol was developed but alternatively spliced transcripts presented a major problem. Therefore, a second mutation detection strategy was developed based on the genomic amplification of twenty-one fragments encompassing all (118) exons and their splice-sites, and subjecting these fragments to fluorescent chemical cleavage of mismatch (FCCM). Although the FCCM is more labour intensive this is outweighed by the significantly higher number of mutations detected. We will present the advantages and disadvantages experienced with both these techniques, which will help in the design of mutation screening strategies for other disorders.