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Preimplantation diagnosis of sex in X linked disease by fluorescent in situ hybridisation

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We have used four approaches to sexing human blastomeres using the technique of fluorescent in situ hybridisation (FISH). The first and second involve using Y and X chromosome specific probes respectively in single hybridisation experiments. Although good efficiency of hybridisation is obtained, each strategy has drawbacks and could lead to males being diagnosed as females because of failure of hybridisation (Y probe) or because of tetraploid nuclei (X probe). The third is a dual FISH approach using two Y chromosome probes and one X chromosome probe. Here the possibility of diagnosing males as females is virtually eliminated, but it takes 24 hours to perform alone and thus does not allow biopsy, spreading, diagnosis, and transfer within the 24 hour period. The fourth approach is a rapid dual FISH sexing strategy taking six to seven hours and giving more reliable results than the above approaches. It is this method which has been put into clinical practice. Ten patients, each carrying X linked disorders, have had preimplantation diagnosis of sex in this way in a total of 12 treatment cycles. In the first eight cycles biopsy was on day 2 post-fertilisation and cells were allowed to divide overnight before FISH diagnosis. In the final four, biopsy, spreading, FISH diagnosis, and transfer were all on day 3. It is the latter strategy which has proven to be the most effective in terms of number of nuclei positively diagnosed.

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A polymerase chain reaction (PCR) protocol with nested primers has been developed for amplification of a fragment including the site of the predominant deletion (AF508) of the CFTR gene causing cystic fibrosis (CF) from single cells. The presence of the deletion in the amplified fragment is then detected by intentional heteroduplex formation in mixtures with previously amplified DNA from normal or homozygous deleted cells and gel electrophoresis. Extensive preliminary analysis with single cells, including blastomeres from cleavage stage human embryos, showed that amplification efficiency was high and, to date, the genotype of the original cell has always been correctly identified when analysed blind. Thus, it is possible to diagnose the AF508 deletion within seven to eight hours and transfer selected unaffected or carrier embryos later the same day as cleavage stage embryo biopsy. Preimplantation diagnosis of cystic fibrosis was attempted in three couples in which both partners carry the AF508 deletion. In vitro fertilisation techniques were used to recover several oocytes from each woman and fertilise them with the husband’s sperm. Normally fertilised cleavage stage embryos were biopsied on the third day post-insemination and one or two cells removed for DNA analysis. In one couple, only two oocytes fertilised normally. DNA analysis failed with one of the embryos and the other was diagnosed as affected so none was transferred. In the other two couples, three unaffected or carrier and several affected embryos were identified. Both couples chose to have one normal and one carrier transferred to the uterus later on the same day as biopsy and diagnosis. One of these women became pregnant and has now given birth to a normal girl, unaffected by CF, and free of both the parental mutant alleles.

Preimplantation diagnosis of cystic fibrosis by DNA amplification from single cells

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The reliability of diagnosis of single cell PCR

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The polymerase chain reaction has been shown to be capable of amplifying a single molecule of a single locus sequence to visibly detectable amounts of specific signal. We have analysed over 500 single sperm for the AF508 mutation causing cystic fibrosis from a known carrier of this deletion. The method of amplification used for the analysis of very small amounts of DNA tends to require two rounds of PCR. In the second round a nested set of primers, internal to those used in the first round, is used to reamplify a small aliquot of the product from the first round PCR. When this method has been successful the product from the second round of PCR is easily visualised after gel electrophoresis under UV light. We have also achieved similar results with a coamplification system, where sex chromosome specific primers are added to the first round reaction. A comparison of the ΔF508 mutation and the normal sequence has shown the incidence to be 45.9% respectively. A comparison of 150 and Y bearing sperm has shown the incidence to be 50:50. The average rate of success for the coamplification of single sperm in our hands is ~65%. There are many possible reasons for this. The accuracy of the FACS sorter adding a single cell to each of a 96 well microtitre plate was shown to be 99%. Cell lysis liberating the genomic DNA for PCR is the first critical chemical reaction and can be hampered if the cell adheres to the plastic wall of the wells or if the sulphydryl reduction is not effective. These data indicate the need for more rigorous testing before single cell PCR can be used routinely for genome analysis, although we have shown there does not appear to be any meiotic drive for CFTR.

Diagnosis of APKD by genetic markers and ultrasonography

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The aim of this study was to assess the effectiveness of ultrasonography and molecular techniques in the presymptomatic diagnosis of APKD. The data were collected from a voluntary registry of 150 affected families. An independent calculation of the age dependent false negative rate of ultrasonography in subjects at 50% risk of inheriting the APKD gene was 3% in the second and third decades. Twenty-two families were informative for multipoint linkage analysis. Using this method we calculate that the upper limit for the prevalence of APKD transmitted at the PKD2 locus is 4.5%. When these figures were included in individual risk assessments, 83% of subjects at 50% prior risk could be assigned a modified risk category with an error rate of 5% or less.
Phenotype and genotype heterogeneity in autosomal dominant polycystic kidney disease

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A study of autosomal dominant polycystic kidney disease (ADPKD) identified 18 families (285 affected subjects) with mutations at the PKD1 locus linked to the αglobin complex on chromosome 16 and five families (49 affected subjects) in which occurrence of this locus could be dismissed (non-PKD1). Comparison of clinical features showed that non-PKD1 patients lived longer (p = 0.005), had a lower risk of developing renal failure (p < 0.05), were less likely to have hypertension (32%, 95% CI 14-82%), and were diagnosed at an older age (p < 0.001), and had fewer renal cysts present at the time of diagnosis (p < 0.01). While the majority of PKD1 families were ascertained through clinical centres treating patients with renal impairment, none of the non-PKD1 families came from this source (p < 0.05). It is likely that the current estimates of the proportion of unlinked based predominantly on families ascertained from dialysis and renal transplant programmes, are underestimates of the true prevalence of non-PKD1.

Detection of germline mutations in the APC gene in FAP families

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Familial adenomatous polyposis is an autosomal dominantly inherited condition predisposing to the development of colorectal adenomas and polyps with the inevitable progression to colorectal cancer. The recent isolation of the causative APC gene has enabled the direct detection of mutations in affected subjects allowing accurate, presymptomatic diagnosis in at risk family members. Worldwide, preliminary studies have identified 50 to 60 different germline mutations in this gene. We have chosen the technique of denaturing gradient gel electrophoresis (DGGE) as the most sensitive with which to screen FAP families for APC gene mutations (Fodde et al, 1992). To date, 71% of the cases have been screened for germline mutations in DNA samples from 16 FAP and two sporadic colorectal cancer patients.

Twelve DGGE variants have so far been identified and, of these, 11 have been characterised by sequencing. Four base substitutions in exons 5, 7, 8, and 10 and seven frameshift mutations, all in exon 15, have been identified; 5 bp deletions at codons 1061 and 1309, since none of the methods could be applied to unrelated families. Preliminary phenotype-genotype correlations between CHRPHE status and the nature and distribution of these APC gene mutations have been made.

Discrepancy in the estimation of relative male and female mutation rates for Duchenne muscular dystrophy

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Analyses of the relative male and female mutation rates giving rise to Duchenne muscular dystrophy have generally concluded that the two rates are equal. We have identified two recent reports from the direct analysis of deletion mutations (van Essen et al, 1992), and an indirect analysis on both deletion and non-deletion mutations (Muller et al, 1992) also concluded that the rates were approximately equal. We have estimated statistically the relative rates in (1) deletion cases, by determining the proportion of carrier mothers having a male fetus compared with a female fetus, and (2) in non-deletion/duplication cases, by determining the grandparental origin of the mutant X chromosomes. In both cases the relative rates appeared to differ significantly from a 1:1 ratio, by finding in (1) significantly fewer mothers of isolated cases to carry a deletion, and in (2) a higher number of mutant X chromosomes to be of grandpaternal origin, when compared with a model of equal male and female mutation. The discrepancy between our data and those of recent studies can be explained by a significant level of mosaicism in the different studies, since our methods was able to take this into account directly. This suggests that a more detailed molecular approach is required to resolve the question of new mutations in DMD.

Fetal echogenic bowel and cystic fibrosis in low risk fetuses

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Sonographic demonstration of increased echogenicity of the fetal bowel in the second trimester is known to be associated with cystic fibrosis (CF). In a series of 200 fetuses at high risk of having CF, ultrasound examination showed echogenic bowel in 50 to 60% of affected fetuses. However, there are several observations of this observation which include an incidental finding, intrauterine growth retardation, congenital infection, and other abnormalities of the fetal bowel. The incidence of echogenic bowel in the low risk population and the relative contribution of the different underlying pathologies is unknown. This makes the initial estimation of risk and counselling of parents following an unexpected finding of echogenic bowel difficult. Until recently the only diagnostic test available for CF in these circumstances was measurement of amniotic fluid microvillar enzymes, but this can be associated with erroneous results, particularly where the prior risk is low. With the identification of the CF gene and mutations within it we are now able to offer a more accurate diagnostic test in 78% of cases. We have experience of eight cases where echogenic bowel was detected with ultrasound in low risk fetuses. The parents and/or fetus were screened for mutations in the CF gene. In three cases the parents were found to be heterozygous for the ΔF508 mutation and subsequent testing showed that the fetus was homozygous. In all cases the parents elected to interrupt the pregnancy. The non-concordance confirmed the presence of a significantly diluted ileum filled with meconium. In the fourth case analysis of a fetal blood sample showed that the fetus carried both the ΔF508 and G54D mutations. At birth the neonate had meconium ileus requiring surgical treatment. Our observations have confirmed the association of echogenic bowel with cystic fibrosis. However, counselling of parents after the incidental finding of echogenic bowel at a routine second trimester ultrasound examination is difficult as there are no low risk population data available. There is clearly a need to establish a study to determine the significance of this finding in the general population.

Glucokinase gene mutation in type 2 diabetes mellitus

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Type 2 diabetes mellitus is among the commonest metabolic diseases but despite much research its aetiology has remained largely unknown. There is a strong genetic component as illustrated, for example, by the high level of concordance between twins and this has given impetus to genetic studies of diabetes. Progress has been made recently by the demonstration of linkage of a marker near the glucokinase gene on chromosome 7p to a subtype of type 2 diabetes known as maturity onset diabetes of the young (MODY) in French and British pedigrees. We have now characterised a mutation in the glucokinase gene in the British pedigree and have found a codon 299 mutation in exon 8
resulting in a substitution of glycine to arginine. Fifty patients previously diagnosed as having classical type 2 diabetes mellitus were screened for this mutation and one further patient was found to have this exon 8 mutation. This patient was a member of a large family with multiple affected members; all the 10 subjects in this family who have inherited this mutation have type 2 diabetes. The discovery that glucokinase gene mutation may cause MODY and a proportion of late onset type 2 diabetes should facilitate further progress in understanding the metabolic and genistic basis of type 2 diabetes.

Cloning of a candidate gene for Menkes syndrome spanning the MTK translocation breakpoint

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The gene responsible for Menkes syndrome has been assigned to Xq13 by a combination of comparative mapping and linkage analysis. A previous report has mapped the translocation breakpoint (Kaput et al, 1986) associated with the disease in a female patient to an interval delimited by PGK1 and a group of six more proximal Xq13 markers, including DXS56. A cosmid (cFl1) located 30 kb from a translocation breakpoint associated with Menkes syndrome in a female patient (Consalez et al, 1992) was used to screen a fibroblast cDNA library. Several positive clones were isolated and found to hybridise a 9 kb transcript on numerous different tissues, including muscle, heart, brain, fibroblast, pancreas, and, to a lesser extent, liver, lung, and kidney. The same clones in turn used to screen a total of five cDNA libraries constructed from fibroblast, fetal brain, human skeletal muscle, and HeLa cells. As a result, a contig of over 30 cDNA clones has so far been developed, spanning the translocation breakpoint and extending over a 250 kb genomic region. A putative promoter region has been identified 350 kb distal to locus DXS56. Automated sequencing of the gene has disclosed a 4 kb ORF directed distal to proximal, encoding a 1300 aa protein, disclosing homologies to cytochromes, ceruloplasmin, and zinc binding peptides. Work is in progress to define the key functional domains and physical structure of this protein.


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The UK born Pakistanis offer an opportunity to investigate the effects of inbreeding in a developed country, and Birmingham is well suited for such a study. A prospective survey started with births in 1986 and the 4934 babies enrolled then have been followed up for five years. These index children were born to 2432 European mothers, 509 Afro-Caribbeans, 625 Indians, 956 Pakistanis, 216 Bangladeshis, and 196 mothers of other races. There were 667 (98%) Pakistani mothers related to their husbands, usually more closely than first cousins. The mean inbreeding coefficient of their children was 0.0086. The offspring of the consanguineous Pakistanis suffered three times the amount of mortality and morbidity compared to the other ethnic groups. Such offspring had a 1% risk for early death owing to congenital anomalies, a 2% risk for death before the age of 5 from other causes, and a 7% risk for a severe chronic illness or disability, which was accompanied by mental retardation in half the cases. Several new socio-economic features of non-consanguineous Muslims were the same as consanguineous Muslims, the excess risk for severe health problems suffered by the latter group is attributable to inbreeding.

A splice site mutation of the COL4A5 gene detected by DGGE causes Alport syndrome

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Alport syndrome is an X linked disorder characterised by sensorineural deafness and nephritis. Electron microscopic examination shows patchy thinning and thickening of the glomerular basement membrane. A new col- lagen (COL4A5) has recently been identified and assigned to Xq22, the same region as Alport syndrome. A small number of dele- tions and re-arrangements of the COL4A5 gene have been reported, but the majority of disease alleles are likely to arise consequent to point or small DNA sequence alterations. We have looked for mutations in 18 patients affected by Alport syndrome and two with the historically distinct disorder, thin membrane disease. The COL4A5 genomic sequence has at least 50 exons. Following computer simulation, GC clamped primers have been generated for a number of exons. Single base changes have been detected by DGGE of PCR amplified genomic DNA. We report a 1% substitution of the last nucleotide of exon 14 (3') in brothers with marked sensorineural hearing loss, haematu- ria, and mild renal impairment at the ages of 17 and 12 years respectively. The base change predicts either a Gly to Ser amino acid substitution or a splice donor site muta- tion. Illegitimately transcribed lymphocyte COL4A5 mRNA from the carrier mother is composed of two populations, one reduced in size compared to the normal. Sequencing will confirm whether this mutant molecule arises through exon skipping.

Medical genetics in the reformed NHS and in Europe

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Although there appears to be support in Britain from the House of Commons, Government ministers, and patient organisa- tions for augmenting genetic services, no genetics centre currently has adequate re- sources and therefore depends on volunteers for its development. The 2% of the population are more than willing to accept genetic counseling and testing, without consultation with the government. The government would be able to stop the circumvention of genetic development in the last two years. Worse, Wales and at least one English region have devolved genetic services to districts, which is contrary to policy stated by ministers. To improve the situation medical geneticists, assisted by the Royal College of Physicians and others, are developing strategies which is hoped will maintain integrated regional clinical and laboratory services and achieve well evaluated develop- ments within the environment of NHS Research and Development. Meanwhile there is increasing interest in the DSFH in surveying manpower and training in the EC and be-
yon, and this will become important in the UK with greater job mobility. We have found that the clinical genetics is officially recognised in only four EC countries (Germany, Netherlands, Portugal, and UK) and in six non-EC countries (Austria, Czechoslovakia, Finland, Israel, Norway, and Sweden). In seven EC and five non-EC countries there is no formal recognition. Procedures for accreditation differ and it is currently impossible to assess comparability of role and training of clinical geneticists (almost nothing is known of the comparability of laboratory medical geneticists). Five EC countries (France, Germany, Netherlands, Portugal, and UK) and six non-EC countries (Czechoslovakia, Egypt, Finland, and Sweden) report training programmes in clinical genetics with a duration varying from two years (France, Germany) to up to nine years (Denmark). There is, however, general agreement about the skills required by clinical geneticists and consequently about the content of training programmes and in more than half the EC and non-EC countries respondents strongly support the establishment of a European diploma in clinical genetics. The ESHG will make recommendations to EC and national governments to seek official recognition and resources.

Characterisation of a 340 kb YAC and cDNA enrichment from a region spanning the locus for retinitis pigmentosa (RP3)

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X linked retinitis pigmentosa has been shown to be genetically heterogeneous with one locus (RP2) localised to Xpl1.4-p11.2 by linkage and another by genetic linkage, while the other locus (RP3) is localised to Xp21.1-p11.4 both by genetic linkage and by the analysis of deletions. Two patients have been identified with deletions in the Xp21.1 region who have retinitis pigmentosa, one of whom (BB) also suffered from DMD, McLeod’s syndrome, and CGD. Analysis of these deletions has suggested that the CYBB locus, responsible for CGD, is the closest distal marker to RP3 and may be close to the proximal BB deletion breakpoint junction (JBB). We have, therefore, isolated a subclone from JBB which is currently being used to characterise this region further. Since CYBB is the closest distal marker it was used to screen the available YAC libraries by PCR. Pulsed field gel analysis and fluorescence in situ hybridisation (FISH) of a 340 kb YAC has confirmed that CGD and JBB lie within 200 kb of each other and suggest that this YAC contains the RP3 gene. In addition, we have confirmed the presence of at least three Cpg rich regions in the YAC that may represent islands. The YAC was used to isolate expressed sequences from the RP3 region by cross hybridisation to retinal cDNA libraries. Purified YAC DNA fragments were annealed to catch-linkers, amplified by PCR, and immobilised on a filter or attached to streptavidin-magnetic beads using biotinylated PCR primers. Amplified cDNA phage clones were annealed to the YAC. Following a series of stringent washes, bound cDNAs were eluted from the YAC and either put through a second round of enrichment or cloned. By this means cDNA sequences contained within the YAC are selectively enriched. cDNAs isolated using this method are currently being characterised.

An RT-PCR-SSCP strategy for detection of mutations in the gene encoding the α1 chain of type I collagen: application to the analysis of four osteogenesis imperfecta patients

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The aim of this research was to develop a general screening strategy for detection of mutations in the gene encoding the α1 chain of type I collagen in patients with osteogenesis imperfecta (OI). The resulting protocol involves five main elements: (1) reverse transcription of total cellular RNA from cultured fibroblasts, (2) PCR amplification from the cDNA of four overlapping segments, each of approximately 1 kb, (3) generation of subfragments of approximately 100 bp to 350 bp in a series of five single or double restriction enzyme digests for each amplified product, (4) analysis of single-strand conformation polymorphisms (SSCP) of subfragments, a technique capable of identifying fragments harbouring mutations; analysis of more than one digest at this stage increases the efficiency of detection of mutations and often allows mapping of the mutation to a small region of the amplified product, (5) DNA sequence analysis of the region of interest to define the mutation. Application of this strategy has led to the identification of mutations resulting in glycin to serine substitutions in the α1 chain of type I collagen in four patients with OI. The substitutions are at positions 247 and 352 in patients with lethal phenotypes (Sillence type I) and at positions 382 and 541 in patients with non-lethal phenotypes (Sillence types IV and III, respectively). These results extend our knowledge concerning the relative importance of domains within the collagen chains with respect to their function.

Elucidation of possible mechanisms of sex chromosome non-disjunction using centromere map analysis

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We have studied 98 sex chromosome aneuploids of maternal origin with 15 or more highly polymorphic probes to investigate if aberrant recombination is involved in the etiology of non-disjunction. We have found that 58 of the non-disjunctional events occurred at meiosis I, 17 at meiosis II, four as postzygotic mitotic errors, while as yet 19 are undetermined. Centromere map analysis has shown highly significant differences in the level and distribution of recombination on the X chromosome between non-disjunctional and normal meiosis. These differences include both complete absence of recombination (Nolullochiasmate non-disjunction) and a very marked increase of pericentromeric recombination.

Investigation of mutations of the p53 tumour suppressor gene in fresh and archival ovarian cancer material

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We have investigated the incidence of p53 mutations in fresh ovarian cancer tissue by a combination of chemical cleavage and DNA sequencing of PCR amplified fragments from exons II to IX; these are the regions of the gene that contain the mutation hot spots. To date we have identified mutations in 6/22 specimens. In five of these specimens the mutation would have produced an amino acid substitution in the p53 protein, and in the sixth case the mutation represented a polymorphism. DNA has now also been extracted from paraffin fixed archival material and is being analysed in the same way. The use of archival material should allow correlation between p53 mutations and a variety of clinical parameters, and may provide an indicator of those patients who will benefit from toxic drug treatment.

A variant of the Li-Fraumeni syndrome?

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We describe a mother and daughter with constitutionally high levels of p53 protein in their normal tissues. The patients are members of a family with multiple cancers, similar to the spectrum of tumours seen in Li-Fraumeni families, many with an early age of onset. Sequencing of the entire p53 gene and detailed protein analysis in the daughter suggests that she is accumulating high levels of wild type p53. We propose that this phenotype defines a familial cancer susceptibility syndrome, similar to the Li-Fraumeni syndrome, which is defined by high levels of p53 expression in normal tissue.
Prenatal sex determination from maternal peripheral blood using the polymerase chain reaction

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We have investigated the use of a nested PCR assay for the detection of a fetal specific Y chromosomal sequence (DYS14) from DNA extracted from unsorted maternal peripheral blood. Serial dilutions of male DNA into female cord blood DNA indicated that the assay could detect an equivalent of a single male cell in 300,000 female cells. The assay exhibited absolute specificity for male DNA with no amplification from a panel of 10 female cord blood DNA. When used on DNA extracted from unsorted peripheral blood from a series of 75 pregnant women, concordance between the assay results and fetal sex was observed in 55 cases (73%). The assay showed positive predictive values of 86%, 67%, and 87% in the first, second, and third trimesters, respectively. The corresponding negative predictive values were 83%, 64%, and 67%, respectively. We have also shown that retesting 44 samples a second time allows detection of a proportion of male bearing pregnancies with a high degree of accuracy in that all 15 women who gave positive signals in two consecutive amplifications had male fetuses. We have also applied the test at eight weeks' postpartum to eight women who had previously delivered male babies and no Y specific signal could be detected in any of them, thus suggesting that most women have cleared their circulation of fetal cells by eight weeks after parturition.

Biochemical screening for chromosome abnormalities and neural tube defects in the first trimester

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We have identified AFP, UE3, intact hCG, Free β hCG, and PAPP-A in pregnan-

cies with various chromosomal and structural abnormalities identified within a series of 14,000 consecutive prospectively collected serum samples at 7 to 14 weeks' gestation. In a group of 16 Down's syndrome pregnancies, median MOMs were: AFP 0.65, UE3 0.67, intgCH 0.97, FβhCG 1.96, and PAPP-A 0.58. Significant correlation was found in the Down's samples for intgCH/FβhCG and for PAPP-A/FβhCG. In a group of five trisomy 18 pregnancies median MOMs were: AFP 0.71, UE3 0.34, intgCH 0.27, FβhCG 0.15, and PAPP-A 0.62. The most effective combination of markers along with maternal age was FβhCG and AFP which gave an estimated 54% detection of Down's syndrome pregnancies at a false positive rate of 5%. None of 13 pregnancies with open NTD from which maternal serum samples were collected at 8 to 13 weeks' gestation had raised (≥20 MoM) MSAFP. However, matched second trimester samples from the same pregnancies at 16 to 20 weeks all had significantly raised MSAFP levels. No significant differences were found for UE3 and intgCH. Thus, biochemical screening for chromosome abnormalities may be practicable at any gestation from 7 to 20 weeks using AFP, FβhCG, and maternal age. However, a separate screening protocol using AFP at 15 to 20 weeks' gestation would still be required for effective detection of NTD.

Preliminary results of a prospective study of Down's syndrome screening using Free β hCG

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Retrospective studies over the past two years have identified that maternal serum Free β hCG, in combination with AFP and maternal age, can achieve Down's syndrome detection rates higher than that achieved by total hCG, AFP, and maternal age combinations. To test the true efficiency of this proposed strategy we have undertaken a prospective study. In our pregnant population of 7864 cases we have observed 10 cases of Down's syndrome, which equates to an incidence of 1.3 per 1000 births. Only two cases were in women over 35 years old. Of the 10 cases, eight were identified by the Free β hCG/ AFP/maternal age protocol with a Down's syndrome diagnosis in 300 or greater. To identify these cases, 5.5% of the pregnant population was identified at increased Down's syndrome risk. Of these women, 89% accepted an offer of amniocentesis. In addition our screening protocol identified with increased Down's syndrome risk, three cases of Turner's syndrome, two cases of hydrops and diagnosis in the third trimester and seven cases of fetal death. One case of tri-

osity 13 was also terminated (in a mother over 35) in which the Free β hCG level was less than 0.65 MoM. We conclude that a Down's screening programme using Free β hCG is the protocol of choice and have shown prospective detection rates of 80% can be achieved with the added benefit of identifying other abnormalities.

Rapid confirmation of trisomy 18 on interphase cells from venous and cardiac blood smears using a directly fluoresceinated 18 centromere specific probe

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We present three cases where in situ hybridisation to whole blood smears using a directly fluoresceinated, centromeric probe, Chromoprobe 18, allowed the detection of trisomy 18 (as determined by the presence of three signal spots) in interphase cells with the minimum of sample and probe preparation time. In two cases postnatal confirmation of trisomy 18 detected at amniocentesis was possible after TOP (case 1: 14/67, control 0/50; case 2: 26/40, control 1/55, three signal spots observed). In the third case a newborn baby with Edwards syndrome requiring surgery was provisionally diagnosed as tri-

sony 18 within 24 hours (case 3: 59/163, control 2/104, three signal spots observed). Conventional cytogenetic analysis at 48 hours confirmed this finding. By minimising sample and probe preparation time and removing the need for post-hybridisation signal amplification, detection of simple tri-

somy is possible within three to four hours of sample receipt using this approach.

Chiasma frequency, quadrivalent orientation, and first meiotic segregation in a human male reciprocal translocation heterozygote t(15q20)(q13;q13.1) analysed by dual colour fluorescence in situ hybridisation

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Chiasma frequency, quadrivalent orientation, and first meiotic segregation has been investigated in a human male with the karyo-
type 46,XY,t(15q20)(q13;q13.1). The rear-
range chromosomes were identified by dual colour fluorescence in situ hybridisation using the whole chromosome library pBS20 in combination with the paracentromeric probe D15Z1. At first metaphase (MI) the chiasma distribution in the quadrivalents was assessed directly. At second metaphase (MII) resolved interstitial chiasmata gave rise to chromosomes bearing one trans-
located and one normal sister chromatid. Interstitial chiasmata were found in 31% of 87 MI and 32.5% of 117 MII cells. Examination of the MII cells was used to deduce the segregation patterns. All types of 2 segregation, that is, alternate (14.5%), adjacent I (16.2%), and adjacent II (39.3%), as well as 3:1 (1.7%) and 4:0 (0.9%) were found. The remaining cells were indistinguishable from
used was that recommended by Huson et al (J Med Genet 1989;26:712–21) that children should be seen twice yearly and adults yearly for a general medical examination to monitor for complications but that no specific screening investigations should be done. One or more family members from 101 families have been seen. The major referral reasons have been confirmation of diagnosis (19%), for genetic counselling (17%), and for specialist advice regarding NF (64%). Eight percent of those with a referral diagnosis of NF1 had a different final diagnosis: these included segmental neurofibromatosis, multiple lipo- matosis, infantile myofibromatosis, and urticaria pigmentosa. The only major disease complications identified through the clinic were plexiform neurofibromas (7%) which were already present but their significance had not been appreciated by the referring clinician. However, major changes in management have been advised; 30% were not under regular follow up before attending the clinic, and in approximately 40% of the children seen, educational difficulties were being experienced and had not been specifically identified as being related to NF1. There have been no prenatal diagnoses for NF1 from the clinic population to date, although three couples have requested informed maternal testing. The clinic experience to date suggests: (1) there is still a need to improve awareness of NF by health professionals; (2) twice yearly routine appointments for children have proved too frequent; (3) even in a specialist clinic population, most complications are relatively infrequent and will require multicentre collaboration to improve knowledge of natural history and assessment of different treatment modalities.

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Gorlin syndrome and congenital anomalies in medulloblastoma

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Gorlin syndrome (GS) is an autosomal dominant disorder characterised by multiple basal cell carcinomas, jaw cysts, and skeletal anomalies. There have been nearly 40 reports of its association with medulloblastomas. We have undertaken the first population based study to assess the true level of its incidence in medulloblastoma; 173 consecutive cases of medulloblastoma registered with the Manchester Children’s Tumour Registry were studied. Case notes, X rays, and health surveys were reviewed. Two of 173 cases had a definite diagnosis of GS and a further case at 50% risk of GS was identified. GS accounts for 1 to 2% of medulloblastoma cases and 3 to 5% of cases of GS develop the brain tumour. Average age of onset of medulloblastoma in GS is two years (cf. 7-26 years). All early onset medulloblastoma cases should be assessed for GS in view of the risk of multiple basal cell carcinomas following radiotherapy and androgenisation. GS has a high incidence of congenital anomalies and genetic syndromes was found in this series (6-8%). One case of type 1 neurofibromatosis was found and also previously unreported association with Rubinstein syndrome.
Terminal deletions of 2q: is there a consistent phenotype?

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Only two cases of deletions of the long arm of chromosome 2 involving band q37 have been reported previously. We present four unrelated subjects with similar terminal deletions of 2q. Their ages ranged from 15 months to 17 years at the time of diagnosis. All four had varying degrees of mental retardation. Consistent dysmorphic features were large, prominent ears with large fleshy lobes and eczema in all four; three had upward slanting palpebral fissures, fine or sparse scalp hair, sparse eyebrows and eyelashes, with normal teeth and nails. Our findings of four terminal deletions of 2q37 among about 2500 cases referred over five years for developmental delay would suggest that this deletion may be underdiagnosed.

Absence of facial involvement may differentiate a scapular onset form of muscular dystrophy from FSH dystrophy

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We have examined 13 subjects in a family of whom seven are affected with a dominantly inherited muscular dystrophy with presentation and course similar to facioscapulohumeral muscular dystrophy (FSHD) except for consistent absence of facial weakness. Onset was in the shoulder girdle muscles in the second to fourth decades. In 2/7 weakness progressed to humeral muscles and to proximal and distal lower limb muscles, affecting ambulation. Contractures, pseudo-hypertrophy, and cardiac involvement were not present. CK was moderately raised in some subjects up to 1.5 × normal limit. Muscle biopsy from the proband showed myopathic changes consistent with muscular dystrophy and nerve conduction was normal. Precise diagnostic classification in this family is difficult. On clinical grounds the condition should be differentiated from FSHD and from known limb girdle, scapulohumeral, and scapuloperoneal syndromes. DNA typing performed at 4x35 loci D4S319 and D4S163, which are known to be closely linked to FSHD (θmax < 0.05), gave a lod score at θ = 0.05 of -1.44 or + 1.95 depending on grandparental haplotyping, and was therefore inconclusive. The identification of a closer marker or of a restriction fragment associated with FSHD would resolve this difficulty and help clarify the importance of facial weakness as a cardinal feature of FSHD.

Two cases of aneuploidy and the fragile X syndrome

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Case 1. A 9 month male infant was referred for chromosome analysis because of developmental delay and dysmorphic features. He had brachycephaly, hypertelorism, and epicanthic folds. Echocardiography confirmed patent ducus arteriosus and mitral regurgitation. His karyotype was 49,XXX,Y,frag(X)(q27.3) (30% expression). Case 2. A female neonate born at 37 weeks’ gestation was referred for assessment. She had dysmorphic features with simple, low set ears, a posterior cleft palate, bifid tongue, and polydactyly. A perimembranous ventricular septal defect was diagnosed on echocardiography. Her karyotype was 47,X,frag(X)(q27.3) +13/28% expression. The concurrence of fragile X syndrome with aneuploidy is well reported, particularly with additional X chromosomes. Both of these children were diagnosed as having fragile X after a few fragile sites were seen on initial unstressed cultures, later confirmed after culture in standard fragile X media. It is important to follow up such findings on unstressed cultures even in the presence of abnormalities sufficient to explain the clinical phenotype, to ensure appropriate genetic counselling for other family members.

X linked adrenomyeloneuropathy misdiagnosed as multiple sclerosis in a manifesting heterozygote

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A 26 year old female was referred for assessment of diplopia, progressive difficulty in walking, and urinary frequency. The second of three sibs, she had been diagnosed as having suffered an episode of transverse myelitis (? multiple sclerosis) with resultant weakness and spasticity in both legs at the age of 13. She was also assessed as having a familial mental retardation syndrome on the basis of mild intellectual impairment (IQ 68 to 78) in common with her older brother. Physical examination at the age of 26 showed bilateral abducting and rotary (on superior gaze) nystagmus, dysarthria, spastic limb weakness, upper and lower limb hyperreflexia (except for absent ankle jerks), extensor plantar responses, and absent vibration sense in the toes. Her gait was ataxic. Neurophysiological examination confirmed the neuropathy as axonal; CSF analysis was normal. Plasma very long chain fatty acid (VLCFA) analysis showed the level of C26:0 VLCFA to be 1.2 µg/ml, the C26:2 ratio to be 0.055, and the ‘discriminant Y’ value was calculated at 1.0205. These results are compatible with a diagnosis of X linked adrenoleucodystrophy/adrenomyeloneuropathy. Review of the family history suggested a further two first cousins may also have the disorder. The proband had recently given birth to a baby girl.

Trends in the number of births with spina bifida

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The incidence of neural tube defects (anencephaly, encephalocele, and spina bifida) in south Wales fell from 8 per 1000 births between 1956 and 1966, to less than 5 per 1000 in 1973. From then the affected 16 week pregnancies dropped to below 2 per 1000 in 1989. Prenatal diagnosis available since 1974, and serum AFP and ultrasound screening since 1976 followed by selective abortion, have all but eliminated anencephalic births and reduced spina bifida and encephalocele births to well below 2 per 10 000. Similar falls in incidence have been reported from elsewhere in the UK. Recurrences are now rare and with all pregnancies in the UK having ultrasound screening and over 80% AFP screening as well, fewer than 80 infants with spina bifida are liveborn per year, with fewer than 50 surviving beyond one year. As screening and surveillance is likely to extend, this number will reduce further unless the trend for termination of pregnancies with malformed fetuses is reversed. Reasons for the fall in the number of affected pregnancies with NTD (as opposed to unexplained congenital hydrocephalus) may include an as yet unexplained secular trend, or more likely, improvement in maternal nutrition and demographic changes. This has implications for the incidence of NTD elsewhere in the world.

POSTERS

Maternal serum screening for Down’s syndrome in East Anglia

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In 1991 maternal serum screening was made available to women of all ages attending five maternal hospitals in East Anglia. Amnio- centesis was offered when the risk of Down’s syndrome at term exceeded 1 in 200, calculated from maternal age and serum levels of AFP (Delfia), total hCG (Delfia), and unconjugated oestriol (Kodak). A total of 12 148 women were screened representing an uptake of 80%. The recall rate was 3.9% in hospitals which offered dating scans before sampling, significantly lower than the 10.1% recall rate in hospitals in which gestation was based on dates (p < 0.005). Amniocentesis was carried out in 599 pregnancies representing 3.3% of women screened; 14 Down’s syn- drome pregnancies were identified. Based
Abstracts

upon an incidence of Down's syndrome of 2 per 1000 in the second trimester, the expected number of affected pregnancies would be 24, giving an estimated detection rate of 57%. With outcomes known for 11 months, only six Down's pregnancies are known to have been missed so that the actual detection rate may be somewhat higher than estimated. If oestriol had not been included in the analyses only 10 Down's pregnancies would have been identified. The other abnormalities detected were trisomy 18 (1%), trisomy 13 (1%), 46,XY.del5p15.1 (1%), 46,XY,t(9;12)(q33;q24) (1), 45,X (1), 48,XXX (1), 69,XXX (3), aneucaryophilia (5), open spina bifida (7), hydrocephalus (5), exomphalos (1), gastrochisis (1), placental sulphatase deficiency (3), missed abortions (3), twin pregnancies (15), and triplets (1).

Biochemical screening for chromosome abnormalities: five years' experience of routine screening in the west of Scotland

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In the west of Scotland, before the introduction of biochemical screening, maternal age screening (>35 years) resulted in an average annual detection rate of 13% for Down's syndrome, less than half the possible detection rate of 30%, owing to a low uptake (<40%) of amniocentesis. Following the introduction of biochemical screening in 1987, an analysis of over 100,000 pregnancies screened routinely shows that 43% of Down's pregnancies were identified within a screen positive group of 6.2% of pregnancies. The overall aneuploidy rate was 42% and the actual detection rate for Down's 28%. In September 1991, hCG analysis was added to the AFP/age combination. A review of the first 11,900 pregnancies of known outcome has shown that 75% of Down's pregnancies were identified within a high risk group of 5.3%. An increased uptake of diagnostic testing (to 65%) has also led to improved screening efficiency with 50% of all Down's pregnancies being diagnosed prenatally.

Molecular and genetic mechanisms in recurrent severe osteogenesis imperfecta

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We have examined the type I collagen proteins and genes in seven families with recurrence of severe osteogenesis imperfecta (OI) and clinically normal parents. Five families were consanguineous and two non-consanguineous. An affected family had one affected child born to consanguineous parents. In the two non-consanguineous families we have identified heterozygous mutations in either COL1A1 or COL1A2 and mosaicism in one parent. Of the consanguineous families, two have shown excessively modified type I collagen but RFLP haplotype analysis indicates non-linkage for COL1A1 and COL1A2. A third was also unlinked by RFLP analysis and showed no overmodification of type I collagen. A fourth showed no protein overmodification but affected children were homozygous at beta COL1A1 and COL1A2. The family with the single affected child showed normal protein but homozygosity for RFLPs at the COL1A2 locus. The last consanguineous family showed overmodified type I collagen and homozygosity for RFLPs at the COL1A2 locus. Extensive DNA analysis showed heterozygosity for a sequence polymorphism in the COL1A2 gene in both affected subjects but failed to identify a causative mutation. Recurrent OI appears to be molecularly and genetically heterogeneous. Mosaicism for dominant mutations is most likely for unrelated parents. In consanguineous families the disease may or may not be linked to the type I collagen genes and may or may not be associated with excess post-translational modification of type I collagen.

Detection of mutations in part of the APC gene in FAP families and sporadic colorectal cancer

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Familial adenomatous polyposis (FAP) is an inherited autosomal dominant disorder characterised by the development of numerous adenomatous polyps in the colon and rectum with rapid progression to colon carcinoma at an early age. The gene responsible (APC) was localised to 5q21–22 and subsequently cloned. The message is divided into 15 exons, the last of which contains more than 3/4 of the coding sequence. In this study, 14 mutations in nine families and 13 sporadic cancers have been found in a small region of the gene between codons 1028 and 1499 using the technique of single strand conformation polymorphism analysis (SSCP). They all generate stop codons, either by point mutations or small deletions or insertions causing frameshifts. One mutation was found in a sporadic colon tumour, a colorectal cancer cell line and four unrelated FAP patients. This 5 bp deletion produces distinctive heteroduplex bands which can be detected easily using a non-radioactive assay.

p53 mutations in ovarian cancer


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Using the SSCP (single strand conformational polymorphism) technique and anti-p53 monoclonal antibodies pAb240 and pAb1801, 66 malignant ovarian tumours have been screened for p53 mutation in exons 5 to 8, and p53 expression, respectively. Thirty-four of the tumours (52%) showed an SSCP band shift in this region of the gene, including six in exon 5, seven in exon 6, 12 in exon 7, and 10 in exon 8 (one of the tumours showed a shift in exons 7 and 8). Monoclonal antibody staining was found to correlate with the presence of mutations detected by SSCP analysis, as was chromosomal allel loss using 17p probes pYNZ22 and pHP53, and 17q probe PTHH9. Presence of p53 mutation did not appear to be prevalent in any particular pathological subgroup of tumours and did not appear to be linked with FIGO staging. Seventeen of the SSCP shifts have been further characterised by DNA sequencing and have all been shown to represent genuine mutations. These include 15 point mutations (14 missense and one nonsense) and two deletions (2 bp deletion introducing, by frameshift, a stop codon further downstream, and an unusual 6 bp deletion made up of separate 2 bp and 4 bp deletions). The point mutations, when taken with other published point mutations in ovarian cancer, showed no prevalence of any particular transversion or transversion type.

Molecular aspects of P53 in lymphoma and leukaemogenesis

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Single strand conformation polymorphism (SSCP) analysis was used to detect P53 mutations (exons 5, 6, 7, and 8) in 30 Hodgkin's and non-Hodgkin's lymphomas. We found 13 mobility shifts, the majority in exons 5 and 7 and none in exon 8. The lymphomas were also analysed by Southern analysis using the probes pBHP53 and pMCT35.1, which map to the P53 locus, and PYNZ22 (telomeric to P53 on chromosome 17p). Allele loss was detected in 6/21 samples all of which had mutations in P53 as detected by SSCP analysis. The myelodysplastic syndrome (MDS) is a clonal disorder of the haemopoietic stem cells which provides an excellent model of leukaemogenesis. Samples representing all five subtypes of MDS were examined for P53 mutations by SSCP analysis. Shifts were found in 12/28 subjects.

Common mechanism of tumourigenesis in five types of von Hippel-Lindau disease neoplasms

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Von Hippel-Lindau (VHL) disease is an...
autosomal dominant familial cancer syndrome with variable expression. The most frequent complications are retinal and cerebellar haemangioblastomas, renal cell carcinoma, and phaeochromocytoma, but more than 20 different tumours have been suggested to be associated with VHL disease. The VHL disease gene has been mapped telomeric to RAF1 in chromosome 3p25-26, and there is statistical and molecular evidence to suggest that the VHL disease gene functions as a tumour suppressor gene. However, this concept has recently been questioned. We have analysed 21 paired tumour/blood DNA samples for evidence of loss of heterozygosity (LOH) for chromosome 3p markers. LOH was detected in five tumour types: renal cell carcinoma (2/11), cerebellar haemangioblastoma (1/8), phaeochromocytoma (1/1), choroid plexus papilloma (1/5), and pancreatic tumour (1/1). Chromosome 3p allelic loss has not previously been reported in VHL associated choroid plexus papilloma and pancreatic tumour. These results suggest that there is a common mechanism of tumourigenesis in VHL disease tumours. Our results are consistent with the VHL disease gene functioning as a tumour suppressor gene (LOH always included the region of the VHL disease locus), but chromosome 3p allelic loss was not restricted to 3p25-26 and we cannot exclude a possible role of other chromosome 3p tumour suppressor genes in the pathogenesis of VHL tumours.

Germline rearrangement at the APC locus detected by PFGE

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The autosomal dominant premalignant condition, adenomatous polyposis coli (APC), is caused by mutation or deletion of a gene which maps to chromosome 5q22. Although analysis of small, nested, molecular deletions in affected patients was instrumental in the isolation of the gene, almost all germline mutations detected so far are point mutations or 1 or 2 bp deletions. Previously the gene MCC (mutated in colon cancer), situated at 5q21 and 150 kb proximal to APC, had been considered a candidate familial polyposis gene by virtue of its high levels of somatically acquired mutations in sporadic colorectal carcinomas. We have investigated the nature of the germline mutation in one APC family with a severe phenotype: early onset of disease and the occurrence of multiple primary cancers. Pulsed field gel electrophoresis of an affected family member has been used to show gross rearrangement around the APC locus. The three rare cutting enzymes, Msi1, Not1, and Sal1, were used in single and double digests of normal and patient blood lymphocytes and probed with cDNAs encompassing the APC and MCC genes. Preliminary data show additional aberrant sized bands in the patient when compared with normal blood lymphocyte DNA. These appear to represent a rearrangement of the locus on one homologue. We propose that this rearrangement represents breakage and inversion of at least 150 kb of DNA close to the APC locus.

Early experience in preclinical diagnosis of familial adenomatous polyposis (FAP) in Wales

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Thirty-three clinically normal at risk members of families with FAP have completed the Cardiff DNA diagnostic programme. Pedigree risks were modified using age of onset data in 21 subjects who had already been screened by sibgnodoscopy and in 12 the prior risk was 50%. Flanking marker (Pz227, C1p1p1, EGB27, and YNS49) and intragenic polymorphism studies (FB54D and the exon 11 Rsal polymorphism) reduced the risk of gene carriage to <1% in 10 subjects. Nine of these wished to continue sibgnodoscopy screening (though on a less frequent basis) and one chose not to be screened again. Using extragenic markers we noted risk instability in one pedigree; a risk of 5-4% in one subject was revised to 38% when two cousins with unknown disease status were typed. Pedigree structure in FAP may predispose to risk instability, the poor prognosis in previous generations frequently leads to phase being unknown, and this problem is compounded by age dependent dependence. Caution should be exercised before modifying accepted bowel screening protocols on the basis of studies with linked markers.

Two cases of 5q deletions in subjects with familial adenomatous polyposis: possible link with Caroli's disease and Beale's syndrome

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Two cases of subjects with deletions of chromosome 5q are reported. Both are affectionate with familial adenomatous polyposis (FAP) and mild mental retardation. In both cases, the colon only had macroscopic polypsis in the proximal colon in adult life (in their thirties), although microscopic adenomatous change was shown on histological examination in the more distal colon, with occasional single polyps. Other features of FAP in both subjects included delayed onset of hypertension of the retinal pigment epithelium (CHREPL). One of the subjects (case 1) has haemorrhoidal polyps and desmoid tumours; the other (case 2) has a marfanoid habitus with an abnormal chest, wasted calf muscles, clawing of the toes, and Caroli's syndrome. His deletion is cytogenetically more extensive than that in case 1. The deletions were: proband 1 46,XX,del(5) (q21.3-q23.1) and proband 2 46,XY,del(5) (q15 q22.3 or 23.1). The paucity of adenomas in the left side of the colon suggests that FAP cannot always be confidently be excluded by sigmoidoscopy examination. The expression of the disease in the colon in cases where the whole gene is deleted could be milder than in the more usual autosomal dominant cases where nonsense mutations and deletions of only a few base pairs rather than deletions of the whole gene are the rule.

The dilemma of two cell populations in Fanconi anaemia

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Fanconi anaemia (FA) is an autosomal recessive disorder associated with diverse clinical manifestations. The heterogeneity in the phenotypic expression, clinical course, and age of onset makes diagnosis on the basis of clinical manifestations difficult and often unreliable. FA patients are currently diagnosed by cytogenetic assay, their cells showing high levels of mutagen induced chromosomal breakage. In lymphocyte cultures most or all cells usually exhibit typical chromosomal breaks and exchanges after exposure to DNA cross linking agents such as diepoxynbutane (DEB). In a series of 25 FA patients diagnosed in our laboratory, four cases were found to have two cell populations in their PHA stimulated lymphocyte cultures. After DEB treatments only a percent of the cells analysed (24, 36, 44, 50%) showed an increased breakage rate (mean breakages per cell in these four cases were 0, 0, 1, 2, 7, 9 mean breakages per aberrant cell were 4, 1, 1, 2, 7, 8 respectively). There were no consistent phenotypic manifestations common to these four patients that could suggest a correlation with the cytogenetic findings, and clinical manifestations were as diverse as in classical FA. In our experience the incidence of the type of FA having two cell populations seems to be higher than that previously reported by Auerbach: 16% compared to 10%. To explain the cytogenetic findings in these FA patients we suggest the following hypotheses: (1) slower cell cycle in cells exhibiting high aberrations in vitro; (2) transfection acquired chimerism; (3) constitutional chromosomal instability; and (4) mosaicism produced by back mutations.

Turner's syndrome detection by MSAFP/Free β (hCG) screening

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Abstracts

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We report findings using a protocol for Down's syndrome maternal serum screening which includes maternal age, AFP, and Free ß (hCG). This protocol is capable of detecting 80% of all Down's syndrome pregnancies when evaluating maternal serum before 17 weeks of gestation. Data presented here are from the respective maternal serum screening. In 11 cases presenting with increased Down's syndrome risk, amniocentesis showed nine cases of 45,X Turner's syndrome and two cases of 45,X/47,XX sex Turner's syndrome. A 12th case presented with both raised maternal serum AFP and Free ß (hCG). Analysis of amniotic fluid cells in this case showed 46,X(Xq) Turner's syndrome. These cases represent a subset of chromosomal abnormalities other than Down's syndrome that are being identified by this new screening protocol. These findings prompt further investigation into the strong association between Turner's syndrome and Down's syndrome and Free ß (hCG).

Analysis of the unstable CTG repeat in myotonic dystrophy

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We have investigated the length of the repeat in 29 DM families by PCR amplification and analysis of the product on polyacrylamide sequencing gels. In unaffected subjects (controls or spouses of DM patients) 26 of 31 (84%) were homozygous for the number of CTG repeats (range 5 to 30 repeats). In clinically affected subjects, 47 of 47 had only one discernible allele on the PCR assay, two had an allele in the normal range and a diffuse high molecular weight band, and four were heterozygous for alleles within the normal range. Repeat sampling and clinical reassessment of the four affected subjects with a 'normal' pattern is in progress. We tested 25 subjects at risk for DM, of whom 17 had a normal pattern, three had one normal allele and a high molecular weight diffuse band and had four with only one allele. One subject, the asymptomatic father of an affected female, had one normal allele and one allele with 43 repeats. A comparative analysis of these data with those obtained on the same samples by Southern blotting is in progress to assess whether PCR analysis can replace blotting as an initial and more sensitive screen for the DM mutation.

X linkage in boomerang dysplasia

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Boomerang dysplasia is a rare form of lethal short limb dwarfism. Four reports of male infants with the distinctive skeletal anomalies, including boomerang shaped long bones in the lower limbs, which was the suggestion of X linked recessive inheritance. We report two affected males in consecutive generations from the same family. A 25 year old woman presented after an unaffected pregnancy which had resulted in a neonatal death at 33 weeks' gestation of a male baby with multiple congenital anomalies consistent with boomerang dysplasia. The mother's younger brother was born at 30 weeks' gestation and died shortly after birth with almost identical abnormalities, also fulfilling the diagnostic criteria for boomerang dysplasia. This report supports an X linked recessive mode of inheritance.

The Martin Bell family revisited

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In 1943, Martin and Bell reported a family with seven mentally retarded male first cousins whose mothers were sisters. Members of this family have been residing in an institution since that time. In 1978 they were cytogenetically tested for the fragile X syndrome and found to be positive. Following publication of the cloning of the FMR1 gene, we returned to the institution and probing remaining members with DNA probes. Two out of the original seven members were still resident and one other had recently died. Blood was taken from one of these two men. Previous cytogenetic analysis had shown 20% fragile X positive cells. Repeat analysis showed only 1%. In our laboratory this is not considered a significant result. DNA analysis using probe OX1.9 showed a clear band of 7 kb and an absent 5 kb band. This confirms that the Martin Bell family does indeed have fragile X syndrome and supports previous reports of a decrease in cytogenetic expression with increasing age.

Fragile X syndrome families in the west of Scotland

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Families with fragile X syndrome have been extensively studied in our area, previously by linkage analysis and cytogenetics and now with one of the new gene probes (OX1.9). We have 49 west of Scotland families on our register from a population of approximately 3 million. There are at least 80 affected males known, giving a minimum prevalence of 1 in 18 750 males. There are 12 affected females, giving a prevalence of 1 in 125 000. However, since current estimates of the true frequency of affected males is 1 in 1250, and affected females is 1 in 2000 (with a gene frequency of 1 in 850), there are clearly many unreported cases of mentally handicapped persons with fragile X syndrome, in particular among affected females. Geographical distribution also indicates uneven ascertainment. Increased ascertainment can only come through educating other professionals of the need for testing all subjects with unexplained mental handicap. Unusual results so far among west of Scotland pedigrees include one family with both normal and handicapped males cytogenetically positive, but normal on probing with OX1.9, and one family with a cytogenetic and molecular fragile X positive male, but a mother apparently normal on both tests.

A clinical and genetic study of incontinentia pigmenti

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IP is a rare X linked dominant condition with male lethality. Present genetic counselling is based on the analysis by Carney (1976) of 464 references. He found significant complications in 80% of cases and notable CNS disorders in 30%. We subsequently found that represents biased ascertainment. Using strict diagnostic criteria 111 subjects (85 familial and 26 sporadic) were included in our study. Over 90% had a blistering rash within the first two months, over 90% had other ectodermal features, up to 30% had ocular involvement, but overall less than 10% had CNS disease. The incidence of severe mental
retardation in familial cases was 3% compared to 15% in the sporadic cases. Chromosomal rearrangements were found in two sporadic cases, with mosaicism in one of these, but neither involved the X chromosome. Linkage analysis using ST14 and F8C in six families supports linkage to Xq28. We suggest that our figures may be more accurate for genetic counselling.

Congenital skull defect and recurrent abdominal desmoids: a variant of familial adenomatous polyposis?

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The proband was born at term with a large skull defect which was repaired. Her parents noted that she had poor enamel deposition of her milk teeth but no other cutaneous problems. Growth and development were normal. At the age of 16 years she developed a desmoid tumour of the transverse mesentry which was surgically treated. Two years later another desmoid arising from the greater omentum was also surgically treated. Two colonoscopies performed (at the age of 16 and 18 years) were normal. She has no CHRPEs (congenital hypertrophy of the retinal pigment epithelium) or jaw osteomas. There is no family history of FAP but her grandmother was noted to have a few rectal polyps (tubulovillous adenoma) at the age of 78 years. The occurrence of desmoid tumours before the development of polyps has been reported in one pedigree where a 1 year old infant had this in association with a hepatoblastoma. Skull osteomas have also been reported frequently but few if any give rise to skull defects. The significance of her grandmother’s diagnosis is uncertain but it raises the possibility that a germline mutation of the APC gene is responsible for this association in our patient.

The scleratrophic syndrome of Huriez

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Huriez (1968) described 44 subjects in three families with a dominant condition characterised by palmoplantar scleratropy, keratoderma, and nail hypoplasia. Skin and bowel cancer was frequent. A further two families have been described in French publications. Linkage was suggested to MNSs (4q28-q31) lod score 4-2 at z = 0-05. In connection with a study of dysmorphic syndromes on 4q we have examined a family from Britain with this condition. Ten subjects were affected in three generations. Clinical features were dry, smooth, palmar and plantar skin, keratoderma predomi- nantly over pressure areas, nail dystrophy, small hands and feet, flexion contractures of the fifth finger, and a pinched nose with telangiectases. The hair and teeth were normal. Two further subjects, now dead, developed squamous cell carcinomas in areas of affected skin, at ages 22 and 45 respectively.

Cone-rod dystrophy associated with subretinal neovascularisation and gingival dystrophy

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We describe three generations of a five generation pedigree totalling 38 patients. A form of cone-rod dystrophy was diagnosed with loss of acuity followed by progressive electroretinography changes and field loss with night blindness in later life. The heritance pattern was autosomal dominant. In most, affected most subjects (11 out of 17) were noted to have frontal gingival atrophy with canines recessed by 30 years of age. All affected subjects seemed to develop subretinal neovascularisation at both maculae by 31 to 39 years of age causing aggra- vated acuity loss. The combination of these three pathologies together has not previously been described, although other retinal dys- trophies have been complicated by subretinal neovascularisation and cone-rod dystrophy with dental amelogenesis imperfect.

X inactivation studies in adrenoleucodystrophy

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Adrenoleucodystrophy (ALD) is an X linked progressive neurological disorder, character- ised by demyelination of the central nervous system and adrenal insufficiency. Manifesta- tion of the disease, in the form of spastic paraparesis, sometimes occurs in female car- riers. We decided to investigate whether skewed X inactivation might account for manifesta- tion in females. We used the probe M278 and the enzymes Psl + HpaI/HpaII on three manifesting carriers of ALD and on nine non-manifesting carriers. Of the mani- festing carriers, two showed random X inactiva- tion and one was uninformative. Of the non-manifesting carriers, four showed skewed X inactivation. There was no associ- ation of skewness with age. These results suggest that skewed X inactivation is not a major factor in the manifestation of ALD.

A search for uniparental disomy in Sotos syndrome

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Sotos syndrome (SS) is an overgrowth syn- drome associated with developmental delay. The aetiology remains uncertain. In our study of 40 patients with SS there are no pedigrees supporting either AD or AR in-

heritance, although paternal age is significa- ntly raised (observed 31.5, expected 28.6; 0.02 > p > 0.01). We report preliminary re- sults of a systematic search for uniparental disomy (UPD) in SS, initially focused on five candidate regions, 3p21, 6p21, 4p, 2p, and 11p15. The first two regions are the break- points in a patient with SS and a de novo reciprocal translocation. The third and fourth were considered following our finding of a mosaic translocation involving 2p and 4p. Finally 11p15 was investigated because of its association with the overgrowth dis- order Beckwith-Wiedemann syndrome. Twenty-four nuclear families have been studied by Southern blot hybridisation with a set of diallelic and variable number tandem (VNTR) microsatellite DNA probes. These included H3H2, pEFID347.4, and pEF641.1 on chromosome 3, pATA and pCZ30 on chromosome 6, pYNH24 on chromosome 2, mCO2C14 on chromosome 4, and CH-RAl and pHIN531 on chromo- some 11. To date we have not detected UPD using these markers.

Ear anomalies, clefting, and limb reduction defects: a new autosomal recessive condition?

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We present a pair of sibs of healthy unrelated parents with an interesting range of clinical manifestations. The male presented at birth, after a normal pregnancy, with extreme macro- rophyelia owing to multicocular porenchymal. His karyotype was normal. He was also noted to have a cleft palate and the family were counselled that this was likely to be the result of a deleterious vascular event. The second child, a female, was born after an unremarkable pregnancy and was noted to have absence of the right arm accompanied by rhizomelic shortening of the left upper limb and camptodactyly of the third finger at the PIP joint. X-CT was observed to be dys- morphic with upward slanting external pal- pebral fissures, a cleft palate, hypoplastic external auditory meati, and bilateral preaur-icular tags. Her CT scan of the brain was normal, the karyotype was 46,XX, and premature cen tromeric separation of the chromosomes was not observed. On re-exami- nation of the index case hypoplastic external auditory meati and bilateral preaur-icular tags were apparent but the facial struc- tures were by now very distorted. An excess of alcohol or vitamin A intake was not a feature during either pregnancy. The sibs most likely represent inheritance of an un- known autosomal recessive gene with an unusual spectrum of manifestation.

Male fertility in Noonan syndrome

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Antenatal carrier screening for cystic fibrosis: comparative evaluation of two approaches

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We are performing a randomised trial of stepwise versus couple screening, incorporating both psychological and economic evaluation. CF carrier tests are offered at the Aberdeen Maternity Hospital to all clinical cases, with those found to be carriers offered testing for both approaches. A comparative evaluation of these approaches is necessary because it has been proposed that with couple screening (1) there is less anxiety for the couple, (2) screening efficiency may be less as relatives of carriers cannot be made aware of their increased risk, (3) uptake rates may be lower, and (4) financial costs may differ. To date, 920 women have been offered stepwise screening. Of these 91% chose to take the test and 30 carriers were detected. Partners of 29 carriers accepted the offer of carrier testing after counselling. Of the two couples of carriers has been found who elected to have prenatal diagnosis. Eighty-five percent of 220 couples offered couple screening took the test. One pair of carriers has been identified; they elected to have prenatal diagnosis. Four carriers have been given a low risk result because of their partner being at low risk of being a carrier. Uptake rates of both approaches are high and not significantly different from each other. Psychological and economic factors may prove most important in determining the best method for antenatal carrier screening.

A pilot population carrier screening programme for cystic fibrosis

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A pilot population carrier screening programme for cystic fibrosis has been carried out in a primary health care centre to evaluate uptake rates for screening and to assess laboratory aspects. Thirty-four carriers were identified in 1040 subjects who were screened for the four mutations detected by the ARMS multiplex assay (Cellmark Diagnostics). Retesting of samples with conventional assays for these mutations showed no false negatives and one false positive result in the ARMS assay. This was a non-pathological sequence change (621 + 3A > G) which resulted in amplification from the mutant ARMS primer. The ARMS assay proved to be a rapid and reliable test for the presence of the four mutations. Screening uptake rates were very dependent on the method of approach used, with the active opportunistic approach being the most productive. Analysis of 172 CF patients in the South East Thames Region for 17 CF mutations accounted for 80% of all CF chromosomes in this region.

An unusual cystic fibrosis family

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We report clinical and molecular results on a family with CF in four members of two generations. They show a wide variation in phenotype and an uncommon mutation inherited from both sides of the family which nearly led to our failure to recognise the adults as being affected. The proband was an infant with congenital heart defect (Falkor's tetralogy) requiring shunting, who had recurrent severe respiratory infections with Pseudomonas and failure to thrive out of keeping with the severity of her heart lesion. Sweat tests gave equivocal results and CF gene mutation analysis showed her to carry both ΔF508 and R117H. Analysis of the immediate family showed that the healthy father carried R117H, the mother carried ΔF508, and the 7 year old brother, who had no bowel symptoms and a single attack of 'croup', carried both R117H and ΔF508. However, although the 30 year old mother was not related to her husband and appeared well, it emerged that she had a history of chest symptoms since her teens diagnosed as bronchopulmonary aspergillosis and further analysis of her DNA showed her to carry R117H in addition to ΔF508. With this result, further discussion indicated that her 24 year old brother had a lifelong history of mild 'asthma' and a two year history of loose bowel motions and abdominal pain diagnosed as Crohn's disease. He also carries ΔF508 and R117H. Thus the clinical spectrum of 'cystic fibrosis' continues to enlarge, needing a greater index of suspicion in both paediatricians and now in adult physicians for possible cases. As sweat tests may be equivocal in these patients and are more difficult to interpret in adults, greater reliance may be placed on the genotype for diagnosis with obvious implications for the DNA laboratories.

Cystic fibrosis in Grampian: a population study

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We have ascertained cases of CF in Grampian region, north-east Scotland, from 1960. The regional incidence was calculated to be 1/2260 and the carrier frequency 1/24. The estimated point prevalence on 1.1.89 was 1/7100 (correcting for cases not yet diagnosed); this is higher than that reported for Britain. One hundred and nineteen of the 120 CF chromosomes known within Grampian on the prevalence date were analysed. A mutation was detected in 94% (82.1% ΔF508, 6.8% G551D, 1.7% G542X, 3.4% 621+1,G-T, 1717-1,G-A, R553X, or Δ1507). No mutation was detected on seven chromosomes despite additional testing for R560T and R117H. The prevalence of ΔF508 (81%) and other identifiable mutations (94%) is significantly higher than that previously published (71% ΔF508, 84% all mutations) for a mainly southern Scottish population. These findings have local implications for the efficacy of carrier screening and the usefulness of DNA testing in cases where the diagnosis of CF is in doubt.

Relative risk and risky relatives
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Cystic fibrosis (CF) is a recessive disorder in which some, but not all, mutations can be identified. Thus, consultants who test negative are at risk of carrying an undetectable mutation. This risk can be calculated by using Bayes’ theorem in conjunction with the results of mutation analyses from the consultant and their relatives. The tabulated results of these calculations provide a simple method for obtaining accurate risk values for a consultant who tests negative in CF gene mutation analyses. A computer program to calculate the probabilities for diseases with any given incidence and proportion of detectable mutations is available from the first author.

Reproductive behaviour after a second trimester termination of pregnancy
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Eighty-four couples were interviewed two years after a second trimester termination of pregnancy for fetal abnormality (TOP for FA). The aims were to assess their reproductive behaviour and to identify those whose desire for children conflicted with their fear of further fetal abnormalities and the personal decisions that would again con-

Abstracts

Ocular motor abnormalities in early Huntington’s disease
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Saccadic abnormalities described in Huntington’s disease (HD) include fixation instability, slow saccades, and impaired frontal lobe guided saccadic tasks. We systematically examined a range of five saccadic paradigms in patients with early HD. A total of 85 families was available, following virtually complete ascertainment in the Northern Ireland population. There were 95 living affected patients and 25 (26%) had early signs of HD. Eighteen (72%) of the subjects with early HD were tested with saccadic paradigms: the age range was 28 to 68 (mean 47). Two failed owing to excessive blinking artefact. Of the 16 successful studies, 15 had quantitatively abnormal results: all showed abnormal antiascades and abnormal remem-

Heterozygote frequency, mutation rate, fertility, and genetic fitness in the N Ireland Huntington’s disease population
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The epidemiology of Huntington’s disease (HD) in the Northern Ireland (NI) population has been extensively studied. Following virtually complete ascertainment, estimates of the heterozygote frequency, direct and indirect mutation rates, fertility, and genetic fitness (W) of the population were made for 1 April 1991, the date of the decennial census in NI. The estimates were found to be signifi-

Prevalence and incidence of Duchenne (DMD) and Becker (BMD) muscular dystrophies in Slovenia
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The prevalence and incidence of DMD and BMD were measured in the state of Slovenia (population 1,999,477 in 1990). Patients were identified from the national register of neuromuscular disorders and from the records of University Paediatric Clinics and Division of Medical Genetics, Ljubljana. Be-

The Emilia Romagna birth defects register: goals and accomplishments
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The Emilia Romagna Register for congenital malformations was established in 1978. During the study period, among 242,696 births with a regional coverage of 90%, 4,610 cases (1.9%) were detected. No cluster in time or space could be demonstrated. In the years
1981 to 1990, 870 babies with multiple anomalies were detected. Among them, there were 390 cases with chromosomal anomalies (19 × 1000), 90 with monogenic conditions, and 26 with an environmentally caused condition (0.1 × 1000). In 105 cases (0.5 × 1000) a known association or sequence was identified. Particular attention was given to the identification of specific syndromes with high recurrence risk (144 cases, 4.7%) and genetic counselling was offered.

**Homozygosity mapping of autosomal recessive disorders and complex consanguinity**

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Mathematical analyses of the power of nuclear families for autosomal recessive disorders have shown that in order to have a high likelihood of showing linkage in gene mapping studies inordinate numbers of families, preferably with multiple affected sibs, are required. Use of homozygosity mapping with affected offspring of first cousins has been advanced as far fewer families are needed to prove the same likelihood of showing linkage. Collection of affected offspring for linkage studies of ostensibly first cousin matings from certain ethnic groups in which consanguineous marriage is common often shows the consanguinity to be much more complex than at first enquiry. Modelling the more common complex consanguineous pedigree structures encountered shows that previous inbreeding patterns, polymorphic marker loci, and disease allele frequencies and possible locus heterogeneity affect the potential power of these families in linkage studies.

**Flanking markers for the gene for the Gorlin syndrome**

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The Gorlin (naevoid basal cell carcinoma) syndrome is an autosomal disorder characterised by multiple naevoid basal cell carcinomas, recurrent odontogenic keratocysts, skeletal anomalies, intracranial calcifications, and developmental malformations. As part of a continuing clinical study of over 70 affected patients, we presented linkage data localising the gene to 9q22.3–q31 in nine families. A further four families have been studied and there continues to be no evidence of locus heterogeneity in our families although there is variability of clinical signs and symptoms within and between families. Zmax is 7.70 with D9S127 at δ = 0.05. Recombinants place the gene between D9S125 and D9S127.

**A linkage study of X linked Charcot-Marie Tooth disease (CMTX) using highly informative loci**

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Previous studies of CMTX have shown linkage of the disease to loci between the centro-mere and Xq13. We are studying a number of X linked families, including a large Scottish family and three smaller British families. These four families were studied using four highly polymorphic probes: M27β (EcoRI digests) Xq1.11.22; HAR (PCR primers and DXS453 (PCR primers) both within interval 7, Xq1.12–q13.1; PKG1 (PstI/XbaI digests) Xq1.3.3. Zmax for locus M27β is 7.42 at δ = 0.10; HAR 5.66 at δ = 0.05, DXS453 7.43 at δ = 0.00; PKG1 5.05 at δ = 0.00. As can be seen these markers are tightly linked to the disease locus. From these results the most likely position of the disease is distal to HAR and proximal to DXS453. However, there is limited information on this area owing to lack of crossovers within these families.

**Linkage studies in X linked Emery-Dreifuss muscular dystrophy: a new microsatellite marker localises a key recombinant**


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X linked Emery-Dreifuss muscular dystrophy (EMD) is a rare disorder characterised by early contractures, slowly progressive muscle wasting and weakness (initially hypertonic), and cardiomyopathy with conduction defects and a risk of sudden death, mapped to Xq28. We have studied eight families (including three not previously published) with DNA markers from distal Xq. Our data confirm the EMD is very close to the factor VIII coagulant gene locus (FBC) with a maximum lod score of 7.86 at 0.03 recombination. Three recombinants support DXS52/DXS15 as proximal flanking markers and one recombinant supports FBC as a distal flanking marker. Multipoint analysis incorporating published data confirms placement of EMD distal to DXS52/DXS15 with the most likely location between the red/green cone pigment genes (RCGP) and FBC. The physical distance from RGP to FBC is about 800 kb and we have excluded YACs from this region into cosmids and screened for microsatellite markers to characterise the flanking recombinants. This has identified a polymorphic CA repeat which is informative in the key recombinant between EMD and FBC providing localisation of this crossover to within 100 kb of the 3' end of the factor VIII gene.

**Genetic localisation of RP2 type of X linked retinitis pigmentosa**

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Heterogeneity analysis has been carried out on 37 X linked retinitis pigmentosa families. Seven families were identified with >70% probability of being RP2 type. Six point linkage analysis was carried out on this subset of families in order to localise RP2. The results show that the most likely order of loci is: †††OTC-0.09–DXS228/DXS7/MAOA-0.03–RP2-0.12–TIMP-0.03–DXS225-0.06–DXS14—cen with a maximum multipoint lod score of 13-46. Locus heterogeneity causes problems in the presymptomatic diagnosis of carriers and in prenatal diagnosis. In small families, the disease locus often cannot be determined. However, provided that no recombinations in the RP2–RP2 interval (about 73% of meioses), it is possible to provide accurate risks by examining markers flanking both disease loci. In such cases, the most accurate calculation is: 0.008 assuming no interference, or 0.008 allowing for interference. Finally, three families with putative X linked RP have been identified that do not show linkage to the RP3 or RP2 regions. Further studies are required to investigate the possibilities of another X linked locus or autosomal dominant RP.

**Identification of a nonsense mutation in the amelogenin gene (AMELX) in a family with X linked amelogenesis imperfecta (AIHI)**

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A previously undocumented family with X
linked amelogenesis imperfecta has been investigated. In some subjects the most noticeable defect was of enamel hypoplasia whereas in others the hypoplastic changes were subtle (and might have been overlooked on cursory examination) and the most noticeable change was of enamel colour, indicating a degree of hypomineralisation. PCR amplification and direct DNA sequencing of exons of the amelogenin (AMELX) gene has been carried out. A nonsense mutation was found in exon 5 involving a single base deletion (CCCC→CCC) in an affected male. Inserting the reading frame and introducing an inappropriate TGA stop codon (an opal mutation) into the exonic sequence of the gene immediately 3' of the mutation. We propose that AMELX is implicated in both the formation of enamel of normal thickness and in the normal mineralisation process.

Screening for occult Y mosaicism in girls with Turner's syndrome

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The percentage of mosaics in fetuses is appreciably higher than in liveborns with Turner's syndrome. This has led to the theory that most 45,X girls are actually mosaics owing to a mitotic error in an early zygote. If the mosaicism is in a small percentage of cells, it may be missed with conventional cytogenetic analysis where only a small number of cells are analysed. Occult Y mosaicism has important clinical implications because of the increased risk of gonadoblastoma. The aim of this study was to look for occult Y mosaicism in girls with Turner's syndrome. Fifty-two girls with Turner's syndrome with a variety of karyotypes were investigated by PCR, using a series of primers from all areas of the Y chromosome. A total of five patients was found to be positive for Y sequences. Two of these had 45,X/46,XY karyotypes and one was 45,X/46,X + t. These three were positive for all sequences analysed. The last two girls had 45,X karyotypes. One girl was positive for Y centromeric sequences and is presumed to have a small fragment and the other positive for long arm material and is presumed to have a translocation. PCR is a rapid method for detecting Y sequences in Turner girls and in view of the relatively high percentage found in this study we would recommend screening of all girls with a panel of Y specific primers.

Screening for the G985 medium chain acyl-CoA dehydrogenase deficiency mutation in Scottish women

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Medium chain acyl-CoA dehydrogenase deficiency (MCAD) is a common inborn error of metabolism which results in defective mitochondrial beta-oxidation of long chain fatty acids. It has been associated with sudden unexplained death in children. A point mutation changing adenine to guanine at position 985 in the MCAD cDNA sequence has been found to be a common mutation accounting for more than 90% of all disease alleles in the MCAD gene. The carrier frequency of G985 by DNA analysis has been reported in two studies. Blakemore et al. found six heterozygotes among 410 neonates in Trent, UK (carrier frequency 1 in 68), and Matsubaru et al. found 12 among 479 neonates in Birmingham (carrier frequency 1 in 40). We have used DNA extracted from clotted blood obtained from our alphafetoprotein screening laboratory and have used a combination of PCR with NcoI restriction enzyme digests to look for the G985 mutation. We screened a total of 542 women and found that two of them were carriers of the mutation, giving a heterozygote frequency of 1 in 271. We conclude that the level is considerably lower than that found in other UK studies, suggesting either that MCAD is not a significant contributing factor in sudden unexplained death syndrome in Scotland, or that another MCAD mutation is more common in this population.

Manifesting heterozygosity in Norrie's disease

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A female child with a characteristic family history of Norrie's disease presented at the age of 2 with leucocoria and a retinal fold. DNA studies of her affected brother detected no deletion with L1-28 (DXS7 locus). Studies of family members with this and other markers known to be linked to the Norrie's disease locus showed that the female child, her affected brother, and an affected uncle had inherited the same maternal X chromosome from the father and the unaffected uncle. Methylation sensitive X chromosome probes showed that in the female child there was a skewed X inactivation pattern favouring expression of the maternal X. These observations are consistent with the hypothesis that the ocular findings in this girl are the consequence of manifesting heterozygosity for Norrie's disease.

Mutation analysis of fragile X families

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We report on our analysis of 23 families with a clinical and cytogenetic diagnosis of fragile X. This used the probes DXS1.9 and DXS0.55. To assess the accuracy of the test, 33 likely female carriers were analysed. Full mutations or premutations were detected in all but one of these. The exception was from a family where no affected male DNA was available to confirm presence of the mutation.

Early experience of fragile X mutation detection in the north-east of Scotland

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The use of DNA analysis and cytogenetics has been examined in patients between the ages of 2 and 5 years referred by the local preschool development assessment centre for developmental delay. Forty-three patients have been analysed cytogenetically and using DNA probes and PCR to detect the unstable microsatellite repeat p(CCG)n at Xq27.3. Mutations were found in three patients (7%) who were cytogenetically negative.

DNA markers for the analysis of the Criiger Najjar syndrome, type I

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Criiger Najjar syndrome type I (CNI) is a lethal, recessively inherited metabolic disorder characterised by birth un conjugated hyperbilirubinemia. Glucuroridation, essential for detoxification and excretion of bilirubin, is catalysed by two members of a family of hepatic isoenzymes, the UDP-glucuronosyl transferases (UGT-1 gene, EC 2.4.1.17). Several isomorphs of the enzyme are encoded by a single gene with different functional specificity being achieved by the joining of unique exons which encode the amino-terminal portion of each isoform and exons which encode the common c-terminal portion. Normal levels of liver and bile bilirubin UGT activity is absent in patients with CNI and previously no prenatal diagnosis has been possible. Informative DNA markers for the UGT-1 gene have been developed and are shown to co-segregate with CNI in sibship analysis of affected families. These data confirm the autosomal recessive nature of CNI and identify markers for both carrier detection and prenatal diagnosis.

Visualisation of microsatellite repeats by silver staining

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We have developed a protocol to allow the detection of microsatellite repeats non-isotopically, using silver staining. Amplified PCR products are run on 1 mm thick, 8 to 10% (9/1) polyacrylamide gels. Microsatellites up to 140 bp in size are run during the day on 12 cm minigels, larger products either cut down using an appropriate restric-
A strategy for the investigation of Duchenne and Becker muscular dystrophies in the diagnostic genetics laboratory based on accurate carrier detection

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We describe a strategy for the investigation of DMD which incorporates two techniques that provide accurate carrier diagnosis. The techniques are analysis using dinucleotide repeat markers and pulsed field gel electrophoresis (PFGE). The location of highly polymorphic CA repeat markers from a region of the DMD gene which is prone to deletion means that analysis using these markers yields information on the carrier status of women from approximately 30% of families. Where the above analysis is uninformative or not appropriate, PFGE analysis is performed. Altered sized restriction fragments which result from deletions/duplications can be detected and used as markers of the disease mutation in approximately a further 30% of families. By adopting the above strategy, we are able to provide accurate carrier diagnosis in approximately 60% of DMD families where an affected male is available for analysis. Many women are given negligible carrier risks and require no further laboratory investigations. Even in families where no affected male is available for analysis it is often possible to characterise mutation and identify a marker which will enable accurate prenatal diagnosis.

The experience of DMD/BMD carrier detection by in situ hybridisation

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Our experience of carrier detection by in situ hybridisation initially involved the cosmid CPT1 which contains exon 45 of the dystrophin gene. Twenty-eight percent of our families had a suitable deletion involving all of this cosmid, and we were able to give definitive carrier risks (bearing genotyping mosaicism) to all these women in families. This included several women who had intermediate risks after DNA analysis. Where the deletion did not include all the cosmid, the interpretation of the results became subjectively different. This has implications for the use of FISH as a general method for carrier detection in families with a deletion. This method of carrier detection is very useful in conjunction with DNA analysis and we are actively working upon other cosmids to make it applicable to more families.

Different molecular mutations in male sibs with Duchenne muscular dystrophy

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We describe two brothers from an Afro-Caribbean family affected by DMD. DNA analysis with cDNA probes and SSCP and showed that two boys had inherited different maternal X chromosomes. Analysis with probe pERTW-8 showed a junctional fragment in one of the affected boys which was also present in the mother and grandmother. The other affected boy did not show the junctional fragment and had the same haplotype as his unaffected brother. This shows that the mutations causing the disease in the affected sibs are different, which explains their differing clinical symptoms. Our findings are similar to those reported recently by Miciak et al who described three related DMD males each carrying a different mutation on a different X chromosome. We also speculate that a transposon may be involved in disrupting the dystrophin gene in the family described here.

Mutation detection in rhodopsin and peripherin genes in the Scottish retinitis pigmentosa population

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Using single stranded conformation polymorphism (SSCP) analysis, we have analysed the candidate genes rhodopsin and peripherin in affected subjects. 17 autosomal dominant retinitis pigmentosa families and 17 isolated cases from the Scottish population. Mobility shifts in rhodopsin were detected in exons 13, 15, 17 and 19 and seven subjects respectively. Shifts were detected in five subjects in exon 1 of peripherin. The nature of the shifts is at present being determined by direct sequencing, where initial results have shown that a mutation in exon 3 of rhodopsin segregates with the disease phenotype in an early onset dominant family. In seven simplex cases, the shifts are due to a polymorphism outside the coding region. Further sequencing studies will show the nature behind the remaining shifts. It is assumed, in line with current evidence, that additional rhodopsin and peripherin mutations will be detected in dominant families, and perhaps in isolated cases where they may represent new, dominantly inherited mutations.

Predictive testing for Huntington's disease: experience with the multiallelic probe pPK1.65

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Analysis of families with a history of HD currently involves the use of closely linked DNA markers of which the VNTR probes such as pKP1.65 (Langford et al 1988) has proven to be the most informative. We have analysed 29 families and of the 20 families for which informative data have been obtained to date, 13 have been with pPK1.65. This high level of informativity has been achieved by extended electrophoresis on 2% agarose. Optimisation of band separation has also shown the presence of further alleles in addition to the four commonly reported for this probe. We have currently detected at least eight alleles with this approach. We are confident that improved band resolution as achieved here could also be applicable to other VNTR probes and would greatly improve analysis of HD families.

Predictive testing in a one generation Huntington's disease family

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We were presented with a predictive test request for a member of a one generation HD family. Analysis of the affected sibs combined with anonymous testing of a number of further sibs with the VNTR probe pKP1.65 identified all four parental markers including the high risk allele. This provided the basis of a predictive test as requested while maintaining confidentiality of other family members' results. Resolution of the high risk allele was achieved by extended electrophoresis on 2% gel. This was confirmed by combining DNA from both affected subjects resulting in three bands of which only one was common to both. We have successfully analysed a number of families that do not exhibit the ideal three generation pedigree structure, including several two generation families and one family in which DNA was not available from any affected subject.
Wolf-Hirschhorn syndrome: paternal origin of a submicroscopic deletion of chromosome 4

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Wolf-Hirschhorn syndrome (WHS) is a multiple congenital anomaly syndrome associated with the deletion of chromosome 4, band p16. An 11 year old female is described with severe pre- and postnatal growth deficiency, microcephaly, seizures, severe mental retardation, and prominent kyphosis. The characteristic facies was evident. High resolution karyotype showed no visible abnormality of chromosome 4. Southern DNA analysis using probe p157 showed that she had not inherited a paternal allele at locus D4S111. Probing with p252 (D4S115) and pBS731 (D4S98) was uninformative, but probes YNZ.32 (D4S125), pBS674ED (D4S95), and KPI.65 (D4S43) were characteristic. The proximal end of the deletion therefore lies between D4S111 and D4S43, about 2 Mb from the telomere. This patient further exemplifies that in WHS, de novo deletions predominantly affect the chromosome 4 of paternal origin. In cases with familial translocations, published reports suggest that either parent may transmit the deleted chromosome. Investigation of a patient with suspected WHS should include DNA based analysis, as submicroscopic deletions are not infrequent.

Molecular genetic studies of Angelman syndrome and Prader-Willi syndrome patients

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DNA samples from AS or PWS patients and their parents were tested with the probes D15S9, D15S10, D15S11, and D15S12 by Southern analysis, and GABRB3 using PCR. Six out of nine AS patients were found to have one chromosome 15 homologue deleted for the probes tested, with the origin of deletion being shown to be maternal in all cases. One case was shown to result from paternal isodisomy, and no abnormality could be detected in the remaining two patients. Eleven out of 17 PWS patients were hemizygous for all probes tested, with the paternal origin of the deleted 15 being confirmed in nine cases. One patient was found to have one 15 deleted for GABRB3, D15S9, and D15S10, but to be heterozygous for D15S11 and D15S12. Saioto et al recently reported a three generation family deleted for D15S10 and GABRB3, affected by AS but not PWS. No abnormality was detected in four of the remaining PWS patients, and the results from the final patient indicate maternal heterodisomy, though this is yet to be confirmed.

Mutation analysis in families with hyperkalaemic periodic paralysis

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Members of a large family and a sporadic case of hyperkalaemic periodic paralysis (HYPP) have been examined for the presence of either of two described mutations in the gene coding for the α subunit of the adult skeletal muscle channel (SCN4A). Assays involving PCR amplification and restriction digestion of products were used, and in the absence of samples known to carry the mutations, internal controls were incorporated into the assays to ensure the reliability of the results. In one case the presence of the mutation creates a restriction site, while in the other a modified primer was used which, in the presence of the mutation, introduces a restriction site into the product of PCR. The results obtained by PCR assay were confirmed by sequencing. The affected members in both families were each found to display one of the two mutations, suggesting that these are relatively common mutations in this disorder. Additionally, the mutation present in the large family was found to be associated with a phenotype of interarterial muscle weakness, as described in all the previously reported cases with this mutation.

Congenital myopathy with fibre type disproportion: a family with a chromosomal translocation 10(17) may indicate candidate gene regions

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A child with severe congenital myopathy with fibre type disproportion was found to have an apparently balanced translocation t(10;17)(p11;q25). The same translocation was found in the clinically healthy mother, who nevertheless on EMG showed discrete signs of myopathy. Maternal uniparental heterodisomy was excluded by RFLP analysis of DNA markers on chromosomes 10 and 17. The regions are investigated further as candidate regions for a myopathy gene.

The screening of the fibrillrin gene FBN1 for causal mutations in Marfan syndrome

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Marfan syndrome is a connective tissue disorder with an autosomal dominant pattern of inheritance manifesting as cardiovascular, skeletal, and ocular anomalies. It has been linked to the gene for the microfibrillar protein fibrillrin and several missense mutations have previously been identified within this gene. Screening of the available gene sequence for further causal mutations has been carried out on a total of 16 patients by searching for single stranded conformational polymorphisms (SSCP) and PCR amplification on PCR amplified fibrillrin mRNA isolated from either fresh blood lymphocytes or cultured skin fibroblasts. Two patients were found to be heterogeneous for point mutations that would result in missense mutations within two separate EGF-like repeats of the fibrillrin protein: a cysteine to tyrosine substitution at codon 325 and an asparagine to serine change at codon 1246.

Molecular analysis of the allelic expression of the cartilage specific type II collagen gene COL2A1 in osteoarthritis

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Osteoarthritis (OA) is the most commonly diagnosed musculoskeletal disorder and is characterised by a progressive degeneration of articular cartilage. The major protein of cartilage is type II collagen which is encoded by the COL2A1 gene. Recently, COL2A1 has been linked to OA in two pedigrees. We have set out to determine whether a loss or reduction in the expression of COL2A1 in cartilage, with a subsequent reduction in the levels of type II collagen, could be a contributing factor in the aetiology of OA. Genomic DNA and RNA were extracted from the knee cartilage of OA patients undergoing knee replacement. By direct sequence analysis of reverse transcription-PCR (RT-PCR) amplification genomic DNA, we assessed which patients were heterozygous and homozygous for two sequence dimorphisms, one located in exon 50 and the other in the 3' UTR of COL2A1. The COL2A1 cDNA regions encompassing the sequence dimorphisms were then amplified by reverse transcription-PCR (RT-PCR) of cartilage RNA. The expression of each dimorphic allele in heterozygotes was then determined by restriction digestion and primer extension analysis. Using this technique, we have observed differential expression of COL2A1 alleles in the cartilage of some OA patients.