Abstracts of the Joint British Medical Genetics meeting organised by the Clinical Genetics Society and the Clinical Molecular Genetics Society held on 19 and 20 September 1991 at the University of Bristol

Recurrence of Wolf-Hirschhorn syndrome owing to a submicroscopic 4p deletion detected prenatally by molecular analysis
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Wolf-Hirschhorn syndrome (WHS) is a clinical pattern associated with deletions of the tip of the short arm of chromosome 4. Affected children are mentally retarded, growth retarded, and dysmorphic. The diagnosis was suspected in a 2 year old girl with developmental delay, height and weight on the third centile, and subtle dysmorphic features. However, cytogenetic analysis of the child and her parents was normal. Analysis using DNA probes from 4pter showed apparent non-maternity indicating a submicroscopic deletion. A maternal copy of F5.53 (E Bakker) was present localising the phenotype to monosomy for genes distal to D4S10. The couple started a second pregnancy and were offered chorion villus biopsy. The fetus had no maternal copy of probes distal to D4S10 and the pregnancy was terminated. Fluorescent in situ hybridisation using probes from the D4S10 locus has shown that the mother carries a 4;10 translocation. Five other cases of WHS have been referred to us where the chromosome 4 deletion was not identified on the first karyotype. In four of these the deletion is now known to be the unbalanced form of a familial translocation and hence had a high recurrence risk. An apparently normal karyotype in a child with features of Wolf-Hirschhorn syndrome is an indication for further molecular genetic analysis.

Imprinting: a mechanism in tuberous sclerosis?
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Tuberous sclerosis (TS) is an autosomal dominant disorder known to show variable expressivity and reduced penetrance. It has recently been suggested that evidence of genomic imprinting might be expected in TS on the basis of maternal imprinting occurring in the region of the mouse chromosome homologous to the region of the human chromosome 9cen–q34, the probable location of one of the gene(s) responsible for TS. In addition, both maternal and paternal imprinting is seen in the region of the mouse chromosome homologous with the other region of the human chromosome, 11q13–q21, which is the likely location of another gene responsible for TS in some families. We have reviewed the records of all the families seen in the clinics of the Yorkshire Regional Genetics Service with a diagnosis of tuberous sclerosis over the last six years. A complete screening protocol carried out on both parents of 27 apparently sporadically affected families identified one of the parents to have features of TS in nine in which the proband could be described as being severely affected by virtue of both mental retardation and dysmorphic features. The former often being intractable and the latter usually being moderate to severe. In six out of these nine, the mother had findings consistent with having the gene for TS. In addition, we have identified in five families in which the family history at the time of presentation showed the disorder to be obviously familial, nine out of the 10 persons with TS were severely affected and all of them inherited the TS gene from their mother. In a sixth family in which the disorder affected 17 subjects in four generations, all but one person were mildly affected (that is, absence of fits or mental retardation), the gene having been passed on by the mother in 12 instances and by the father in four. We suggest that genomic imprinting may be an important mechanism determining the expression and penetrance of the TS gene(s) and may be an important factor to consider when counselling couples about the recurrence risk in instances of TS. It should be considered as further evidence of genetic heterogeneity in linkage studies.

Linkage studies in spinal muscular atrophy
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Childhood onset proximal spinal muscular atrophy is a disorder characterised by selective loss of the cell bodies of the alpha motoneurons of the spinal cord. Three forms can be distinguished on the basis of severity of disability and age at which such disability manifests. All three types show autosomal recessive inheritance and have been mapped by linkage analysis to probes at 5q11.2–q13.3, between D5S66 and D5S39 (Brustowicz LM, et al. Nature 1990;344:540–1; Melki J, et al. Nature 1990;344:767; Gilliam TC, et al. Nature 1990;345:823; Melki J, et al. Lancet 1990;336:271). We have expanded the original linkage analysis by the use of a highly polymorphic (dC–dA)/(dG–dT), repeated associated with D5S112, a locus which lies between D5S66 and D5S39. This repeat, defined by primers JK53CA1 and JK53CA2, was isolated from a plasmid library constructed from an 850 kb YAC containing D5S112. PCR screening of 132 unrelated CEPh subjects yields 12 alleles, with a heterozygosity of 77%. Typing in 31 SMA pedigrees shows no evidence of linkage disequilibrium of any allele with the disease. We have also screened CEPh and SMA pedigrees with microsatellites from the D5S39 locus (DSS204) and DSS125, which maps telomeric to D5S66. Multipoint analysis gives the most likely order as: cen-D5S66-D5S125-JK53CA1-D5S112 (-D5S39-DSS204)-tel. The SMA locus lies in between the D5S39 and JK53CA1/D5S112. In situ analysis of cosmids from the flanking markers confirms the order from genetic studies.

Emery–Dreifuss muscular dystrophy: progress in gene mapping
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Emery–Dreifuss muscular dystrophy (EMD) is characterised by (1) early contractures of the Achilles tendons, elbows, and spine; (2) slowly progressive muscle wasting and weakness with a predominantly humeral and peroneal distribution; and (3) cardio-myopathy with cardiac conduction defects and risk of sudden death. Inheritance is usually X linked recessive but can be autosomal dominant. Studies in five X linked pedigrees confirm our earlier report of linkage to Xq28 markers (JK53CA1/D5S112–JK53CA1) and put EMD distal to STd4 (DXS52) and very close to the factor VIII coagulant gene locus, F8C (lod score 5.02 at zero recombination). Additional families are being sought to provide the linkage data needed to refine the
Non-syndromic cleft lip and palate and transforming growth factor alpha: association and linkage studies

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Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder that has an estimated prevalence of 4 per 6000 people, with 25% of cases resulting from new mutations. The condition is characterised by abnormalities of the eye, aorta, and skeleton, is clinically heterogeneous, and shows great intramafial variability of expression. Recently, linkage analysis has shown a locus for MFS on chromosome 15. Fibrillin, a glycoprotein of the microfibrillar fibre system, is found in all tissues involved in MFS and immunostaining studies of cultured fibroblasts from MFS patients have shown defects in the synthesis, secretion, or extracellular assembly of fibrillin in 70% of cases. At least two fibrillin genes have been identified, one of which has been localized to 15q21.3 (Fib5) and the other to 5q23-3q1 (Fib5). To test Fib5 as a candidate gene for MFS, linkage analysis of 27 classically affected Marfan families was performed, using VNTR markers within the Fib5 and Fib5 genes. No recombinants were observed between Fib5 and the MFS phenotype, and a maximum lod score of 24.85 at 0=0 was obtained. In contrast, discordant segregation was observed with Fib5 excluding Fib5 as a candidate gene. This strongly suggests that Fib5 is the MFS gene and that there is no gene linkage between Fib5 and Fib5 markers.

Genotype prediction in the fragile X syndrome using the new DNA marker Ox1.9

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The fragile X syndrome is the most common inherited cause of mental retardation and is associated with the expression of a fragile site at Xq27.3 when lymphocytes are grown under conditions of thymidine stress. We have recently shown that fragile X affected males are hypermethylated in a region near the fragile site so providing evidence for the role of genomic imprinting as predicted by Laird. Using a DNA probe adjacent to this imprinted locus (Ox1.9) we have shown that 77/normal transmitting males and 136/143 (95%) fragile X positive, mentally retarded males also have an insertion or amplification of DNA sequences in this region, owing to varying numbers of a CGG trinucleotide repeat. This can be detected by Southern blot analysis as a restriction fragment length polymorphism event. Two percent show no evidence of the amplification event and are either extreme mosaic subjects or could be mutations elsewhere in this region. Detection of...
mutated fragments in carrier females is more variable and it is not possible to predict the phenotype from the genotype. Analysis by \textit{Pst}I digestion and hybridisation to the DNA probe Os0.55 identifies a small fragment crossing the region where mutations are occurring. Analysis of this fragment, therefore, allows a more accurate analysis of the insertion/amplification event. Normal subjects have allelic lengths between two and 50 copies of the CGG repeat. This is increased to between 55 and 125 copies in normal transmitting males, and still further in affected males. Carrier females generally have mutant alleles in all mitotic chromosomes. Analysis of DNA markers linked to the disease locus (a microsatellite marker and a locus on the X chromosome) suggests that the most common factors involved in induction of non-disjunction. If the trisomic state is involved in the pathogenesis of leukaemia, its effect may be because of (1) extra doses of gene products, (2) homozygosity for a recessive gene which confers a proliferative advantage on the cells, (3) isodicisomal diploid cells which have lost the normal homologue present in the original trisomic cell line. Further effort is required to elucidate the causes and effects of trisomy in both constitutional and acquired conditions.

Towards the single cell molecular diagnosis of fetal disorders

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Three requirements must be met for single cell fetal diagnosis to be achieved: a source of fetal cells, proof that such cells are fetal, and a diagnostic test that operates on single cells. We use fluorescence activated cell sorting to enrich for glycophorin positive, transferrin positive cells from the mononuclear cell layer derived from maternal peripheral blood that has already been depleted of T lymphocytes and monocytes. Such cells have the characteristic hetero-antisera of fetal nucleated erythrocytes. Single cells of fetal or polar body origin may also be obtained from the manipulation of human oocytes or pre-embryos. We believe that the origin of single cells can be proven if their molecular analysis is us to be used diagnostically, whether the cells are obtained by sorting from maternal blood or by microdissection of cells, in situ hybridisation, PCR, gel electrophoresis, etc. We have developed a multiplex polymerase chain reaction (PCR) approach to achieve this, based on the identification of autosome and sex chromosome polymorphisms. This approach can be augmented for the PCR diagnosis of Mendelian genetic disease. We hope to incorporate the study of chromosome 21 polymorphisms to provide an exclusion test for most trisomy 21 conceptions.

The use of preserved tissue DNA from deceased relatives to estabish phase in familial adnomatous polyposis (FAP) families

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Hippel-Lindau disease is a dominantly inherited cancer syndrome with variable expression. Major complications include retinal, cerebellar, and spinal haemangio- blastosoma, renal cell carcinoma, and pheochromocytoma (Maher et al. J Med Genet 1990;27:1512). We have previously identified linked DNA markers which flank the VHL locus (Hosoe et al. Genomics 1990;8:634-40; Maher et al. Genomics 1991;10:95-60) in a total of 47 families. To determine accurate estimates of marker recombination frequencies (and confidence limits) and to include locus heterogeneity, we have analysed the genotypic data on these 47 families using common age at onset data (Maher et al. J Med Genet 1991;28:443–7). Significant linkage was detected with RAPI (Zmax = 9.0 at 0-0.06, CI 0-02-0.11), D3S18 (Zmax = 3.9 at 0-0.02, CI 0-005-0.06), and D3S191 (Zmax = 8.9 at 0-0.09, CI 0.03-0.17). Formal heterogeneity testing gave no evidence of locus heterogeneity. These results were used to predict the risk of 15 at risk relatives from a further 11 families with suitable structure, studied in Cambridge. The combined use of DNA marker and age at onset data (Maher et al. J Med Genet 1991;28:443–7) enabled the risk for 7/15 subjects to be reduced to <2%. This approach will increase the efficiency of screening in VHL disease by concentrating resources for screening on high risk subjects.

Trisomy in haematological malignancy. A United Kingdom Cancer Cytogenetics Group (UKCCG) Collaborative Study

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There are many well established associations between acquired cytogenetic abnormalities and haematological malignancies. In order to gain further insights into the clinical significance of acquired, single trisomies as primary neoplastic events, the UKCCG collected data from contributing laboratories over a three year period. A total of 74 cases was recorded (excluding cases of trisomy 8 in myeloid disorders and trisomy 12 in lymphoid malignancy, where the non-random findings are uninterpreted). The majority of cases were reported from institutions of myeloid lineage. The most common trisomies to be reported were for chromosomes 21, 11, 9, and 13. The occurrence of trisomy 21, 13, 8, and 9 as constitutional abnormalities suggests that there may be commonly factors involved in induction of non-disjunction. If the trisomic state is involved in the pathogenesis of leukaemia, its effect may be because of (1) extra doses of gene products, (2) homozygosity for a recessive gene which confers a proliferative advantage on the cells, (3) isodicisomal diploid cells which have lost the normal homologue present in the original trisomic cell line. Further effort is required to elucidate the causes and effects of trisomy in both constitutional and acquired conditions.
and in order to form a strategy for prenatal diagnosis by direct detection of mutations they could be divided into three groups according to their incidence. The first group consisted of IVS-1 position 5 (G-C), the 619 bp deletion, codons 8/9 (+G), IVS-1 position 1 (G-T), and codons 41/42 (-C TT) which accounted for 93% of the β thalassaemia alleles in this population. A regional variation in the distribution of these mutations was observed with the codon 8/9 (+G) mutation being predominant in north-west Pakistan and the 619 bp deletion and the IVS-1 position 1 (G-T) mutation being predominant in the polytransfusion regions of Sindh, Gujarat, and Punjab. The second group which included codon 15 (G-A), codon 5 (C-T), IVS-1 minus 1 (G-C), codon 16 (-C), and IVS-2 position 837 (G-T) accounted for 5/7% of all β thalassaemia genes, and the last group consisted of cap site 1 (A-C), IVS-2 position 1 (G-A), IVS-1 position 110 (G-A), IVS-1 minus 1 (G-A), -88 (C-T), and codon 88 (+T) which accounted for 1/5% of all alleles. This comprehensive description of the spectrum of β thalassaemia mutations in the Asian Indian population will be valuable for prenatal diagnosis of the disease.

A 16 Mb YAC contig at the FAP locus (5q21-22) contains the genes for MCC and APC


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Evidence from loss of heterozygosity in sporadic colorectal tumours and genetic linkage studies of familial adenomatous polyposis (FAP) families has previously shown the presence of a putative tumour suppressor gene, involved in both sporadic and hereditary colon cancer, on chromosome 5q21. The markers YNS 48 and YNS 64 are known to be markers close to and flanking the FAP gene and recent data indicate that the anonymous markers L5.79 and EF5.44 also flank the gene and are within these two markers. We have constructed six contigs of yeast artificial chromosomes (YACs), totalling approximately 5.5 Mb of genomic DNA, from between YNS 48 and YNS 64. One contig of 1.6 Mb spans the two markers L5.79 and EF5.44. By using the YACs to screen cDNA libraries we have shown that there are at least four genes within this contig. Two of these genes, MCC (mutated in colorectal cancer) and APC (adenomatous polyposis coli), have been shown to be involved in colorectal cancer and one (APC) is the gene for FAP.

Using a system for amplifying the entire adenomatous polyposis coli gene from ectopic transcripts found in peripheral blood lymphocytes, we have isolated microgram quantities of cDNA from a representative set of blood samples from six Duchenne muscular dystrophy patients with no detectable rearrangements (which represent 30% of cases). These have been efficiently screened for sequence variation by chemical mismatchpolymerase chain reaction and yielded a small number of polymorphisms, rare neutral variations, and a candidate deleterious mutation in each case. This approach will allow direct diagnosis to be applied to virtually all cases of DMD and BMD, and to examine carrier diagnosis in female relatives. It will also provide functional dissection of the dystrophin protein through study of genotype/phenotype correlations.

A comparison of approaches used in community screening for Tay–Sachs carriers

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In the course of offering a national screening service for Tay–Sachs disease, 200 people completed questionnaires, before and after undergoing carrier testing. Subjects were recruited from two sources: (1) from those seen individually at a genetic counselling clinic, and (2) people attending a mass public screening who did not receive individual pretest counselling. This study shows that carrier screening, even during pregnancy, does not raise anxiety levels as long as a normal result is obtained. Measuring people's knowledge levels indicate that mass community screening can increase people's understanding of recessive inheritance when compared to individual counselling. Mass screening always runs the risk of being confusing to people who are uninform ed and unprepared. Before screening 33% of people said that even if they were not a carrier themselves, they would be concerned or prefer not to receive a carrier result. A normal result this concerned proportion remained at 23%. There may be adverse labelling of carriers and the possibility of creating a stigmatised group of single carriers argues for caution before screening teenagers and young adults.

Oromandibular hypogenesis syndromes, limb reduction defects, and chorion villus sampling

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We have recently reported on four babies with oromandibular–limb hypogenesis syndromes among 289 pregnancies in which chorion villus sampling (CVS) was carried out at 55 to 66 days' gestation. (Lancet 1991;337:762–3). A fifth baby had a transverse limb reduction defect. Estimates of the birth prevalence of these defects derived from the British Columbia registry are 0.057/10 000 livebirths for oromandibular–limb hypogenesis syndromes and 1.8/10 000 livebirths for transverse limb reduction defects. This high incidence raised the possibility that CVS was the cause of the severe abnormalities in these pregnancies. A further four babies with oromandibular–limb hypogenesis syndromes after CVS at 8 to 9 weeks' gestation have subsequently been reported (Mastroiacovo and Cavalcanti, Hsieh et al. Lancet 1991;337:1091–2; Myny et al. Lancet 1991:338:1425–4; Firth et al. Lancet 1991;338:51) and such cases have been brought to our attention (Burton, personal communication) providing strong evidence for an association between oromandibular–limb hypogenesis and early CVS. Following our report some centres have also found an increased incidence of limb reduction defects whose mothers have undergone first trimester CVS (Mastroiacovo and Cavalcanti, Hsieh et al. Lancet 1991;337:1091–2; Burton, personal communication). Others have not found an increased incidence (Monni et al. Lancet 1991;337:1091; Mahoney, Jackson et al. Lancet 1991;337:1422–3). The correlation
between timing of CVS and the severity of defects, earlier CVS (8 to 9 weeks) with major limb reductions (Haisch et al.), and later CVS (10 to 11 weeks) with digital reduction defects (Burton, personal communication) is further evidence that CVS may have an aetiological role in these abnormalities.

Familial transmission of outflow tract defects of the heart

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We present four families with transmission of outflow tract defects of the heart. Family A includes three children, one with a membranous VSD and clear aortic arch, and one with aortic coarctation. and one with interrupted aortic arch who also had DiGeorge syndrome. Family B comprises a mother and son with tetralogy of Fallot. In family C a mother with a patent ductus arteriosus and right aortic arch has a child with pulmonary atresia and a VSD and also had a child with truncus arteriosus and hypoparathyroidism who died. Family D includes mother with a history of congenital heart disease, the nature of which is uncertain, and a child with pulmonary atresia and a VSD. A cytogenetic interstitial deletion at 22q11 was found in all affected subjects and the mother in family A. This was confirmed by the deletion of a DNA probe from this region of the genome. In family B there was a visible interstitial deletion at 22q11 in the son. Molecular analysis showed a DNA deletion in both the mother and the child using a probe with known karyotypes but all had a molecular deletion. Deletions at 22q11 are known to be associated with DiGeorge syndrome. These families show that such deletions are also associated with apparently isolated congenital heart disease. Subjects with such deletions represent a high risk group for recurrence of congenital heart disease.

Towards isolating the gene for von Hippel-Lindau disease I. Genetic linkage analysis

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Von Hippel–Lindau (VHL) disease is a dominantly inherited cancer syndrome with a minimum incidence of 1/36 000 (Maier et al. J Med Genet 1991;28:443–7). Major complications include retinal, cerebellar, and renal cell carcinoma and phaeochromocytoma (Maier et al. J Med Genet 1997;7:1152). The VHL disease gene has been mapped to 3p25–26 by genetic linkage studies and flanking markers identified (Hosoe et al. Genomics 1990;8:634–40; Maier et al. Genomics 1991;10:957–60). As part of a programme to isolate and characterise the VHL gene we have constructed a long range physical map around the VHL locus using pulse field gel electrophoresis and situ hybridisation. The map contains four loci linked to VHL disease (RAFI, D3S601, D3S18, D3S732). Clustering of rare cutter enzyme restriction sites suggesting a CpG island was detected close to RAFl and D3S18. The construction of a long range physical map in the region of the VHL locus will provide a basis for screening affected patients for deletions, and facilitate the mapping and ordering of DNA sequences cloned from this region.

X linked ocular albinism: an assessment of clinical methods of carrier detection

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In families with X linked ocular albinism (XLOA) carrier females may be identified by the presence of iris translucency, characteristic 'mud splattered' fundus appearance, and the presence of macromelanosomes on skin biopsy. However, in some families obligate carriers may have normal irides and fundi.

Towards isolating the gene for von Hippel-Lindau disease II. Construction of a long range physical map around the VHL locus

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Closely linked polymorphic probes for the predictive diagnosis of familial adenomatous polyposis

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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 has been localised to 5q21–22 by linkage and several probes have become available for presymptomatic diagnosis. Twenty-five different families from Leeds, Liverpool, and St Mark's Hospital, London, have been analysed for linkage using the probes p227, C11H11, ECB27, YN5.48, and MCV6.1. Results obtained showed that none of these markers

Abstracts

We have assessed 17 families affected by XLOA in the UK, together with an extensive pedigree in Newfoundland, to determine the relative values of clinical examination and skin biopsy in carrier detection. Three hundred and sixteen persons have been examined, including 73 affected males and 77 obligate heterozygotes; 93.5% of obligate heterozygotes had a 'mud splattered' fundus appearance with hyperpigmented streaks in the periphery and 76% had marked iris translucency. Skin histology showed macromelanosomes in 86%. Thirty-eight of 92 (41%) females at 50% risk of being gene carriers had definite signs of the carrier state and 31 (34%) were unequivocally normal but a further 23 (25%) had mild retinal pigmentary abnormalities, often with iris transillumination defects, of uncertain significance. In the UK families, 30 of 32 obligate heterozygotes with affected sons were unaware of their carrier state before the birth of the affected child and 16 of the 27 at risk females with definite carrier signs (59%) had been totally unaware of their carrier status before being examined in this study. XLOA is underdiagnosed within the population. Fundus examination will detect most females but will not enable reliable genetic counselling for all at risk females, even when supplemented by skin biopsy.

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Molecular genetic evidence for the existence of a severe form of adenomatous polyposis coli which results from fresh mutation

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Adenomatous polyposis coli (APC), also known as familial adenomatous polyposis, is the result of a dominant mutation in a gene which has been mapped to chromosome 5q22. Typical APC patients develop an average of 1000 colorectal adenomas and extra-colonic neoplastic lesions are frequent. Owing to the high number of adenomas present the lifetime risk of colorectal cancer is 100% although for each adenoma the chance of progression to malignancy is low. Based on the number of isolated cases, it is frequently stated that the mutation rate in APC is high. However, in untreated patients the average age at death from carcinoma is 40 years by which time most would have reproduced. If, however, a proportion of patients is prone to malignancy of early onset then this may preclude reproduction. We present evidence for the existence of a more severe form of APC in two patients, both of whom appear to be new mutations. The first patient presented with symptoms at the age of 15 and in the second, at 23, two carcinomas were found. Japanese (Miyaiki et al. Cancer Res 1989;49:7166–73) and our own molecular data have confirmed that in APC carcinomas a second event has occurred, with loss or inactivation of the remaining normal allele on chromosome 5. However, DNA analysis has shown that, as expected, allele loss is usually a rare event in APC adenomas. Using genetic markers on chromosome 5 we have found evidence of allele loss in two out of three patient families from Japan (Rees et al. Br Cancer 1989;59:361–5) and four of 10 from the second patient. This high frequency of specific loss suggests that these patients have a more severe form of APC.

Incidence and significance of congenital hypertrophy of the retinal pigment epithelium (CHRPE) in familial adenomatous polyposis coli (FAP)

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Most patients with FAPC have multiple areas of CHRPEs, but the exact incidence has varied between studies. We have studied 32 affected patients and 39 at risk relatives from 29 kindreds with FAPC to determine (1) incidence of CHRPEs and evidence for inter-familial differences, (2) reliability of CHRPEs for presymptomatic diagnosis, and (3) relationship between CHRPE and other phenotypic expressions of the APC gene.

There are quantitative and qualitative differences in the incidence of CHRPEs between normal controls and affected patients: 26 of 32 patients had one or more CHRPEs and 19 (59%) had >2. There were significant inter-familial differences in predisposition to CHRPEs. When the index patient had >4 all other affected relatives (n = 5) had >4 CHRPEs, and when the index patient had 0–3 CHRPEs all other affected relatives (n = 3) had 0–3 CHRPEs (p <0.02). There were no significant differences in mean age at diagnosis (27 ± 5 years, incidence of colorectal [2/19 ± 5/13]) or extra-colonic (1/19 ± 2/13) malignancy, and Gardner phenotype (8/19 ± 3/13) between patients with ≥4 and those with 0–3 CHRPEs. In families with typical APC (>100 polyps) the presence or absence of CHRPEs does not appear to be associated with prognostic implications. Because of inter-familial differences in predisposition to CHRPEs it is important to establish the CHRPE status of individual FAP families before the results of ophthalmoscopy are used to predict the genotype of at risk relatives.

De novo chromosome 5 translocation associated with adenomatous polyposis coli

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Following the report of a mentally retarded patient with APC and an interstitial deletion of chromosome 5 (Herrera et al. Am J Med Genet 1989;26:61), the APC gene was mapped to chromosome 5q by linkage analysis (Bodmer et al. Nature 1987;328:614). Further reports of retained APC patients with chromosome 5 deletions have refined the localisation of the APC gene to 5q21–q22 (Hockey et al. J Med Genet 1989;26:61–8). Cross et al. J Med Genet 1991;28:564. We report a mentally retarded man with APC, minor dysmorphic features, and multiple CHRPEs (>10), but no family history of APC or mental retardation and no osteomas or cutaneous cysts. Cytogenetic analysis showed an apparently balanced complex rearrangement involving chromosomes 5, 9, 11, 12, and 16. The breakpoint on chromosome 5 was at 5q21–q22. In situ hybridisation indicated that the cosmid probe YN5.48 (D5S51) mapped close to and telomeric to the translocation breakpoint on 5q. Parental chromosomes were normal and neither had evidence of CHRPEs. Our findings support the results of genetic linkage studies which localise the APC gene centromeric to D5S51 (Dunlop et al. Br J Hum Genet 1990;47:982–7), and are compatible with the translocation breakpoint disrupting the APC gene.

Oestrogen receptors in neurofibromatosis

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In neurofibromatosis (NF1) the number and size of neurofibromata may increase in pregnancy, at puberty, and possibly with the oral contraceptive pill. It is possible that the development of neurofibromata is modified hormonally and if this were correct hormonal therapy may be beneficial. Neurofibromata from four patients (including one pregnant patient) were biopsied and assayed using the Abbott ER-EIA monomolar antibody test for oestrogen receptors. Normal skin biopsies, a lipoma, and an oestrogen receptor positive breast tumour were used as controls. All the neurofibromata showed insignificant levels of oestrogen receptors, while the breast tissue confirmed high levels. Levels below 20 fmol/mg cytosol protein are usually regarded as negative.

Fragile X syndrome and idurionate sulphatase activity

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Fragile X syndrome has perplexed geneticists since it was first described. Even the recent discovery of the mutation only sheds a little light on the strange inheritance pattern observed in this syndrome. Laird has suggested a model that fragile X is caused by a mutation of the X chromosome that prevents the reactivation of inactivated (‘Lyonised’) genes around the fragile X site in oogenesis. There has, however, been no direct evidence
to support this idea. We have examined the pattern of three loci flanking the fragile X site in 16 affected males and 12 normal males. Blood levels of coagulation factors VIII and IX were found to be normal but serum iduronate sulphatase activity was significantly lower, 0.73 ± 0.01 nmol/mg protein (p < 0.001). The idurionate sulphatase gene is the closest known gene distal to fragile X and therefore the most likely to be affected if Laird's model is correct. Further work will be required to relate this finding to the molecular structure of the fragile X site.

Location of the gene for Marfan syndrome

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Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder with an estimated prevalence of about 4 to 6 per 100,000 persons. It is a systemic disorder of the connective tissue and imparts significant morbidity and mortality. The characteristic manifestations are those of the musculoskeletal, cardiovascular, and ocular systems. The diagnosis of the disease is entirely dependent on internationally agreed clinical criteria (Beighton et al., 1986). Diagnosis can be difficult owing to the variability in expression both within and between families. The need for a biochemical or genetic marker is therefore essential. The protein fibrillin has been shown by immunohistochemical studies to be present in decreased levels in about 70% of MFS affected patients, but decreased levels of this protein are also found in subjects affected by other connective tissue disorders (Godfrey et al., 1990). The biochemical test is therefore not a definitive marker for MFS. The location of the gene for MFS was first assigned to chromosome 15 by positional mapping in five Finnish families. Confirmation of this finding followed after fine mapping around the locus identified 15q15.2-2.1 as the location of the gene for MFS (Tsipouras et al., 1991). Part of the fibrillin gene (FIB15) has now been isolated and the gene has been mapped to the 15q15-2.1 region (Lee et al., 1991) shown to be the location of the MFS gene by positional mapping (Tsipouras et al., 1990). Linkage of the fibrillin gene to MFS has been shown by two groups, Lee et al. (1991) and Dietz et al. (1991). The International Marfan Syndrome Consortium, of which our laboratory is a member, has generated and submitted for publication further data which confirm the linkage. Here we report two of the families which contributed to the consortium data, one which shows linkage in a classically affected family and another which shows linkage in a family with no ocular involvement.

Absence of close linkage between renin gene and susceptibility to eclampsia and pre-eclampsia

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The hypothesis that susceptibility to pre-eclampsia and eclampsia is closely linked to the maternal renin gene was tested. Blood samples from many Icelandic families with at least three affected females in two or three generations were collected. The proband had eclampsia or severe pre-eclampsia (BP 160/100 ± proteinuria) and the daughters and granddaughters eclampsia or pre-eclampsia (BP 140/90 ± proteinuria). DNA was digested with BglII and HindIII restriction endonucleases and subjected to electrophoresis. Southern blots were made on nylon membranes. The blots were hybridised with two labelled renin gene probes. For linkage analysis, lod scores were calculated by the LIPED program for the following loci: BglII 90-50 kb alleles (exon 1) and HindIII 90-62 alleles (exon 2-9) was found to be 0.67 ± 0.33 and 0.71 ± 0.29 respectively. No significant difference was found between spouses and affected females. Lod scores were calculated for various combinations of inheritance patterns and gene frequency. A value of −2 which conventionally excludes close linkage was found. This excludes the renin locus as the putative susceptibility gene for pre-eclampsia and eclampsia and further candidate loci are currently being explored in this group of families.

Linkage analysis of an X linked rod and cone dysfunction

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Aland Island eye disease (AIED, MIM 300600) is an X-linked disorder that primarily involves dysfunction of both the cones and rods. We have studied a large six generation family with nine affected males and 14 obligatory carriers. The affected males have subnormal visual acuity, nystagmus, high axial myopia, foveal hypoplasia, and protanomalous colour vision deficiency associated with minor fundal pigmenta-

Linkage heterogeneity in X linked hypophosphataemia

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Linkage heterogeneity is one of the most intractable problems in human gene mapping. When there is one major established locus for a disease, flanking markers can be used to define apparently doubly recombinant meioses. Such meioses strongly suggest the disease in that family does not map between the flanking markers. Probabilities can be calculated by Bayes or using LINKAGE. We show a family with hypophosphataemia including four apparent double recombinations for disease versus markers which flank the X chromosome HYP locus. LINKAGE gave odds of 4862:1 against this family's disease mapping to HYP. This method is much more powerful than using HOMOG with a single marker, but it requires a well established marker framework.

Linkage analysis in Peutz-Jeghers disease

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Peutz-Jeghers syndrome (PJS) is a rare, dominantly inherited tumour syndrome characterised by hamartomatous polyps of the small bowel and an unusual pigmentation of the skin. Affected subjects are also at increased risk of malignancy, including cancers of the small and large bowel, breast, pancreas, ovary, and testis. It is now clear that genes producing rare tumour syndromes through inheritance of germline mutations may also be involved in the development of sporadic cancers as a result of stochastic mutation. As part of a programme to identify and isolate such genes we have initiated an attempt to localise the PJS locus by linkage analysis. Five informative pedigrees have been identified from the St Mark's Hospital Polyposis Register and other sources, and RFLP typing is proceeding for a large number of loci. Two point linkage analysis has so far excluded the PJS locus from two candidate regions identified from a karyotypic abnormality, and efforts are continuing to increase coverage of the remainder of the genome.

Dominantly inherited spinocerebellar ataxia (SCA1): linkage studies

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The late onset autosomal dominant ataxias are a heterogeneous group of disorders which have been classified into four types. In general, degeneration in the cerebellum, spinothalamic tract, and posterior column systems lead to ataxia (shaky movements and unsteadiness gait) and dysarthria (poor pronunciation) with onset usually in the third decade. Linkage to markers on chromosome 6p has been shown in some families (Ranum et al, 1991; Zoghbi et al, 1991) and excluded in others (Sasaki et al, 1988; Auburger et al, 1991). It has been shown in some of the HLA linked families that the disease gene (SCAI) is located near the D6S89 locus (the known location of SCA1). The remaining four families are too small to be informative but linkage to D6S89 is not seen in family 4 or 6. It may become possible to correlate some aspect of phenotype with the involvement of SCA1 as more families are examined for their linkage with D6S89.

Cystic fibrosis: how much do teenagers know and how much do they want to know?
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Population screening for carriers of a mutant recessive gene aims to identify at risk subjects and couples before the birth of an affected child and so screening programmes are directed towards persons of reproductive age. In the UK, where 1 person in 23 in the Caucasian population carries a mutant gene for cystic fibrosis, there will be a large variation in the basic level of awareness of the disorder and in understanding the implications of carrying a mutant gene. We have compared two questionnaires given to a group of teenagers (aged 14 to 18) in a boarding school in Bristol, we have assessed, first, their background knowledge of the disorder, and subsequently, after an explanation about the nature of the disease, their attitudes to carrier detection, family planning, and prenatal diagnosis. Although initially most knew very little, attitudes were very receptive to the information that was given. The majority would wish to know their carrier status and believe this knowledge will influence their attitudes to family planning. Because teenagers can easily accept information about CF, we conclude that such information should be incorporated into the secondary school curriculum and that a suitable time to screen persons for carrier status could be immediately upon leaving school.

Neonatal screening for cystic fibrosis
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We have instigated a two year pilot scheme to assess the viability and cost effectiveness of screening the Guthrie blood spots, which show positive on initial immunoreactive trypsin (IRT) testing, for the cystic fibrosis (CF) mutation ΔF508 (by PCR and PAGE analysis) in order to predict a moderate risk of having the disease before repeating the IRT analysis and subsequent sweat test. Our preliminary results confirm the feasibility of substituting DNA analysis on the initial dried blood spot for IRT estimation on a second blood sample. In the first six months of the project, 34 235 initial IRT samples were screened; 104 samples were above the mean 110 nmol/ml threshold and referred for DNA analysis. Of these six were homozygous for ΔF508, 10 were heterozygous, 85 homozygous for the normal alleles, and three failed. Of the two heterozygotes, five were diagnosed as CF by repeat IRT and sweat test analysis while the other five are presumably carriers. These results suggest that repeat Guthries could be reduced by nearly 90%. The acceptability of a DNA based second stage on initial IRT screening will ultimately depend on the perceived social cost of obtaining the second blood sample, and the rate of repeat IRT at SCA1. The remaining four families are too small to be informative but linkage to D6S89 is not seen in family 4 or 6. It may become possible to correlate some aspect of phenotype with the involvement of SCA1 as more families are examined for their linkage with D6S89.

Screening for cystic fibrosis mutations
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Fourteen mutations in the CFTR gene have been screened for in 131 cystic fibrosis (CF) patients from the south-east Thames region; 71-4% CF chromosomes carried the most common CF mutation, ΔF508, and the other CF mutations were present in 28-6% of tests. Among the 131 patients, 6% carried ΔF508, G551D, G542X, 621+1G>T, Marker haplotypes include X2C and KM19. Statistical analyses are by χ² test and Mann-Whitney U test as appropriate. For age and dependent sex analyses, have been done in five year bands. So far we have studied 179 patients, using ΔF508 (178 patients), KM19, and X2C (140 and 136 patients). Full clinical data are not available yet for all patients. Eight patients were pancreatic sufficient (confirmed by three day faecal fat excretion or other tests) and none of these is homozygous for ΔF508. At present, no other consistent association has been shown between any of the genotypes and phenotypes. We plan to expand the study to include a more substantial number of CF families in Wales, to incorporate the data into the follow up of the neonatal screening survey which was conducted in Wales and the West Midlands (1985-1989), and to collaborate with other CF centres in the UK, using the same database and methods of analyses.
Genotype analysis for ΔF508 allele in cystic fibrosis in relation to the occurrence of diabetes mellitus

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Carbohydrate intolerance and frank diabetes mellitus (DM) has been recognised frequently in association with cystic fibrosis (CF). No absolute cause for the carbohydrate metabolism abnormality has been documented. However, secondary damage to the islet cells owing to pancreatic fibrosis is the most common explanation. A possible relation between genotype and the occurrence of diabetes mellitus was examined among 21 cystic fibrosis patients attending the outpatients’ clinic at Addenbrooke’s Hospital. Blood samples were studied for the presence of the most common mutation (ΔF508) and the results compared to the patients’ degree of carbohydrate intolerance. We found that in the adult CF patients (over 18 years of age), there is a greater frequency of diabetes among those homozygous for the ΔF508 allele than among heterozygotes (p<0.05). Those homozygous for ΔF508 were diagnosed as CF at an earlier age than the heterozygotes (p<0.05). Both diabetic and non-diabetic adult CF patients have a similar mean age and the female/male ratio in the diabetic group are similar to that of the whole group. The prevalence of the ΔF508 allele in this group was not different from what is expected in a random sample of East Anglian cystic fibrosis patients. These data suggest that the occurrence of diabetes mellitus in patients with cystic fibrosis may be related to the genotype which could be secondary to the way the genetic factors influence the degree of pancreatic disease and its rate of progression.

Strategies used for detecting CF mutations in the Welsh population

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A study on 186 cystic fibrosis families in Wales has shown that the most frequent CF mutations are ΔF508=71.8%, ΔS549N=6.2%, ΔS549R=2.4%, and ΔS553X=1.1%. We discovered a relatively high frequency of 621+1G>T in our population, which warranted its incorporation into a multiplex system developed by St Mary’s, to allow the simultaneous detection of 621+1G>T and ΔF508, ΔS547 (0.5%), ΔS551D, and ΔS553X. We are running this system in parallel with Cellmark Diagnostics ARMS multiplex which detects ΔF508, ΔS542X, 621+1G>T and ΔS551D. In addition, we have screened for some of the rarer CF mutations (R117H, 1717-1G>A, R560T, N1303K, W1282X, and V520F) pri-

Racial incidence of Duchenne muscular dystrophy in the West Midlands

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There was an impression in the West Midlands of an increased incidence of Duchenne muscular dystrophy (DMD) in Indians, which was strengthened by the unusual occurrence of three different mutations of the dystrophin gene in a single Sikh family (Miciak et al. J Med Genet 1992;29:123-6). We therefore carried out a population study to discover whether DMD is unexpectedly frequent in Indians. Population statistics for children aged 0 to 4 years were taken from the 1981 Census (West Midlands data) and proportions in different races were obtained. All cases of DMD known to our department born 1970 to 1985 were enumerated and their racial group ascertained. There were 176 cases of DMD. Based on the Census data we would have expected 6.9% (12 cases) to be Indians, 4.1% (seven cases) to be Pakistanis, 2.6% (five cases) to be Afro-Caribbeans, and 86.5% (152 cases) to be from other races. In fact there were 24 Indians, three Pakistanis, one Afro-Caribbean, and 148 from other races. The excess of Indian boys in our group was significantly higher than expected (p=7.1, p<0.001), but the apparent deficit of Pakistani and Afro-Caribbean boys was not statistically significant. Could this excess of cases be the result of (1) a lack of genetic counselling? (2) the West Midlands attracting disproportionate numbers of handicapped immigrants? (3) disproportionate numbers of English patients referred out of the region? (4) dystrophin gene mutations more commonly causing DMD than Becker muscular dystrophy in Indians? (5) an effect of transfusible elements? We found no evidence for explanations 1 to 3.

Xp2.1 muscular dystrophy in Scotland, 1951–1987

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There has been active DMD research in the two main centres cited, since the 1960s. Disease registers have been maintained and then amalgamated into a computerised data-

base in the mid-eighties with the centralisation of DNA analysis to Glasgow. In addition to referrals from our genetic and medical colleagues in Aberdeen, Dundee, and Inverness, ascertainment was from the records of the Muscular Dystrophy Group and the Scottish Muscular Dystrophy Association. Articles in the national and local press and radio caused many unknown families to seek genetic advice. Lastly, copies of all death certificates listing muscular dystrophy were obtained from the General Register Office. Diagnostic criteria of all cases were further investigated. A total of 367 DMD pedigrees were found (BMD 72); 487 subjects were affected by DMD (BMD 112) of whom 320 were born in Scotland from 1951 to 1985 (BMD 44 from 1951 to 1970). Using the χ2 test and regression analysis there were no significant differences in five yearly incidences. The overall incidence covering 1 526 036 male births was 209/100 000 (BMD 1951–70, 392/100 000 male births, 44 3/105). The availability of genetic counselling has not affected the incidence of DMD in this period. The poten-
tially preventable cases are those in which the diagnosis has already been made, and therefore relative before their conception; 34 (or 10.6% of total cases) are in this category and there has been no significant drop in inci-
dence. In 1961 to 1965, five such cases were born after mother was counselled as to the possibility that they were carriers before conception. The median age at diagnosis in the first case in a family is 5.2 years; if neonatal screening, for genetic counselling purposes, had been available over this period, a further 23 cases (7%) would have had a diagnosed relative before conception.

All-Wales neonatal screening programme for Duchenne muscular dystrophy

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Newborn screening for Duchenne muscular dystrophy (DMD), by blood kinase (CK) assay, was introduced in Wales on 1 July 1990 funded by the Muscular Dystrophy Group. It is offered as a parental option with the routine PKU/hypothyroid screening programme with a view to give families choice in future pregnancies. Over the last 15 years DMD screening programmes have been introduced in a number of countries; however, the Welsh initiative is the first to incorporate a social research programme. The programme specifi-
cally addresses the issues of whether neo-
natal screening is something that families and health professionals want introduced on a permanent basis and whether there is any evidence of increased trauma in families who experience this much earlier diagnosis of their son’s disorder. A leaflet, 'A New Test for Baby Boys – Do You Want It?’, available in nine languages, was devised to give famil-
ies information before choosing whether or not to have the muscular dystrophy as a 1 in 4000 risk of an affected child. Over 65% have accepted the offer in the last year, of which have been confirmed by subsequent venous CK assay. Of these seven deletions.
Abstracts

have been found in four. Two families have opted for muscle biopsy. All seven families have requested genetic counselling, in four cases initiated by the extended family. The social evaluation, by way of extended unstructured interviews, will focus on all families with a positive screening test, a subset of affected, carriers, health professionals, and families who receive the later traditional diagnosis during the course of the programme. This three year programme aims to explore both principles and practice. To date follow up practice has been considerably modified as a result of feedback from families and health professionals. The overall response of requesting an abnormal screening test has been very positive. One family, six months after the screening test, still feels the knowledge is too difficult to handle.

Detection of two polymorphisms in the dystrophin gene by the polymerase chain reaction

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Two published nucleotide sequences for exon 48 of the dystrophin gene (Chamberlain et al, 1988; Rosenthal et al, 1989) show a single base mismatch from the sequence reported by Koenig et al (1988). The discrepancy involves a C to A transversion at nucleotide position 7304 in the cDNA. This base change could represent either a polymorphism or be the result of a cloning artefact. Analysis using amplification and mismatch detection followed by direct sequencing showed seven out of 10 males to have the C nucleotide, suggesting the change to be a polymorphism. The C to A base change creates a MstI restriction site which can readily be analysed using the polymerase chain reaction. We have found this polymorphism to be inherited in a Mendelian fashion, and 30% of 27 unrelated females were found to be heterozygous. The restriction site was found to be present in 74% of a total of 98 X chromosomes studied. This new polymorphism should prove useful in linkage studies, and particularly in determining carrier status for relatives of the 25% of deletion cases that involve this exon. Additionally, we have sequenced around a polymorphic marker, J66H-1, that was previously typed by the method of Southern blotting. This has enabled us to amplify the polymorphic region and analyse it in families by the method of PCR. J66H-1 is an insertion/deletion type polymorphism located towards the 3' end of the dystrophin gene, in intron 60, with a haplotype of ref/ref, ref/hom, hom/hom. Owing to its location, this polymorphism will be very useful as a 3' marker in the linkage analysis of Becker/Duchenne muscular dystrophy. Selection of new polymorphisms and conversion of existing markers to typing by the method of PCR will greatly reduce the workload and the reporting time involved in diagnostic analysis.

Direct carrier detection for DMD mutations using PCR

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A quantitative, non-radioactive PCR technique has been investigated for the detection of female carriers of deletions and duplications in the dystrophin gene. Two multiplex Southern blot reactions were developed to detect 98% of deletions (Abbs et al, 1991) are used under stringently controlled conditions to quantitatively amplify from a measured amount of genomic DNA, under the conditions used, we have shown the amplification at 23 cycles to be well within the exponential phase of the reaction by measuring the incorporation of radioactive label. Sufficient product is available at this stage to analyse the dosage by visual inspection of an ethidium bromide stained gel. In a blind trial performed on 20 obligate deletion carriers and 20 normal controls, the status of all samples was correctly determined.

Investigation of a female manifesting Becker muscular dystrophy

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Females manifesting Becker muscular dystrophy (BMD) are even more rarely observed than for the allelic condition, Duchenne muscular dystrophy. The 24 year old male proband has typical BMD with greatly raised CK activity and a myopathic muscle biopsy. His mother experienced walking difficulties from age 35 years and eight years later the condition has progressed such that she can only mobilise with assistance. She has a proximal myopathy, marked calf hypertrophy in association with a raised CK, and myopathic muscle biopsy. There are no other affected family members. Further muscle was obtained for dystrophin analysis from both the proband and his mother. Immuno-blotting showed a protein of normal size but of reduced abundance in both subjects. Immunocytochemical analysis in the proband showed that the majority of the fibres showed weak dystrophin labelling. His mother had a more severe pattern with both dystrophin positive and dystrophin negative fibres present. Non-random X inactivation, using locus DXS255, was observed in DNA isolated from peripheral lymphocytes of the proband and his mother. An extended multiple PCR performed on DNA from the proband did not show a deletion in the dystrophin gene, suggesting that the mutation responsible for BMD in this family may be a point mutation or be located in the promoter region for the dystrophin gene or involve an abnormality in transcription/post-translation.

Mutation screening in X linked disorders using single strand conformation polymorphism analysis

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In X linked disorders, carrier detection and prenatal diagnosis using restriction fragment length polymorphism analysis is limited by the high rate of new mutations, the possibility of germline mosaicism, and occasional diagnostic difficulties. Thus efficient screening methods for the direct detection of mutations are required. A survey of exon structure in genes causing important X linked disorders shows that single strand conformation polymorphism analysis (SSCP) of each exon is potentially feasible. We have applied SSCP analysis to cases of ornithine carbamoyl transferase (OCT) deficiency and Peli- zaus–Merzbacher disease (PMD). SSCP analysis of exons 7 and 8 in 29 OCT patients and exon 6 in 8 patients shows sequence changes segregating with the disorder in two families with mild OTC. The OCT gene also contains four TaqI sites, which show a high mutation rate and can be screened by PCR amplification and digestion. One such change, an Arg to Gln at amino acid 109, was found in an affected female. In addition we found one mutation in an Md4 site in exon 7. Classical PMD can be caused by mutations of the proteolipid protein gene though a further autosomal gene has been suggested. SSCP analysis of six exons in three families has shown a sequence change in exon 4 in two obligate carriers and a predicted carrier in one family and a change in exon 5 in the mother and two affected sons of a second family. We thank the Research Trust for Metabolic Diseases and the MRC for generous support. SS is in receipt of an MRC/HGM studentship.

Use of tandem repeats in two loci in the search for myotonic dystrophy

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The tandemly repeated sequence (dGdF)n, (dGdA)n is widely distributed in the human genome. One is found in the first intron of the CEA (carcinoembryonic antigen) (19q13.1–13.2) and another in the first intron of the APOC2 gene (19q13.2), in the order CEA ... APOC2.DM where each ' is 0.5–1 Mb. We have used the PCR and PAGE approaches described by Weber and May (1989) and Litt and Luty (1989) to examine the number, size, and frequency of repeats near and 11 alleles found in normal and myotonic dystrophy pedigrees from Eng- land, Canada, New Zealand, and Finland. We present data on linkage and evidence for an allelic association within the English
Sexing by PCR with an internal control

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A method has been developed for the sexing of DNA from prenatal samples using the polymerase chain reaction (PCR) with an internal control. There has been concern over the sole use of Y specific primers for this purpose as a negative result could either result from the sample being from a female or failure of amplification. Ellis et al. (Nature 1990;344:663–5) developed primers which amplified the pseudoautosomal boundary of the sex chromosomes individually. These primers have now been used in one reaction mix. Three primers are used; a common primer from the pseudoautosomal region plus an X specific primer and a Y specific primer. These yield different size bands, so if the sample is female one band will be amplified but if the sample is male there will be two bands. This reaction has been used for the sexing of prenatal DNA, identification of a marker chromosome from a fetus which was mosaic 45,XO/46,XX+, +mar, and identification of incorrectly labelled samples from a husband and wife. It has proved to be a fast and reliable means of determining fetal sex in cases of X linked disorders when further molecular tests need to proceed as soon as possible.

Prenatal diagnosis of the fragile X syndrome by direct analysis of the premutation in chorionic villus DNA

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The fragile X syndrome (FRAX) is the most common familial form of mental retardation. Affected boys are clearly an X linked condition, the inheritance pattern in fragile X is not simple. Unaffected males who transmit the fragile X to their daughters are common, and about one-third of carrier females show mental impairment. The families pose serious problems for genetic counselling of FRAX families. Recent findings (Yu et al. Science 1991;252:1179–81, Oberle et al. Science 1991;252:1097–102, Vertek et al. Cell 1991;65:905–14) have shown that the basic defect in FRAX is instability in a DNA fragment at the fragile site. Unaffected carriers and normal transmitting males show a small increase in the size of this fragment, while in affected subjects the fragment is greatly increased in size. We have used newly isolated probes pfx3a and Ox1.9 to determine the FRAX genetics. Three fetuses of a pregnancy in the mother of an affected boy. Fetal sexing by PCR and fluoresce induced indicated the presence of one male and one female fetus. Using probe pfx3a, the mother was found to have a PstI fragment of 1.3 kb in addition to her normal fragment at 1.0 kb. The fetal female had only a 1.0 kb fragment, while the male fetus had only a 1.2 kb fragment. In males, fragments of less than 1.0 kb are indicative of a ‘normal transmitter’. Fetal blood sampling was carried out on the male fetus at 20 weeks’ gestation. The fetus was found to be cytogenetically normal, and the same 1.2 kb fragment was detected in DNA from the fetal blood. Analysis of the pregnancy using linked markers indicated that the female fetus had inherited the low risk allele while the male has inherited the high risk allele. Application of the direct test to this pregnancy has thus resulted in the prevention of the termination of what is predicted to be a phenotypically normal male.

Can prenatal diagnosis be offered in neonatal lethal spinal muscular atrophy (SMA) with arthrogryposis and fractures?

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Following the localisation on 5q (5q11.2-q13.3) of the gene for acute infantile SMA (Werdnig-Hoffmann disease) and later presenting forms of recessive SMA, prenatal diagnosis can now be offered in these conditions. Concern has been expressed as to whether families will accept the prenatal neonatal presentation of SMA associated with arthrogryposis and fractures may have a separate genetic disorder, possibly even of X linked recessive inheritance. We have studied a consanguineous Caucasian family, in which two of five sibs presented at birth with SMA and arthrogryposis. Both affected sibs were male, but the parents were informative for CEA-APOC2. DNA typing at polymorphic loci D5S526 and D5S112, known to be closely linked (3 cM) to the 5q SMA locus, showed recombination with the disease locus in this family, and failed to support the possibility at this site of homoygosity by descent, since a minimum of three recombinants/14 meioses would have had to occur. It therefore seems very unlikely that the disease gene in this family could be 5q SMA, and plans for a chorion villus biopsy in the current (sixth) pregnancy were cancelled.

Prenatal diagnosis of chromosome abnormality by culture of fetal pleural effusions

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Non-immune fetal hydrops, a condition with heterogeneous aetiology, may be identified in the second or third trimester of pregnancy by routine ultrasound scan. Many affected fetuses have been found to have a chromosomal abnormality, making rapid karyotyping important in the management of the pregnancy. Often the non-immune hydrops is associated with a structural effusion, which may be aspirated as a therapeutic measure. Lymphocytes from the fluid, cultured by standard tissue culture methods, yield chromosome preparations suitable for high resolution analysis. We have cultured the pleural effusions, and obtained good quality karyotypes on five; one small sample failed to grow. Attempts to culture an ascitic and a cystic fluid have failed to produce karyotypes, each of these samples being small and containing few cells.

Prenatal diagnosis in von Hippel-Lindau disease

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Von Hippel-Lindau disease (VHL) is a dominantly inherited cancer syndrome with variable expression. Major complications include retinal, cerebellar, and spinal haemangioblastomas, renal cell carcinoma, and phaeochromocytoma (Maher et al. J Q Med 1990;77:1151), and the effects of this disorder can be devastating. Flanking DNA markers have been identified (Hosoe et al. Genomics 1990;8:634–40, Maher et al. Genomics 1991;10:957–60) and presymptomatic and prenatal diagnosis is now possible. We have performed the first prenatal diagnosis of VHL disease using linked DNA markers. A 34 year old primigravida requested prenatal diagnosis. She was found to have renal cell carcinoma aged 33 years and other relatives had died from or been disabled by VHL disease at an early age. Molecular genetic analysis showed that the current pregnancy was not informative for prenatal diagnosis at D3S18 (phase was established from an affected and an unaffected sib). The D3S18 locus shows tight linkage to VHL disease with a recombination fraction of 0.02. Risk analysis with the MLINK program showed that prenatal diagnosis would reduce the risk to the fetus to 5% or increase it to 95%. After
careful counselling the parents decided to proceed with prenatal diagnosis and a chorion villus biopsy was performed. A high risk result was obtained and the pregnancy terminated. This case represents the first prenatal diagnosis for VHL disease. Although prenatal diagnosis for VHL disease is now available, the likely demand is uncertain.

Molecular genetic analysis of spinal muscular atrophy

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The spinal muscular atrophies (SMA) are a group of inherited disorders characterised by degeneration of the anterior horn cells of the spinal cord, resulting in muscle wasting and chronic childhood-onset forms show autosomal recessive inheritance, and have been mapped to 4q12–q13 by linkage analysis. We have established a service for prenatal diagnosis of the early onset (types I and II) forms of SMA, using three linked polymorphic DNA markers (D5S6, D5S112 and D5S39). In all 13 families studied, both parents were heterozygous for at least one marker, and both were heterozygous for flanking markers in three out of 13 completed linkage studies. Prenatal diagnosis was carried out for three pregnancies at risk for SMA type I. The risks to the fetuses were calculated to be 5-6%, 5-9%, and 6%, using MLINK, and all three families elected to continue the pregnancy. One pregnancy spontaneously miscarried at 22 weeks and the others are continuing. The service is being restricted to confirmed classical early onset SMA at present, in view of possible genetic heterogeneity in later onset and atypical forms.

Prenatal hearing tests

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Diagnosis of deafness is problematic in newborn and young infants. It is realised, however, that compensatory communication behaviours are best instigated as soon as possible, preferably at or immediately after birth. A technique which enables the diagnosis of deafness before birth would thus be extremely valuable. This paper reports the successful detection of deafness before birth. Prenatal diagnosis of deafness has to overcome the problem of false positive results, in particular owing to the fetus being in a non-responsive behavioural state. Here we report that a combination of sound and light stimulation can overcome this problem and has been used to detect deafness successfully in two cases. Furthermore, use of pure sound and vibratory stimuli may enable the differentiation of conductive and sensorineural deafness. The quickness and ease of use of the technique means that it could be used as a general screening procedure in cases where deafness resulting from genetic factors or environmental insult, for example, rubella, is suspected.

Trisomy 18: anomalous fetal behaviour

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This paper discusses the movement patterns of a 34 week fetus diagnosed as trisomy 18 and compares this to movement patterns of similarly aged normal fetuses. This fetus exhibited a number of differences in its behaviour not seen in the normal fetus. The gross movements of this fetus were highly abnormal involving rotation about its longitu- dinal axis. Its single eye movements showed the complete reverse pattern of those seen in normal fetuses, the majority of eye movements being in the vertical plane. The cyclic activity of the fetus also differed from that usually seen; the characteristic burst/ pause pattern of normal fetuses was not observed. The paper concludes that this anomalous behaviour may be a useful marker for the presence of trisomy 18 and may indicate the necessity for further genetic investigations.

A comparison of three cytogenetic measures of chromosome instability in ataxia telangiectasia

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Seven families with eight members with ataxia telangiectasia were studied. In one case of Nijmegen breakage syndrome we investigated to compare three different cytogenetic methods of assessing chromosome instability. Original diagnosis was based on clinical phenotype and inheritance pattern. Cytogenetic analysis was undertaken by the following methods: (1) spontaneous rearrangements, particularly involving chromosomes 7 and 14, (2) chromosome aberrations following X irradiation in G2 phase of the cell cycle, (3) chromosome aberrations induced by the chemical mutagen adriamycin in G1/S phase of the cell cycle. All methods were found useful, with no overlap between controls and AT patients. However, the range of values suggests that each test might not always provide clear diagnosis on its own. Spontaneous rearrangements were detected at a mean level of 12% in AT patients (range 6 to 32) compared with 0-3% in the controls (range 0 to 2). X irradiated AT cases (100 Cgy) had a mean number of aberrations per cell of 1 03 (range 0-6 to 1 65) compared with control levels of 0-19 (range 0-15 to 0-45). Adriamycin treated AT patients (0-01 μg/ml) gave a mean number of 2-52 aberrations per cell (range 1:1 to 4:15) as compared with a mean of 0-35 in controls (range 0-25 to 0-65). These results suggest that sensitivity to adriamycin might be a useful additional diagnostic test for AT, especially if X irradiation is difficult to arrange. Scoring of chromosomal aberrations is a valuable and available screening test for ataxia telangiectasia, but at least two methodologies should be used for definite diagnosis.

Eight cases of 7p deletion: clinical features, cytogenetic findings, and molecular studies

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Craniosynostosis or premature closure of the cranial sutures is a common malformation occurring in approximately 1 in 1000 children. To date more than 200 cases of 7p monosomies have been described, approximately half of which have been associated with craniosynostosis. There is a considerable variation in the severity of the deleted segment. However, craniosynostosis appears to be consistently associated with either a deletion or partial deletion 7p11-7p22 or a deletion of 7p14. Analysis of a panel of eight 7p deletion cases (three with craniosynostosis) was undertaken using informative DNA probes, in order to characterise and define the extent of the deletions at the molecular level. There were six de novo deletions, one de novo unbalanced translocation, t(7p;20p), resulting in an interstitial 7p deletion, and one 7p deletion resulting from the unbalanced product of a paternal unbalanced insertion. Parental DNA was available for analysis in four out of six de novo deletions; in two the deletion was found to be of paternal origin, and in two parental origin could not be determined. The results of the DNA studies, together with a summary of the relevant clinical and cytogenetic findings, are presented. Our findings have enabled us to define a number of these deletion cases, to localise and relative position of five probes and two loci. (1) p5-11, previously mapped to 7p14-pter, does not extend proximally to p14 as previously suggested. (2) TM102L is heterozygous for this probe. (3) TM102L, previously mapped to 7p14-pter, does not extend proximally to p14 or terminally to pter, as case 7 (del 7p15-p22) is deleted for this probe and is heterozygous for MS31 (pter). (4) CRI-R944 and CRI-P137, assigned to pter-q22, have been previously shown to be tightly linked and to flank the Greig-cephalopolysyndactyly (GCP) translocation breakpoint. However, case 3 (del 7p13) is deleted for CRI-R944, but heterozygous for CRI-P137, indicating that these two probes are not cM apart and that CRI-R944 is restricted to 7p13 only, whereas CRI-P137 maps more distally. (4) TCRG, previously mapped to 7p15, maps more proximally, as case 2 (del 7p12-2-p14.2) is deleted for this probe, but case 3 (del 7p13) is heterozygous. This suggests that the TCRG gene is located at 7p14.1–p14.2. (5) As craniosynostosis is an occasional feature in GCP, it has been considered possible that the CRS2 and GCPS loci were one and the same, but the results of case 4 (del 7p13-p15) suggest that they are distinct. Cases 6 (del 7p15-p21.2) and 8 (del 7p22.1-pter) do not have craniosynostosis and therefore the
CR1 locus is most likely to be in 7p21.2 or 7p21.3. Case 8 is also heterozygous for the probes, with no evidence of complete penetrance. Onset of mild pvelvohumeropero- neal weakness occurs at 3 to 5 years and although only slowly progressive (the oldest, 56 years) is still able to walk.

Trisomy 22: in situ hybridisation and molecular studies
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We present a case of a baby girl with the following clinical features: micrognathia, cleft lip, broad nasal bridge, ocular ptosis, epicantlic folds, preauricular pit, posteriorly rotated ears, short neck, widely spaced nipples, hypoplastic nails, and joint contractures. Birth weight and length were below the 3rd centile. The baby died at 8 months of age. Cytogenetic studies using GTG banding of lymphocytes and skin fibroblasts showed a chromosome complement of 47,XX,+22. This finding was confirmed using in situ hybridisation with a chromosome 22 library (pBS-22). DNA samples from both parents and the affected baby were analysed with a minisatellite probe pMS619, which recognises a VNTR polymorphism. The baby inherited three alleles, two identical to the two maternal alleles and one identical to that of the father. We therefore surmise that non- disjunction occurred during maternal meio- sis. Trisomy 22 is the second most frequent autosomal trisomy detected in spontaneous abortions but only a handful of cases of liveborn infants have been described.

Assessing the utility of fibroblast karyotyping
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A request for a fibroblast karyotype represents a significant and expensive cytogenetic investigation. In the light of an increasing demand for this investigation over the last several years an analysis of the previous five years of skin chromosomal analysis was undertaken in an attempt to evaluate its success in the clinical setting. Each request was classified with respect to the blood and skin karyotype into one of six classifications: blood normal/abnormal/equivocal versus skin normal/abnormal. A total of 54 requests from 45 (non-moribund) subjects cases was received over this time period. Of these 10 were reported as abnormal (18.5%) when a previous blood karyotype had been normal. This compared favourably, for a second line investigation, to the mean over a similar time period of 13.7% abnormality rate for blood samples processed in the laboratory. This audit shows that following whether to proceed a skin biopsy in order to exclude a chromosomal aetiology.

Behavioural Science and Genetics Group
JOSEPHINE M GREEN AND OTHER MEMBERS OF THE BEHAVIOURAL SCIENCE AND GENETICS GROUP
UK – nationwide.

Since January a group of social scientists including psychologists, sociologists, and health economists with interests in genetics, as well as a clinician-generaton, has been meeting informally, approximately three times a year. The group exists primarily to allow its members to keep up to date with each other’s research and to exchange ideas and information. A secondary aim is to inform non-social scientists of research on social and psychological aspects of genetic screening and related issues. Aside from meetings we have organised joint sessions, written some joint papers, and conducted some research together. Some members of the Group were invited to give presentations at the recent MRC Workshop on cystic fibrosis carrier screening. Future plans are to hold a sym- posium for geneticists and behavioural scientists to consider both the structure and content of future collaborative work.

Patient satisfaction: a pilot study
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A postal questionnaire was sent to a sample of families attending the Genetic Counselling Clinic in late 1990. Forty families responded (77%); 90% were very satisfied while the remaining 10% were satisfied most of the time. Many respondents, written some joint papers, and conducted some research together. Some members of the Group were invited to give presentations at the recent MRC Workshop on cystic fibrosis carrier screening. Future plans are to hold a symposium for geneticists and behavioural scientists to consider both the structure and content of future collaborative work.

PKU in south west England: a new mutation in the leader sequence of the phenylalanine hydroxylase gene and more similarity with the French Canadians
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To date, about 40 mutations have been defined at the phenylalanine hydroxylase (PAH) locus which give rise to phenylketo- nuria (PKU). Some are unique to individual races or ethnic groups and are in linkage disequilibrium with specific haplotypes. Others are more widespread and several have been found on a variety of haplotype back- grounds. We report a CAC to CCC transition in the leader sequence of the PAH gene,
70 bp upstream from exon 1. The mutation was identified on a rare haplotype 34 in a child who has a severe form of PKU and whose parents are first cousins. Using dot blots and ASO probes, 86 mutant PKU genes were screened and the mutation was found in one other affected unrelated child who also carries a haplotype 34 on one allele. The 165T mutation originally reported in the French Canadian population on haplotype 9 also occurs in the PKU population in the south-west of England. Using SSCP we have found this mutation on other haplotype backgrounds, one of which probably has arisen from an intragenic crossover between a mutant haplotype 9 and a normal haplotype 4 chromosome. The haplotype/mutation associations seen in the south-west of England and in the French Canadians suggest a common ancestry between the two populations through invasions and migrations from northern France.

Trends in the number of births with neural tube defects
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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, has all but eliminated anencephalic births and reduced spina bifida and encephalocele births to well below 2 per 1000. 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 less 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 uncomplicated congenital hydrocephalus) are suggested, which may include an as yet unexplained secular trend, or more likely it is due to improvement in maternal nutrition and demographic changes. This has implications for the incidence of NTD elsewhere in the UK.

Use of sib pair analysis in the identification of genetic determinants of blood pressure
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We have previously described the Ladywell Family Blood Pressure study which was designed to identify genetic determinants of blood pressure. Subjects were divided into four groups depending on their own and their parents' blood pressure measurements, to allow comparison of biochemical variables and RFLPs at candidate gene loci, between groups with contrasting predisposition to developing high blood pressure. The study has now been extended to allow not only an association study but also sib pair analysis of the data. There were 25 sib pairs among the original groups sampled, and a further 42 sibs whose blood pressure measurements placed them in one of the four groups but who were not originally sampled have been traced. This now allows us to analyse 54 sib pairs and trios. They and their parents have been studied with respect to those genes which emerge as candidates from the analysis of our original biochemical data, including glucocorticoid receptor, adrenoreceptors, and insulin. No significant differences were found in the population association study except for the glucocorticoid receptor; this has been further studied by analysis of blood pressure score against genotype showing a definite effect from the larger allele of the RFLP. The sib pair analysis shows a trend towards increased allele sharing of 'like' sibs with GRL and the adrenoceptors which is significant at the 5% level for the beta-1 adrenoceptor.

The epidemiology of Huntington's disease in Northern Ireland
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As part of a genetic and epidemiological study of Huntington's disease (HD) in Northern Ireland, we have attempted a complete ascertainment of HD and have now compiled a patient database which includes data on family members and affected patients. To date, by using multiple ascertainment methods, we have identified 82 families with a history of HD. Currently, we have seen 88 affected patients. A minimum prevalence rate of 5.7/100 000 has been calculated. We estimate an accuracy of ascertainment of at least 90% which would give a total estimated frequency of 6.3. In a population of 1 500 000, therefore, we would expect five new cases per year. The prevalence figure is comparable to values estimated in the UK and Europe and this uniformity would support the hypothesis that HD has originated from more than one original mutation.
Abstracts of the Joint British Medical Genetics meeting organised by the Clinical Genetics Society and the Clinical Molecular Genetics Society held on 19 and 20 September 1991 at the University of Bristol

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