Clinical Genetics Society

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Epidemiology of motor neurone disease
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Motor neurone disease (MND) is a progressive neurological disorder in which there is a generalised degeneration of the motor neurones. The aetiology of the disease is unknown. An hypothesis that MND is caused by a slow virus or bacterial agent contracted in the pre- or postnatal period was proposed and tested using two methods. Firstly, the distribution of birth dates of 662 MND cases treated in Scottish hospitals during the years 1968 to 1979, inclusive, was compared to the distributions of annual incidences of various infectious diseases over the same range of birth years. Secondly, 27 deceased cases of MND diagnosed in Edinburgh, Scotland were analysed for clustering in time and space using birth date and birth place as time and space variables, respectively. There were no apparent correlations for the distributions compared, nor was there support for clustering. Thus, no evidence was found for the stated hypothesis. However, the infectious aetiology cannot be discounted, though it is suggested that the mechanism involved is not as simple as assumed by these two methods.

Fluorimetric electrophoretic assay for creatine kinase in dried blood samples
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The electrophoretic separation of creatine kinase isozymes and subsequent fluorimetric measurement of enzymic activity with the substrate in an agarose matrix has been found to be a suitable method for neonatal screening for Duchenne muscular dystrophy (DMD). The assay conditions and substrate concentrations were varied to determine the optimum conditions for measuring creatine kinase isozyme activities in samples of whole dried blood. The assay would seem to provide a sensitive, simple, and relatively inexpensive method for screening newborn males as a first step in preventing a proportion of cases of DMD. Clearly such screening would be especially important once an effective treatment is found.

Autosomal recessive muscular dystrophy in childhood
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Progressive muscular dystrophy, comparable in severity to the X linked Duchenne type, is rarely seen in girls with a normal karyotype. This autosomal recessive disorder has not been satisfactorily named, or described in enough detail to allow identification of isolated cases in boys. Mistakes in genetic counselling may result. To collect enough cases for analysis, we made a national request through the British Paediatric Association in 1979. Thirteen of 40 cases submitted were accepted, including two brothers of affected girls. All were examined and their notes and biopsies reviewed. The course was only a little less severe and the serum creatine kinase levels were only a little lower than in Duchenne MD. The pattern of selective muscular weakness was like that of DMD but with more weakness of the deltoids. The calves were hypertrophied in 11 cases. Muscle biopsy findings in several cases differed slightly from those typical of DMD in showing better differentiation of the histochemical fibre types, less severe interstitial fibrosis, and more conspicuous large isolated foci of necrotic fibres. These features in a boy, though not fully reliable, should alert one to the possibility of autosomal recessive inheritance. We are very grateful to the many paediatricians and pathologists who co-operated in this study.

Primary prevention of neural tube defects in mice and men by vitamin therapy
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Around 60% of the mouse mutant, curly-tail, have neural tube defects (NTD) and they are a good animal model for human NTD. In humans, a collaborative study involving periconceptional supplementation with Pregna- vorite F, a multivitamin compound, of around 400 women at risk for NTD has largely prevented the recurrence of NTD. In the curly-tail mouse, various in vivo and in vitro experiments investigating the effects of vitamin A on the developing embryonic neural tube are described, from which inferences may be drawn which might have relevance to the human prevention programme. The applicability is not that vitamin A is considered to be the curative agent in humans, but rather that the interaction of an environmental agent with the maternal and fetal genome can be dissected and studied in the mice.

Serum thyroxine binding globulin and the outcome of threatened spontaneous first trimester abortion
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Thyroxine binding globulin (TBG), the principal thyroid hormone binding protein, is increased in concentration in the presence of raised oestrogen levels. We measured
maternal serum TBG by specific radioimmunoassay as an indicator of feto-maternal-placental function in 36 normal first trimester pregnancies and 18 emergencies with threatened spontaneous first trimester abortion, matched for age and duration of pregnancy. TBG (mean±SEM) was significantly higher (39.4±1.5 mg/l) in normal pregnancy than 15 of the emergencies who aborted (22.9±1.0 mg/l, p<0.001). Three threatened abortions settled and produced normal live births. TBG levels at the time of threatened abortion were 32.0, 35.0, and 35.5 mg/l, all in the normal pregnancy range and above the highest value (28.0 mg/l) of the 15 emergencies who aborted. Thyroid function was normal. One patient had a stillborn anencephalic fetus at 32 weeks and the lowest TBG concentration (18.0 mg/l) in the first trimester. Maternal serum TBG assay may be helpful in indicating the outcome of threatened spontaneous first trimester abortion.

An immunoassay for carrier detection in cystic fibrosis
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An immunoassay for a cystic fibrosis (CF) related antigen in serum samples has been developed to a point where it makes quantitative distinction between patients, heterozygotes, and normals. A related immunoradiometric assay allows antiserum to be used at 1:5000 dilution. In a panel of 14 CF patients, 29 heterozygotes, and 23 controls correct assignments of genotype were made in 95% of samples by either assay. However, unresolved problems remain in the correct handling of serum samples before assay.

Fetoscopy and fetal blood sampling in the management of a twin pregnancy with amniotic fluid cell mosaicism and a suspected fluid sampling error
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Amniocentesis was performed on a 37-year-old woman because of maternal age. Ultrasonography demonstrated a twin pregnancy with a clearly defined septum and an attempt was made to sample fluid from both sacs simultaneously. Amniotic fluid cell cultures indicated that both fetuses were 45,XO/46,XX mosaics. In order to verify the mosaicism, and to exclude a sampling error, fetoscopy and fetal blood sampling were performed. The fetal lymphocyte karyotype of one twin was 45,XO/46,XX, but of the other was 46,XY. The parents elected to continue the pregnancy, which resulted in the birth of a normal healthy boy and a healthy, phenotypically normal girl, in whom mosaicism was confirmed. Fetal blood sampling may help in the management of mosaicism, or where a sampling error in twins is suspected, and thus may prevent termination of a normal fetus.

Antenatal detection of a 45,X/46,XY, 6q+ chimaera
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An amniotic fluid sample received at 16+ weeks’ gestation for chromosome analysis for maternal age (38 years) revealed two abnormal cell lines: 60% 45,X and 40% 46,XY,6q+ which were confirmed with G banding. The extra material attached to the long arm of chromosome 6 was of unknown origin. The fetus was terminated at 20 weeks and found on examination to be phenotypically a normal female. Pathological examination revealed normal ovaries and female genitalia. No internal abnormalities were observed. Examination of the placenta, which appeared pale, showed signs of degenerative change with some blood clot over the villi. Chromosome analysis of skin and muscle confirmed both cell lines in the former and the 46,XY,6q+ cell line in the latter. The findings suggest that the two separate chromosome abnormalities in this fetus may be the result of zygotic chimaerism. The sex chromosome abnormality had given rise to a phenotypically female appearance at 20 weeks’ gestation.

Down syndrome in Northern Ireland
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Two hundred and sixty-two cases of Down syndrome (DS) have been ascertained in Northern Ireland during 1974 to 1979, giving an overall incidence of 1.66 per 1000 livebirths. Chromosome studies were available on 182 (70%) of children, although in the last 2 years of the study the figure rose to 80%. Of the children with chromosome studies, 166 (91%) had trisomy 21, eight (4%) had an unbalanced translocation, and eight (4%) had mosaicism. There was an excess of male children (152 : 109). Maternal age was examined at yearly intervals; at age 35 the incidence was 2.94 per 1000 and at age 40 it was 10.46 per 1000. For mothers 19 years or less the incidence was 1.05%. The origin of the additional chromosome was studied in 42 trisomy 21 Down syndrome patients born in 1980 to 1981. In 28, the additional chromosome was maternal, in five it was paternal, and in the remaining nine families the parental origin could not be determined.

Fetal alcohol syndrome. Experience with 40 children from the west of Scotland
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Clinical experience of fetal alcohol syndrome (FAS) reported from Britain has been negligible and it has been suggested that the problem is insignificant in this country. In an attempt to assess the prevalence of the disorder in the west of Scotland, an area of established endemic
alcoholism, consultant paediatricians at the Royal Hospital for Sick Children, Glasgow and in the surrounding district paediatric units were canvassed and asked to provide details of children under their care. Forty children, including one sib pair, with clinical features consistent with FAS were ascertained and their case records reviewed. In addition, maternal, psychiatric, medical, and demographic data were collected. Results suggested that maternal alcohol abuse during pregnancy is a significant cause of childhood (and maternal) morbidity and mortality in the west of Scotland, reflecting the known problem of alcoholism in the area.

A genetic counselling problem: misleading antenatal diagnostic tests
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The consultands were referred for counselling following the therapeutic termination of two consecutive pregnancies where the fetus, although believed to have a high risk of neural tube defect, appeared to be normal. In the first pregnancy routine maternal serum AFP at 16 weeks was 3.4 × median. Amniotic fluid AFP was 4.7 × median. There was no significant blood staining. Retrospective AChE showed a well defined second band. In the second pregnancy maternal serum AFP was 2.3 × median. Amniotic fluid was 7.1 × median, and AChE again showed a clear second band. Ultrasound examination failed to show NTD. Fetal chromosome complement was 46XX,NAD. The matter was discussed by the Society. Although no convincing explanation for these abnormal antenatal diagnostic findings was given it was thought, without dissent, that AFP and AChE assays should be ignored in future pregnancies and Mr and Mrs X given strong reassurance as to outcome of pregnancy.

A Bayesian approach to prenatal diagnosis of neural tube defect
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Results of analysing 3658 fluids for the prenatal diagnosis of neural tube defect (NTD) were reported. The use of amniotic AFP alone led to 2 to 3% of uncertain results owing to borderline AFP levels. When the prior risk was taken into account, and combined in a Bayesian manner with a conditional risk based on the AFP level, the uncertainties were much reduced. The introduction of a second set of conditional probabilities derived from the qualitative acetylcholinesterase (AChE) test further improved the certainty of prediction. For these purposes it was assumed that serum AFP, amniotic AFP, and AChE are independent and uncorrelated. When both AFP and AChE are used, the laboratory results are usually ambiguous and do not demand a statistical treatment, but it remains a useful and logical method of dealing with uncertain cases.

SYMPOSIUM ON DNA TECHNOLOGY IN CLINICAL PRACTICE

Human chromosome specific libraries
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The availability of cloned genes has rapidly expanded our knowledge of the fine structure, organisation, and expression of sequences in the human genome and provided insight into the molecular basis of diseases such as the thalassaemia syndromes. In addition, the application of recombinant DNA technology to human genetics has played an important role in attempting to bridge the gap in resolving power between DNA sequence analysis and classical cytological approaches to mapping and gene linkage. Recent studies have shown that highly polymorphic loci can be identified from human genomic libraries. Using such probes the construction of a fine structure linkage map would be particularly useful in cases where the chromosomal location of the locus associated with a genetic disease is known. In this context a general method for rapidly isolating a wide spectrum of DNA probes for specific chromosomes would be an invaluable aid to human gene mapping.

We have devised a technique for the high resolution separation of human chromosomes and the cloning of a DNA library from relatively small amounts of sorted chromosomes. Human chromosomes 21 and 22 were isolated from a suspension of metaphase chromosomes using a fluorescence activated cell sorter (FACS II). Two recombinant DNA libraries, representing chromosomes 21 and 22, were constructed into the vector gt WES B. Twenty clones selected at random from the chromosome 22 library hybridised to EcoRI digested human DNA, and five of these clones hybridised to single bands identical in size to the phage inserts. These five single copy sequences and a clone coding for an 85 RNA isolated by screening the chromosome 22 library for expressed sequences were characterised in detail. Hybridisation of all six clones to a panel of sorted chromosomes and hybrid cell lines confirmed the assignment of the sequences to chromosome 22. The sequences were localised to regions of chromosome 22 by hybridisation to translocated chromosomes sorted from a cell line having a balanced translocation t(17;22)(p13;q11) and to hybrid cell lines containing the various portions of another translocation t(X;22)(q13;q11-2). Five clones reside on the long arm of chromosome 22 between q11.2 and qter, while one clone and an 18S rRNA gene isolated from the chromosome 22 library reside between pter and q11-2. The construction of chromosome specific libraries by this method has the advantage of being direct and applicable to nearly all human chromosomes and will be important in molecular analysis of human genetic diseases.
The use of X chromosome specific DNA probes for the study of sex linked diseases

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Chromosome specific DNA fragments cloned in bacterial vectors can be isolated which reveal variations in DNA sequence between people (DNA sequence variants). These DNA sequences can be used as genetic markers in standard linkage analysis for segregation with a given monogenic disease and thus permit the localisation of a phenotype to a particular region of a chromosome. Collections of DNA sequences ('libraries') derived from a single purified human chromosome can now be prepared by purification of the human chromosomes by flow cytometry (B D Young, this meeting). We have cloned a library of 50 000 individual recombinants from the human X chromosome which is sufficient to cover most of the DNA sequences of the chromosome. Single copy sequences isolated from the library have been characterised and localised along the X chromosome by hybridisation to human rodent hybrid cell line DNA containing human X chromosome translocations. Some of the sequences have been localised to bands on the chromosome by direct hybridisation in situ to metaphase spreads (D Hartley and G Casey, unpublished).

Several chromosome specific clones have been used to investigate the presence of DNA sequence variations in the genome. DNA prepared from leucocytes with a selection of restriction enzymes. The Mendelian inheritance of the DNA variants has been demonstrated (M Hill et al, Hum Genet, 1982). One of the clones is localised at Xp21→Xp22.3 (H Ropers, unpublished) and shows a TaqI polymorphism in 27% of the population (J Murray, unpublished). We are studying the segregation of this marker in Duchenne muscular dystrophy families. Linkage between DMD and a short arm polymorphism would provide an unambiguous localisation of the Duchenne locus and a closer linkage could then be obtained by taking clones from the same region of the X chromosome. As DNA sequence variants occur approximately once in every 100 to 200 bp in the human genome (Jeffreys, 1977), it should be possible to isolate enough closely linked markers to map the whole length of the human X chromosome.

Once a phenotype has been localised to a region of the chromosome by linkage analysis, chromosome specific libraries can be used to screen cDNA libraries derived from a tissue in which the defect is expressed to investigate the transcription of genes localised in the affected region of the chromosome. cDNA clones also provide single copy markers for further linkage studies and walking along the chromosome.

In situ hybridisation, immunoglobulin genes, and leukaemia

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The genes for immunoglobulin κ light chains have been localised to chromosome 2 in normal lymphocyte and fibroblast chromosomes by in situ hybridisation with (125I)-labelled DNA probes containing 16 to 18 kb of human genomic DNA, each including a single Vκ coding sequence, or with (3H)-CRNA probes made by transcription of the cloned DNA segments. The localisation was achieved by counting grains (after radioautography) over chromosomes in a number of karyotypes. Chromosome 2 was found to hybridise the V-gene probes on a region of the short arm close to the centromere (2cen→p12). The results were confirmed by in situ hybridisation to phytohaemagglutinin stimulated lymphocytes from a balanced translocation carrier 46,XX,t(2;16)(q13;q22) in which the genes were localised to the same region in both chromosomes 2 and 2;16. The genes for the Kidd blood group (Jκ) are closely linked to immunoglobulin κ-chain constant genes and, assuming close linkage of V and C regions, Jκ may also be assigned to this region.

In Burkitt's lymphomas, translocations between chromosome 8 and chromosomes 14, 2, and 22 are observed. These chromosomes all carry immunoglobulin genes, heavy chain (14), κ-chain (2), and λ-chain (22). The κ genes are localised in the region of the breakpoint observed in Burkitt's lymphoma associated specific translocations.

Gene transfer into the mouse germ line

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A cloned rabbit adult β-globin gene has been introduced into the mouse germ line by microinjection into the pronuclei of fertilised mouse eggs. Southern blot analyses demonstrated that nine out of 24 mice (38%) that developed from injected eggs contained the injected gene in liver DNA in multiple copies per cell. All the copies in each cell (between about five and 50) appear to be contained in a head-to-tail tandem array, and the majority of copies are intact and not detectably rearranged. The site of chromosomal integration has been identified by in situ hybridisation to metaphase chromosome spreads in four of the nine mice. In each of these four mice the rabbit globin DNA has integrated into a different chromosome (the integration sites are in chromosomes 1, 3, 12, and 14), indicating that integration does not occur by a mechanism requiring extensive sequence homology. Eight of the nine mice have been shown to transmit the exogenous DNA through the germ line to their progeny, and the frequencies of transmission in most cases approximate 50%. Transmission to third and fourth generation mice has also been demonstrated, and the exogenous DNA appears to be stable in structure and copy number. This suggests that permanent strains of mice carrying foreign genes in different chromosomal locations can be estimated using this technique. Analyses of globin protein chains and RNA transcripts in erythroid cells of these mice have failed to detect any rabbit β-globin mRNA or protein, indicating that the foreign gene is not being correctly regulated. However, transcripts from the rabbit β-globin gene were detected in an 'inappropriate' tissue, skeletal muscle, in one of the lines of mice. These transcripts have a correct 3′
terminus and are polyadenylated, but the 5' terminus has not yet been mapped. They are present in a few copies per cell in muscle cells, but not in any other tissue tested, and not in any of the other lines of mice. Transcription of an exogenous gene in a given cell type might result from integration into a chromosomal locus that is transcriptionally active in that cell type.

**Shotgun genetics**

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It is now possible to identify, isolate, breed, and characterise isolated segments of DNA. This allows genic variants to be characterised directly or, in unique mutants, to be defined by neighbouring variants. These variants may also be used as in 'markers' in pedigree studies, and may have their chromosomal position directly defined, opening up the prospect of integrated mapping studies as used in *Drosophila*.

Little consideration appears to have been given to the numerical aspects of these studies. The ordering of cloning linked loci requires recombinants, and since a centimorgan represents about a million base-pairs, hundreds or thousands of families will be needed to locate a probe within walking distance by pedigree methods. This can be reduced by related population studies. The optimal allocation of resources between clinical surveys, population studies, cell banking, restriction enzyme analysis, and the integration of results will be considered.

The large number of possible markers (there are over 10 million restriction sites, of which many thousands are likely to be highly polymorphic) and the fact that the information in any pedigree is proportional to almost the square of the number of markers, makes it essential to standardise the target families by banking and making them available to other laboratories. It is also uneconomical not to define such families by the established genetic markers. This should lead to a several-fold increase in information per unit cost whether in blood or gold.

The analysis of such multipoint data is theoretically intractable, but efficient intuitive methods have allowed the mapping of all species except man. It is not necessary that more than a minority of families studied should have the disorder being mapped. Large families have no particular virtues, and where very close linkage is being studied, are relatively inefficient, since no sibship, however large, is compounded from more than two parents.

**The transition from the models (haemoglobinopathies) to other inherited diseases**

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The various haemoglobinopathies, and in particular sickle cell disease and the thalassaemias, illustrate well the application of molecular biology to an inherited disease. The DNA sequence defect is always in the globin gene locus and may be a deletion, a base change, or a rearrangement. In each case, a characteristic molecular pathology ensues: no gene is present, transcription does not occur, processing either is faulty or blocked, or a defective protein (too short, too long, or with a change in sequence) results. The level of the defect can be assessed accurately using a gene specific probe; if the cloned gene probe recognises the sequence that has mutated, such a gene specific probe is also disease specific. Probes such as those for the globin gene are powerful tools in studying the inheritance of gene defects within a family, directly or by linkage, and it is now becoming possible to use this approach for diseases where the biochemical change is not understood.