RECENT ADVANCES

Advances in genetics

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The contribution of genetics to the understanding of paediatric disease has increased dramatically over recent years. The identification of disease genes and the understanding of disease processes at the molecular level have implications for screening, diagnosis, and therapeutic manipulation. Inevitably, complex ethical issues have arisen from such a rapid rate of scientific progress, and many ask whether society is prepared for the consequences of such advances.

This review aims to cover areas of progress within the field of clinical genetics over recent years, and also to discuss future prospects, possibilities, and perceived difficulties.

Human Genome Mapping Project

The Human Genome Mapping Project (HGMP), an international project whose ultimate aim is to sequence the entire human genome, will lead to understanding about chromosomal structure, the organisation of the genome, regulation of genes, disease susceptibility, and normal and abnormal human development. The projected completion date is between 2001 and 2005. The human haploid genome consists of around three billion base pairs of DNA, encoding approximately 50 000 to 100 000 genes. One major goal of the HGMP is to produce a map of the human genome, containing 30 000 ordered markers, spaced approximately 100 000 base pairs apart.¹ Expressed sequence tags, which are complementary to the 3' untranslated region of mRNA, are also being developed as markers for individual genes.² These resources will aid positional cloning, where a gene is cloned using information as to its location on the chromosome, as well as being a foundation for the sequencing of the entire genome.³ Other projects involve the sequencing of model organisms such as bacteria, yeast, nematodes, and mice. DNA sequences that share a structural similarity between different species suggest a common ancestral origin. The comparison of human genes with the genes of simpler model organisms gives insight into genome structure, and provides valuable homology data. Having identified a mutant gene in a 'simple' organism such as the fruit fly *Drosophila*, a short cut to finding human genes exists by searching for sequence similarities through computer databases such as FlyBase.⁴ Surprising insights into mammalian develop-

ment and human disease have been gained in this way. An example of this is the homology of DNA repair mechanisms between yeast and humans.⁵⁻⁷

Mutation detection

Once a gene has been isolated, the mutation in the gene resulting in the clinical phenotype in an individual or a family can be sought. Mutation detection remains an expensive and time consuming activity in the majority of cases, but it is one that can confirm a clinical diagnosis, enable prenatal and presymptomatic diagnosis of an individual in an affected family, and provide carrier testing. In some cases a specific test can be used to detect a known common mutation, such as in Apert's syndrome, achondroplasia, and cystic fibrosis. In cystic fibrosis four known mutations account for 87% of mutations in the northern European population. Detection of these mutations is efficient and inexpensive to perform, and can be carried out on a routine service basis. Other disorders amenable to routine testing include those due to a common mutational mechanism, such as deletions in Duchenne muscular dystrophy, and duplications in Charcot-Marie-Tooth disease. However, in many diseases the causative mutations are unique to each family, and mutation detection techniques have to be used for each new case. If the gene involved is large, and mutations occur anywhere on that gene, then localising the mutation may be time consuming. The gene for Marfan's syndrome, fibrillin-1 (FBN1), is located on chromosome 15q21 and spans approximately 110 kb. Mutations in FBN1 were first detected in patients in 1991, and over 50 distinct mutations have since been reported,^{8,9} each generally unique to individual families. Identification of an FBN1 mutation in a new case is therefore difficult, and this technique cannot be used as a diagnostic test. A further example where individual mutation detection in a known gene has proved difficult is provided by the neurofibromin gene in neurofibromatosis type 1 (NF1), which spans over 350 kb of genomic DNA in chromosomal region 17q11.2. Over 100 disease causing mutations have been identified so far,¹⁰ but collectively these are only responsible for approximately 40% of patients with NF1.¹¹

Conditions may show genetic heterogeneity, where mutations at a number of different loci

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result in the same phenotype. Two loci for adult onset polycystic kidney disease have been mapped: polycystic kidney disease 1 gene (PKD1) on chromosome 16 accounting for around 85% of cases, and PKD2 on chromosome 4,¹² accounting for the majority of the remaining cases. Two genetic loci have also been confirmed for tuberous sclerosis, with approximately 50% of familial cases being linked to a locus on chromosome 9q34 (TSC1), and other cases linked to a locus on 16p13.3 (TSC2), closely linked to the PKD1 gene.^{13,14} The PKD1 and TSC1 genes have been isolated but both are very large. Therefore, in isolated cases with either of these disorders, one cannot be certain that one is searching the correct gene for the responsible mutation.

Unusual inheritance patterns

A number of diseases have been found to result from an unstable expansion of a triplet of bases within or around a gene, so-called trinucleotide repeats. The normal number of repeats is polymorphic (meaning the existence of two or more alleles with different repeat lengths at significant frequency in the population), but a repeat number above a certain threshold results in the clinical phenotype. Fragile X syndrome, Huntington's chorea, and myotonic dystrophy all result from such an expansion.^{15,16} Friedreich's ataxia, an autosomal recessive condition, has also recently been found to be caused by a trinucleotide repeat expansion.¹⁷ In this disease, a GAA repeat number of between 200 and 900 within the frataxin gene on chromosome 9 is seen in 95% of affected individuals, while the normal copy number is between 10 and 21. These diseases are all amenable to direct molecular diagnostic testing by assessment of the expansion size.

A number of conditions result from uniparental disomy of an imprinted gene. An imprinted gene is one which is only expressed from *either* the maternal *or* the paternal copy, that is the gene expression is monoallelic. Uniparental disomy occurs where both alleles originate from the same parent. Prader-Willi syndrome results from maternal disomy of chromosome 15q11.13 in 20–25% of cases, and deletion of the same region of the paternal chromosome 15 in the other cases. Paternal disomy of this region results in Angelman syndrome in a small number of cases (<5%), but more commonly Angelman syndrome arises from the deletion of part of the maternal 15q11 band (60–75% of cases). At least 20% of affected individuals have normal chromosomes and no evidence of disomy. These cases may be due to mutations in the imprinting mechanism^{18–20} or possibly arise due to point mutations in the Angelman gene itself. Differential methylation of genes within the critical region is used as the basis of molecular testing of both disorders.²¹ A number of cases of Beckwith-Wiedemann syndrome have been described in association with uniparental disomy of the paternal copy of distal 11p, possibly related to overexpression of intrinsic growth factor 2 (IGF2).^{22,23} Maternal uniparental disomy of

chromosome 7 has been reported to be associated with short stature,²⁴ and has also been identified in a number of patients with Russell-Silver syndrome.²⁵

Mutations in mitochondrial DNA (mtDNA) can be pathogenic in man.^{26,27} One example of a disease caused by mtDNA mutations is Leber's hereditary optic neuropathy (LHON), which is transmitted maternally. Difficulties arise, however, in the genetic counselling of this disorder due to the incomplete penetrance, variable age of onset, and preferential occurrence in males. A number of LHON pedigrees are heteroplasmic for the pathogenic mtDNA mutation, where the mutant allele coexists with the normal allele in an individual.²⁸ The mutant allele frequency varies between individuals within a family, giving rise to further counselling difficulties. It has been suggested that >70% mutant allele frequency is required for a significant risk of visual loss.²⁹ Autosomal DNA mutations can give rise to mtDNA rearrangements, such as in autosomal dominant progressive external ophthalmoplegia, a disorder with ptosis, weakness of the eye muscles, and generalised muscle weakness. Affected individuals have multiple mtDNA mutations, with a pattern of inheritance indicating a nuclear gene defect.³⁰

Fluorescent in situ hybridisation (FISH)

FISH is a cytogenetic technique that detects specific DNA sequences, chromosomal subregions or entire chromosomes during metaphase or interphase of cells, by the use of fluorescently labelled complementary DNA sequences.³¹ The technique has been enhanced by the introduction of multicolour probes—allowing simultaneous examination of a number of sites, and chromosome painting—where consecutive probes spanning an entire chromosome are used. The technique is being explored for the rapid prenatal diagnosis of aneuploidies, by analysis during interphase, giving a result within 48 hours. Some clinical phenotypes result from the microdeletion of a chromosome, resulting in the loss of a number of genes that are located consecutively on the chromosome. The phenotype in these 'contiguous gene syndromes' varies depending on the extent of the deletion, and therefore which genes are missing. FISH techniques are commonly used to determine the presence of microdeletions, which are not visible by conventional cytogenetic means. Examples include deletion of the elastin gene on 7q in Williams syndrome,³² 22q deletions in velocardiofacial / DiGeorge's syndrome,³³ 4p deletions in Wolf-Hirschhorn syndrome, 17p11.2 deletions in Smith-Magenis syndrome, and 17p13.3 deletions in Miller-Dieker lissencephaly. Many probes used in the clinical setting are available commercially. 'FISHing' for microdeletions requires a specific request, appropriate to the clinical findings. As the HGMP progresses and chromosomal regions are delineated by a greater number of probes, and advances are made in the computerisation techniques enabling improved image analysis,

the applications of FISH techniques are likely to expand.

Prenatal diagnosis

SCREENING

Routinely available prenatal screening in low risk pregnancies includes serum screening and ultrasonography for fetal anomalies. Second trimester serum screening for the detection of Down's syndrome measures the serum levels of α -fetoprotein, human chorionic gonadotrophin, and unconjugated oestriol. These parameters are analysed in conjunction with gestational age to predict high risk pregnancies where diagnostic amniocentesis can be offered. A 75% detection rate of Down's syndrome was obtained in a study where over 40 000 pregnancies were tested, with a false positive rate of 4%.³⁴ The detection rate in women over 35 was even higher. Studies have indicated that there is a first trimester increase in level of free β human chorionic gonadotrophin in trisomic pregnancies.³⁵ However, first trimester serum screening remains at a research stage.³⁶ Fetal nuchal translucency (a measurement of the amount of fluid present at the back of the fetal neck, made by ultrasound scanning at 10–12 weeks' gestation) of equal to or more than 3 mm has been reported to be a first trimester marker for chromosome abnormality. In one specialist centre 84% of trisomic pregnancies were detected in this way, with a false positive rate of 4.5%.³⁷ However, for this to be used as a screening test, standardised results need to be achieved by general hospitals providing antenatal care.³⁸

The Royal College of Obstetricians and Gynaecologists currently recommends routine scanning for fetal abnormality at 18–20 weeks, although its value measured in terms of reduced perinatal morbidity and mortality is controversial.^{39,40} The sensitivity of ultrasound in the detection of abnormalities before 24 weeks varies. One study reported a 40.9% sensitivity,⁴¹ while another reported a 16.6% sensitivity.⁴²

CHORIONIC VILLUS SAMPLING (CVS) AND AMNIOCENTESIS

Tissue suitable for prenatal diagnosis, can be obtained in a number of ways. CVS performed at 10–12 weeks' gestation, and amniocentesis, performed from 14 weeks' gestation, are both widely used. Fetal karyotyping, molecular genetic and biochemical tests can be performed on the sample obtained. However the methods described are invasive, and still carry an associated miscarriage rate of around 2% and 1% respectively.⁴³ There have also been reports of limb reduction defects associated with early CVS (<9 weeks),^{44,45} although the significance of this finding has been disputed.^{46,47}

Less invasive techniques are being developed. Trophoblast cells have been successfully cultured from endocervical washing during the first trimester,⁴⁸ though further testing is required to determine the risks. Isolating fetal cells from the maternal circulation, present as early as 5–6 weeks' gestation⁴⁹ could also

potentially provide an early, non-invasive means of prenatal diagnosis.^{50,51} At present, technical problems exist with the isolation and enrichment of such cells.⁵² However there has been some success, particularly with the isolation of fetal nucleated red cells. Once detected, the application of polymerase chain reaction (PCR) or FISH techniques has so far allowed the determination of fetal sex (useful in X linked disorders), aneuploides, and certain specific mendelian disorders.^{50,53,54} At present confirmation of the diagnosis by CVS or amniocentesis is required, but clinical trials are underway to determine sensitivity and specificity of these relatively non-invasive methods.

PREIMPLANTATION DIAGNOSIS

This method of diagnosis involves the removal of one or two cells from the six to 10 cell embryo generated by standard in vitro fertilisation techniques, at around day 3 after insemination, followed by analysis of the cells by PCR or FISH techniques. It was developed in order to provide the option of healthy embryo selection, rather than later termination of abnormal fetuses.⁵⁵ The technique is established for diagnosis of cystic fibrosis using PCR to determine the presence of known parental mutations.⁵⁶ Other conditions which have also been detected at the preimplantation stage include Tay-Sachs disease, Lesch-Nyhan syndrome, and Duchenne muscular dystrophy. Diagnosis of some of the triplet repeat expansion diseases has been reported, including fragile X and myotonic dystrophy.⁵⁷ Although so far there is no evidence for either a reduction in the success of the implantation of the embryo, or interference with the development of the embryo, the numbers studied are small.⁵⁶

Gene therapy

Although much optimism surrounds this potentially exciting area, technical and ethical problems have been encountered when attempting to manipulate genes. The first genetic disorder reported to be treated by gene therapy was severe combined immunodeficiency due to adenosine deaminase deficiency (ADA deficiency). Retroviral vectors have successfully been used to transfer the human ADA minigene, *ex vivo*, into bone marrow cells and peripheral blood lymphocytes.^{58,59} Significant effort has been directed towards cystic fibrosis, where the defect lies in the chloride conduction channel.⁶⁰ Adenovirus vectors were considered suitable vectors to deliver the normal gene to the lungs, but clinical trials have been complicated by a high incidence of mucosal inflammation when high viral doses were used, with no associated evidence for an improvement in the chloride channel defect.⁶¹

The gene for Duchenne muscular dystrophy was cloned in 1987, but slow progress is being made in gene therapy. As the disease gene is large, it cannot be accommodated by any known viral vector, and attempts have been made at transplanting myoblasts. The myoblasts from affected individuals have a low proliferative capacity, and therefore allogenic myoblasts have been used in conjunction with

immunosuppression. However the uptake by the host has not only been poor, but the cells have not migrated from their site of injection.⁶² One way to get around this problem has been explored by injecting a recombinant adenovirus containing a human dystrophin minigene, minidystrophin, into a mouse model. Although this minidystrophin encodes a truncated protein, its expression has been shown to protect the muscle fibres against degeneration.⁶³

Alternative approaches of gene therapy are currently being developed. A retroviral vector, expressing the fumarylacetoacetate hydrolase gene (Fah), infused into the portal vein of a mouse model of the human liver disease tyrosinaemia type 1 (HT1), has resulted in Fah expression in >90% of parenchymal hepatocytes and functional correction of the diseased liver. The healthy hepatocytes have a high regenerative potential, and are selected in vivo over the mutant cells.⁶⁴ This work has promising implications for hepatic gene therapy.

Databases

Knowledge about genetic disease, including new syndrome diagnoses, chromosomal localisation of disease causing genes, mutation analysis, and genotype-phenotype correlations increases daily. The most efficient way to obtain access to such information is via regularly updated computer databases now available through the World Wide Web (WWW).⁶⁵ Examples of databases of use to the paediatrician include the On-line Mendelian Inheritance in Man (OMIM) database and the Genome Data Base (GDB), which are international on-line databases of human genetic data. These allow the users to search for phenotypic descriptions, inheritance patterns, gene localisation information, and specific mutations. Links to genetic databases are available through the UK Medical Research Council HGMP Resource Centre WWW 'home page' (<http://www.hgmp.mrc.ac.uk/>).

Future advances

With the help of the HGMP, determining all of the genes resulting in single gene disorders will be a relatively straightforward process. The next challenge is to elucidate the genetics of common multifactorial disorders, such as heart disease, cancer and schizophrenia, where multiple genes interact with environmental factors to produce the disease phenotype. Significant progress is being made in unravelling the genetic predisposition to insulin dependant diabetes mellitus.⁶⁶ Using genome-wide search methods on affected sibling pairs, a susceptibility locus for Crohn's disease has recently been mapped.⁶⁷

The genetic basis of behaviour is currently stirring much interest. One study suggested linkage between monoamine oxidase deficiency and aggressive behaviour.^{68 69} The study of the genetics of homosexuality has aroused debate. Evidence exists to suggest that male homosexuality is influenced by a gene or genes on Xq,^{70 71} although whether genetic factors

influence female sexual orientation remains unclear. Behavioural genetics is a controversial area that is only beginning to be studied at the molecular level. It is not easy to predict changes in social attitudes to specific behaviour patterns, such as criminality, if these are shown to have a significant genetic aetiology.

Such knowledge raises many ethical and social issues. To what extent should couples be allowed to choose the genetic make-up of their babies? Is presymptomatic testing and carrier detection for adult onset disease appropriate in children?⁷² Will the ability to screen for 'abnormal' or 'socially undesirable' genes lead to eugenic policies on the part of governments? Should insurance companies have the right to insist on genetic tests, or to load the policies of people known to carry specific deleterious genes? How will the increasing knowledge of behavioural genetics affect our concept of free will?

All these issues must be the subject of informed public debate if the obvious benefit of the HGMP are not to be overshadowed by inappropriate use of this powerful technology.

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