Clinical genetics
A review

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This review of clinical genetics is aimed at clinicians whose medical school education contained little reference to genetic disease and for whom the subject is an amalgam of Mendel’s pea plants, fascinating medical rarities and the awesome complexity of gene probes. The changing pattern of medical practice makes it essential that this perception is changed.

The decline in infectious disease and malnutrition together with the increasing longevity of the population has transformed the pattern of disease in Western countries. A little over a hundred years ago, one in three British children died by the age of twelve and it was usual for couples to plan a large family. We are now faced with the general expectation that every couple should be able to produce two healthy children who will survive to old age. Genetic disorder poses a major obstacle to this aspiration. If single gene diseases, chromosome disorders and structural malformations are grouped together, they account for more than half of all miscarriages, a quarter of perinatal deaths and three-quarters of severe handicap. Even among those who reach adult life, one in 80 will suffer the consequences of a late onset genetic disorder such as polycystic kidney disease or Huntington’s chorea, to say nothing of the genetic contribution to diabetes, coronary artery disease and cancer.

Another important factor in the potential workload involved in genetic counselling for a whole population is what might be called ‘the ripple effect’. A single individual with a severe handicap or degenerative genetic disease can have an enormous impact on even quite distant relatives. For example, while the number of people with Huntington’s chorea in any one region will be numbered in hundreds, the relatives at greater than one in ten risk of developing the disease in that region will number in thousands. Very often, a simple lack of understanding of the pattern of inheritance of a particular disease could lead to a permanent blight on the life of an unaffected relative; a typical example would be the niece of a boy with Duchenne muscular dystrophy who fears that she may be a carrier despite the fact that she is the daughter of the affected boy’s normal brother and therefore cannot share her uncle’s defective X-linked gene.

With fewer than 30 consultant clinical geneticists in Britain to date and a medium-term target of one consultant per million population, it is apparent that even with support from specialist genetic nurses and junior staff all families who could benefit from genetic advice cannot and should not receive this from a consultant geneticist. The potential demand is too great. The ability to offer genetic counselling must be regarded as a basic skill for all clinicians.

Despite the enormous variety of genetic disease and the very large numbers of people who might seek advice it is possible to distil the usual anxieties into four questions:

1. Why did it happen?
2. Will it happen again?
3. Will it be as bad?
4. Are there any tests?

‘Tests’ either may refer to the ‘consultand’ in terms of carrier tests or may refer to prenatal diagnosis with a view to selective termination. The term ‘consultand’ is rather clumsy but emphasises that the great majority of people seeking genetic advice are not patients in that they are not sick in the conventional meaning of the word, though the psychological consequences of genetic disease in the family may have a not inconsiderable impact on their well-being. Genetic questions will often be intermingled with direct concern for the well-being of the affected individual. A clinical geneticist may well be consulted about the prognosis for a particular rare condition and the possibility of therapy now or in the future. Such considerations of prognosis and therapy will very often be the primary aspect of a consultation with other clinicians. The central issue to all these questions is the need to make an accurate diagnosis. No amount of sensitivity in counselling can repair the damage when the advice was wrong.

Equally important is the need to calculate the risk for the particular individual; this may be straightforward in the case of parents whose first child had a recessive gene defect or may be very complex as in the case of carrier tests for X-linked diseases and when there is need to make allowance for age of intervening relatives in the late onset dominant disorders.

Having achieved a diagnosis and established the risk for the individual, the third component of counselling is explanation in terms which the family can understand and, if necessary, pass on to other relatives.

The final component of counselling is the most difficult to define; it is inevitable that the translation of genetic risks must involve interpretation by the clinician of the magnitude of the risks involved and the merits of the various options open to the family, but it is crucial that such interpretation takes account of the beliefs and values of the family concerned. A popular phrase in genetic
circles is ‘non-directive counselling’. It is not uncommon for families to be ‘sent’ for counselling because their doctor feels they should be told not to have children. Such interference in the rights of the individual is generally regarded as unacceptable but, equally, it is very difficult to avoid colouring any advice given, particularly when families seek guidance through a complex range of options. It is essential that clinicians involved in genetic counselling remain sensitive to the feelings of the family and the rights of the individual.

The four elements of genetic counselling may, thus, be summarised under four headings:

Comprehension
Calculation
Communication
Compassion

Comprehension: the need for accurate diagnosis

Any requests for genetic counselling should begin with a history and examination. One of the commonest reasons for a request for genetic advice is the birth of a child with a serious physical or mental defect. Depending on the definition of birth defect, approximately one in 40 of all children born has a serious abnormality. Very often the precise causes of such abnormalities are not apparent and the counsellor must rely on empiric recurrence risks derived from the study of families with similar abnormalities. For example, a couple whose child is born with a neural tube defect faces a recurrence risk of approximately 2 per cent, though this figure will vary with the background incidence of the disorder. Before resorting to such empiric figures it is important to identify the subgroups where a more precise explanation can be offered.

Pregnancy history is of great importance; parents will often attribute an abnormality to some trivial event during the pregnancy and on some occasions an important teratogenic exposure will be discovered. A history of drug ingestion or maternal illness such as diabetes would represent obvious factors for consideration as causes of fetal abnormality. Maternal alcohol ingestion during pregnancy may not be volunteered and is often overlooked.

Physical examination of the child may provide the clue to damage of a genetically normal embryo by events during pregnancy. Such abnormalities are more accurately called disruptions. Figure 1 shows the hand of a child born with terminal limb defects. This mechanical disruption resulted from amniotic bands becoming entangled with the limbs of the fetus. In this particular case the diagnosis was indisputable as the fragments of amniotic bands are still visible and, more important, the middle digit was normal and returned to its normal position once the skin band tethering the second and fourth digits was released surgically. Such an abnormality could not have arisen as a primary malformation. For the most part, such mechanical disruptions are sporadic and highly unlikely to recur. Physical abnormalities resulting from abnormalities of the mother’s uterus represent an important exception. With chemical disruptions such as mater-
Chromosomes

The 23 pairs of chromosomes contain the 50,000 or so genes which make up the human genome. A useful analogy in counselling is to regard individual genes on chromosomes rather like beads on a string. The genes are made from DNA and each chromosome contains a single molecule of DNA coiled in a complex fashion to the point at which it becomes visible under the microscope. At one of the stages of cell division known as metaphase, chromosomes become extremely condensed and more easily visualised. These techniques were developed in the late 1950s to the point of clinical application when it was recognised that in Down’s syndrome an extra chromosome, number 21, is present. The numbering of the chromosomes is in descending order of size and centromere position from 1 to 22 with the 23rd pair being the sex chromosomes X and Y. On the basis of shape and length, the 22 pairs of autosomes (those other than the sex chromosomes) form seven groups labelled A to G. The X chromosome forms part of the C group, and the small Y chromosome is in the G group with the 21 and 22 chromosomes. The ability to recognise individual chromosomes came with the development of Giemsa banding and other similar techniques in the early 1970s. These methods, together with the ability to examine chromosomes in a less condensed form, have resulted in the recognition of an increasing range of chromosome abnormalities in handicapped individuals.

Translocation

When the boy in Fig. 4 was born, the paediatrician noted several dysmorphic features and raised the possibility of Down’s syndrome. A chromosome analysis, however, revealed 46 chromosomes in each cell. That was in 1974, just prior to the introduction of banding techniques in the local cytogenetics laboratory. Figure 5 shows one of the C group chromosomes from that original preparation. When his mother became pregnant again, unexpectedly, in her late thirties, she requested amniocentesis to exclude Down’s syndrome, in view of the increased risk in older mothers of having a child with Down’s syndrome. Figure 6a shows the preparation obtained. Again only 46 chromosomes were seen but on this occasion, thanks to the availability of banding techniques, it was possible to see that on the end of one chromosome 9 there was additional material which represented a significant abnormality. In
view of the family history, chromosome samples were obtained from the mother and subsequently from her handicapped son, her sister and her handicapped niece. Both handicapped individuals were found to have the same additional band on chromosome 9 as the fetus. Figure 6b shows the relevant chromosomes from the mother’s blood. She also had additional material on one of her number 9 chromosomes with a corresponding piece missing from one of the number 20 chromosomes. It was thus possible to say that there was a balanced 9;20 translocation being transmitted in this family.

Chromosomes are prone to breakage and if two break simultaneously it is possible for the broken pieces to be attached to the wrong chromosomes. In this case a large piece from number 20 had become attached to number 19 with presumably a tiny piece of number 9 attached to 20. In the carrier of this rearrangement there was no adverse effect since all the genes were still present. The two handicapped children and the fetus had received from their mother the normal number 20 chromosome together with the abnormal 9. The inheritance of a normal 9 and normal 20 from the father had left them with three copies of part of the short arm of chromosome 20, i.e. two normal number 20 chromosomes plus the additional piece on chromosome 9. This unbalanced translocation accounted for the handicap in the two older family members and indicated that similar handicap would be present in the fetus. The mother therefore elected to request a termination. This case history illustrates the practical application of the elegant cytogenetic techniques now available and the advances which have been made in the last two decades in this branch of genetics.

It is likely that the miscarriage indicated in Fig. 3 had resulted from a similar chromosome imbalance since the majority of chromosomally abnormal fetuses are lost through spontaneous abortion. In any family where there has been multiple miscarriage or several handicapped children linked through apparently healthy individuals it is essential that a chromosome analysis be carried out. In many cases, as was the case here, the exact nature of the chromosome abnormality only became apparent when the carrier of the balanced translocation was examined.

In general terms, chromosome imbalance which involves loss or gain of a visible amount of chromosome material will result in handicap and is usually associated with dysmorphic features. In view of the genetic implications for other family members it is important that all dysmorphic children should have a detailed chromosome analysis, even if they are stillborn or liable to die soon after birth.

Cytogenetics laboratories with highly trained staff and a unified system of quality control are now available throughout Britain. As with so many aspects of modern medicine, the pace of technical advance has resulted in a continuing strain on available resources as the examination of chromosome patterns remains a highly skilled and labour intensive technique. One particular development which has added to this strain is the recognition of fragile X mental retardation.

Fragile X mental retardation

In 1977, Sutherland found that by using folate deficient medium it was possible to demonstrate 'fragile sites'. Using this technique a fragile site at the end of the
long arm of the X chromosome can be identified in up to 10 per cent of mentally handicapped males. Figure 7 illustrates a young boy with this abnormality with his parents and normal brother. Unlike most chromosome anomalies, fragile X mental retardation is not associated with major dysmorphic features. There are certain minor features, such as a relatively long face with tall forehead and rather long ears. After puberty the testes are abnormally large. This X-linked disorder was first described by Martin and Bell in 1943, and subsequent review has shown that that original family did indeed have fragile X mental retardation. The so-called fragile site involves 'unravelling' of part of the long arm of the X chromosome so that the end piece appears detached from the main body of the chromosome. Recognition of the fragile X (Fig. 8) is time consuming and is made more so by the fact that in some cases only 3 or 4 per cent of cells will show the marker. It is necessary, therefore, to examine 50–100 cells in order to exclude its presence. Recent studies have shown that approximately one in 1,000 of all live births are handicapped as a result of this condition, making it second only to Down's syndrome as a cause of mental handicap. The clinical burden of fragile X is greater than that of Down's syndrome, since affected individuals have a normal life span and, being X-linked, it is liable to be transmitted to other family members.

Fig. 7. A handicapped boy with fragile X syndrome together with his normal brother whose karyotype was shown to be normal at 20 weeks gestation using fetal blood sampling. Reproduced by courtesy of the Sunderland Echo.

Fig. 8. A fragile X in a typical metaphase spread from a leukocyte cultured in folate deficient medium.

The situation is complicated by two unusual features: approximately one-third of girls who carry this rearrangement are also handicapped, though generally to a lesser degree than the affected males whose mean IQ is 50, and family studies have shown that the fragile X can be transmitted through apparently normal males. The reason why some males are unaffected is the cause of much debate. Whatever the explanation, the consequence is that within pedigrees the presence of handicapped females and transmissions through normal grandfathers can conceal the fact that this is an X-linked condition. Even with the best cytogenetic techniques only about a half of female carriers can be shown to have the fragile X. This makes counselling difficult, particularly when the female carrier is herself mildly handicapped by the condition. The complexity of the condition has hampered the development of prenatal diagnostic techniques since the fragile X cannot, as yet, be reliably identified either in DNA or chromosome preparations from chorion villus sampling at 10 weeks or on fibroblast culture at 16 weeks.

In the family described above, the mother was already pregnant when the diagnosis of fragile X mental retardation was made in her first son. After much consideration she opted for fetal blood sampling which involves removal under ultrasound guidance of a small sample of blood from the fetal umbilical cord. Examination of the lymphocytes from this sample in three separate laboratories showed no evidence of the fragile X and she went on to produce the normal son shown in Fig. 7.
Single gene defects

Single genes are beyond the resolution of the microscope. Recognition of single gene defects has, until the recent development of molecular genetic techniques, rested on the pattern of recurrence within families, the recognition of a characteristic pattern of anomalies or syndrome in an individual, the demonstration of defective gene products, or a combination of these approaches. The process of syndrome recognition or dysmorphology is of particular interest to clinical geneticists. Accurate identification of one of the recognised patterns, currently estimated to number 1,700, is a clinical art which relies heavily on the experience obtained most easily in the genetic clinic. Computerised syndrome databases, such as that compiled by Winter and Baraitser of the Institute of Child Health in London are of great value in this difficult area.

The demonstration of metabolic defects due to enzyme deficiencies, the so-called inborn errors of metabolism, has been one area of major advance. Nearly 200 enzyme defects are now recognised, for which prenatal diagnosis is possible in a substantial proportion. Haemoglobinopathies, such as sickle cell disease and thalassaemia, are another category where the deficient gene product may be demonstrated in the laboratory.

Dominant and recessive inheritance

The concepts of ‘autosomal recessive’, ‘autosomal dominant’, and ‘X-linked’ inheritance, illustrated briefly in Fig. 1, are worthy of closer examination. Genes on the autosomes are, like the chromosomes which carry them, inherited in matching pairs, one from each parent. The term ‘recessive’ simply means that an individual with a single defective copy is judged to be normal and capable of normal growth and development using the single remaining gene or allele. In this situation, disease results when an individual inherits a defective copy of the gene from both parents. Such an individual is said to be ‘homozygous’ for the gene defect. The healthy carrier is ‘heterozygous’. In many cases, it is likely that an affected individual has inherited differing abnormalities in the same gene from the two parents, in which case the affected person should more correctly be called a compound heterozygote. An example would be haemoglobin SC disease resulting from inheritance of the sickle cell gene from one parent and haemoglobin C from the other.

If both copies of a particular gene are essential to normal growth and development, a single defective allele will result in disease. In that case, the gene defect is termed dominant because it becomes evident despite the presence of the normal copy. A parent transmits to offspring a copy of one of each pair of chromosomes. If one of a pair of chromosomes carries a defective dominant gene, the parent will be affected and there is a one in two chance that any child will be similarly affected.

Mutations

In many cases of dominant genetic disease both parents appear normal. If non-paternity can be excluded such cases may be assumed to result from a fault in copying the gene from parent to child: a new mutation. Dominant gene defects which are not compatible with reproduction must always arise as new mutations. On the other hand conditions such as Huntington’s chorea, with a late onset and an apparently stable form, are in almost all cases inherited from an affected parent.

Myotonic dystrophy

The child in Fig. 9 presented with profound hypotonia at birth due to the congenital form of myotonic dystrophy. This discovery prompted examination of the mother who was found to have classic grip myotonia, indicating that she had the typical adult form of the disorder. Her sister was similarly affected while their mother, who had considered herself entirely healthy, also had grip myotonia and cataracts. Such variation in clinical severity is of major importance in counselling because families are interested in the clinical burden of the disease rather than the numerical risk. If a mildly affected relative is not identified, an affected child may be assumed to be the result of a new mutation, leading to the incorrect advice that the risk of recurrence is negligible.

Myotonic dystrophy is of special interest because the severe congenital form is seen only in the offspring of carrier mothers, suggesting some form of materno-fetal
interaction. The risk of recurrence of the severe congenital form prompted this mother to request prenatal diagnosis, using markers linked to the gene on chromosome 19. These indicated a high probability that she had transmitted the normal gene in the current pregnancy.

**Hypercholesterolaemia**

Another gene on chromosome 19 is responsible for production of the low density lipoprotein receptor. It has been shown that a defect in this gene is responsible for familial hypercholesterolaemia. Individuals with one defective copy of this gene are healthy in early life but are prone to develop coronary artery disease with a high morbidity and mortality in middle age. Children who inherit two defective copies from their parents develop severe coronary artery disease in childhood and rarely survive beyond their third decade.

From the paediatric viewpoint, this may be regarded as a rare autosomal recessive disorder since young carrier parents are apparently healthy. The more common presentation, however, is for the heterozygotes to present with coronary artery disease to the adult physician who would regard this as a very common autosomal dominant disorder. This example illustrates the arbitrary nature of these terms, though in practice it is rarely difficult to allocate disorders to one or other group.

Homzygous familial hypercholesterolaemia affects approximately one in every million births, whereas approximately one in 500 adults is heterozygous for this gene. These two figures show a mathematical relationship in what is called the ‘Hardy Weinberg equilibrium’. One in every thousand genes in the population gene pool is defective. The chance of any individual inheriting two defective copies is, therefore, $1/1000 \times 1/1000$, i.e. one in a million. The chance of any individual having one or other of their gene copies defective is one in 500 since they have two copies of the gene and therefore two opportunities to inherit the defective version. Similarly, a recessive disease which affects one in every 10,000 newborn babies will be carried by one in 50 of that population. The many hundreds of rare recessive disorders collectively affect approximately three in every 1000 children born, from which it may be calculated that, on average, all healthy people carry at least one severe recessive defect. All couples face the risk of having children with recessive disease because, by misfortune, both members of the couple may carry the same gene defect. Certain recessive genes have, for various reasons, become more common in certain populations. For example, among Ashkenazi Jews approximately one in 30 carry the gene for Tay Sach’s disease.

Consanguinity presents a particular problem with regard to recessive gene defects. First cousins have one-eighth of their genes in common. In a cousin marriage, there is a one in eight chance that a recessive defect carried by one partner will also be carried by the other and, since, on average, we all carry one such defective gene, there is a one in 32 chance (an eighth times a quarter) that the couple will transmit the same gene defect to their child.

**Calculation**

The above examples have shown some of the ways in which specific risks may be calculated for individual couples. Such calculations may require specialist help when applied to X-linked diseases.

**X-linked inheritance**

The sex chromosomes differ from the others in that the female inherits two X chromosomes whereas the male has only a single X chromosome accompanied by the very small Y chromosome. The latter contains few functional genes. Thus, if the male has a defective gene on the X chromosome it may result in disease, where a similar defect in the female may have little or no effect due to the presence of a normal copy on the other X chromosome. The picture is complicated by the fact that early in development each female cell inactivates the majority of one X chromosome. If a female carries a defective gene on her X chromosome, approximately half of her cells will express the defective gene and half the normal gene; rarely, a majority of cells will express the defective gene and the X-linked disease will be apparent in the carrier female.

**Duchenne muscular dystrophy (DMD)**

DMD results from a defective gene on the short arm of the X chromosome. Approximately one in 3000 live born males are affected by the progressive wasting of the skeletal musculature which generally results in death by the middle of the third decade. It is estimated that in one-third of affected boys the defect arises as a new mutation and in two-thirds the mother is a carrier. If, however, the mother has also had three normal sons, her carrier risk is reduced, since the chance of a carrier mother having three sons unaffected is $\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2}$, or one in eight. This ‘conditional probability’ is combined with the ‘prior probability’ according to Bayes’s theorem thus:

| Bayes’s Theorem | Carrier | Non-carrier |
|-----------------|--------|------------|
| Prior probability | $2/3$  | $1/3$      |
| Conditional probability: | | |
| 3 normal sons | $1/8$  | $1$        |
| Joint probability | $2/3 \times 1/8 = 1/12$ | $1/3$ |
| Posterior probability of carrier status | $\frac{1/12}{1/12 + 1/3} = 1/5$ | |

Thus, the chance that a woman with one affected son and three normal sons is a carrier is one in 5 and the risk of DMD in a subsequent male birth has fallen to one in 10. Prior to the development of gene probes, the only additional information on carrier status was derived from estimation of creatine kinase activity. This enzyme ‘leaks’
from dystrophic muscle in affected males and to a lesser extent in carrier females. If the mother was found to have a markedly elevated level she could be assumed to be a carrier. If the level fell in the normal range, the lower the level the lower would be the likelihood of carrier status. By reference to a standard curve derived from known carriers a further 'conditional probability' could be introduced into the calculation. The recent characterisation of the DMD gene has enhanced the ability to distinguish carriers from non-carriers of this condition but has not in all cases removed the need for such calculations as will be seen below. Before extending the discussion of DMD the fundamentals of molecular genetics must be reviewed.

The 'New Genetics'

The last decade has seen an explosion of knowledge in the field of molecular biology which has had, and will continue to have, far reaching implications in medicine, not least in the field of clinical genetics. A more detailed account in highly readable form has been provided by Weatherall in his book *The new genetics and clinical practice*. The following examples will provide a brief insight.

**DNA**

It is first necessary to review briefly the nature of DNA. Figure 10 shows the basic ladder-like structure. The side arms comprise phosphate and sugar units while the rungs are made from the four bases adenine (A), guanine (G), cytosine (C) and thymine (T). They are loosely bonded, which allows the molecule to separate easily along its long axis to become 'single stranded'. DNA is the 'molecule of life' because it possesses the ability to split into single strands and use each half as a template to create two molecules from one; this is the fundamental step in cell division. Each of the four bases must always have its appropriate matching partner, A with T and C with G. The 46 chromosomes in the human cell contain within their DNA about six thousand million such base pairs which contain the coding sequences or 'genes'. The coding principle resembles Morse code; instead of dots and dashes for each letter, sets of three bases represent one amino acid. With four bases there are 64 triplet combinations or codons, more than enough to cover the 20 amino acids in the human body. Three of the 64 combinations act as stop codons to mark the end of protein synthesis. The genetic code is common to all life forms, a point of importance in later discussion.

**Introns and exons**

In order to translate a gene into a protein, the DNA splits and the half with the coding sequence, the sense strand, generates a matching single strand of RNA: this differs in having uracil instead of thymine. The coding sequences are not continuous but contain intervening sequences or *introns* (as opposed to coding sequences or *exons*) which must be removed before translation. The non-coding segments of the nuclear RNA are removed and the resulting molecule is further modified and transported to the ribosomes as messenger RNA.

Not only do the genes contain non-coding sections, but the genes as a whole make up only a small fraction of the DNA in each nucleus. If an average gene is of the order of 1,000 bases (or 1 kb) in length, there is room for up to 3 million pairs of genes in the DNA of a single cell. The actual number is of the order of 50 to 100,000 functional pairs of genes. The long intergenic segments show striking variation between individuals since mutations are rarely damaging to function unless they affect the areas responsible for the control of gene expression.

**Gene probes**

A gene probe is a single-stranded piece of DNA, normally made radioactive by incorporation of $^{32}$P into its phosphate backbone. This allows it to be located by autoradio-

**Fig. 10. Simplified view of the ladder-like structure of DNA.** Phosphate (P) and deoxyribose sugar (S) form the side arms. The bases adenine (A), thymine (T), guanine (G) and cytosine (C) are joined by weak bonds to form the rungs. In the piece shown the 'sense' strand is on the left. The five bases are the bases 16 to 20 of the beta globin gene. Bases 16, 17 and 18 form the 6th codon and represent glutamic acid. In sickle cell disease, number 17 is changed from T to A. The new codon CAC puts valine at position 6 and results in the abnormal haemoglobin molecule. Such a change is referred to as a point mutation.
graphy. The need for bases to find their matching partner makes it possible to produce gene probes which will match only one or a few sections of human DNA. By heating and cooling to induce double-stranded DNA to split in the presence of the gene probe, some of the probe molecules adhere to their ‘matching partner’ by competition with the other DNA strand.

Restriction enzymes

The two principal approaches to the production of gene probes are either to work backwards from RNA or to use a ‘library’. Key tools in the production and use of gene probes are the restriction endonucleases. These enzymes cut DNA. They are of bacterial origin and were first identified because of their ability to ‘restrict’ the growth of competing organisms by destroying their DNA. Each of the 200 or so known restriction enzymes recognizes a particular sequence of bases, usually 4 or 6, as a ‘cutting site’. It is useful to think of them as ‘scissors which can read’. DNA from any individual will be cut into millions of fragments of varying size by a restriction enzyme. The smaller the number of bases in the cutting site, the greater the number of fragments. The fragments will vary in size but DNA from one person will always produce the same set of fragments when fully digested. The large numbers of variations in the DNA between individuals mean that cutting sites differ, so the number and size of fragments will also differ from person to person.

Chromosome libraries

The fragments of DNA from one or more chromosomes may be used to generate a ‘library’ within which unique sequences specific to that chromosome may be sought. In order to mass produce a single DNA fragment a vector is used. One such vector is the plasmid, a primitive bacterial parasite which comprises a small circle of DNA. A typical laboratory strain has a single cutting site for a particular restriction enzyme and a means of selection using antibiotic resistance. Since all life forms use the same DNA molecule, it is possible to cut plasmid and human DNA with the same restriction enzyme. As the fragments reassemble, some plasmids will incorporate fragments of human DNA since the ‘cut ends’ match. Introduction of the plasmids into E. coli cultured under selective conditions produces millions of copies of the plasmid containing the human DNA. Using the same ‘pair of scissors’, the human DNA is removed and separated for use as a gene probe.

Complementary DNA (cDNA)

In some cases, the original human copy can be made to match a functioning gene. The enzyme reverse transcriptase, made famous by the AIDS virus, turns RNA into its equivalent DNA. This complementary or cDNA matches exactly the original gene apart from the absence of intervening sequences. Some cells produce few proteins, so relatively pure mRNA can be isolated. Red cell precursors were used to generate cDNA for the beta globin gene on chromosome 11 which was used in the first clinical application of gene probes.

Restriction fragment length polymorphisms (RFLPs)

In 1978 Kan and Dozy used the globin cDNA probe in sickle cell disease. Figure 11 provides a diagrammatic summary of the techniques. Using the restriction enzyme Hpa I, the human DNA was cut and spread by electrophoresis according to fragment size. The DNA was transferred to a cellulose nitrate sheet by ‘Southern blotting’ and the beta globin gene probe added. With heating and cooling some of the probe molecules adhered to the fragments carrying the beta globin gene, marking their position on the subsequent autoradiograph.

In most people, a band appeared at 7.6 kb, ie the cutting sites for this enzyme on either side of the beta globin gene were 7,600 bases apart. In some of the black population a cutting site had been lost due to a mutation in an earlier generation. The next cutting site was a further 5,400 bases along the chromosome, so the fragment carrying the beta globin gene was 13.0 kb in length. This produced a band on the autoradiograph closer to the origin. This harmless variant produced, therefore, a restriction fragment length polymorphism or RFLP. By good fortune for the investigators, the original mutation which produced sickle cell disease in the ancestor of their black population had occurred in a number 11 chromosome with the rarer 13 kb variant. Thus, in most people with sickle cell disease they found only the 13 kb band while carriers typically had two bands, a 7.6 kb band from the chromosome with the normal gene and a 13 kb band from the chromosome with the defective gene. As can be seen in the illustration, it was thus possible to track the defective gene on the basis of this polymorphism and predict, without knowledge of the haematological picture, which offspring would be affected. This provided, for the first time, a means of prenatal diagnosis using a harmless RFLP to track the disease gene in fetal DNA.

Initially, the DNA for prenatal diagnosis was obtained by culture of amniotic cells. The subsequent development of chorion villus biopsy at 10 weeks gestation has meant DNA could be obtained even earlier in pregnancy and an abnormal fetus identified before the disease gene was actually functional. Subsequent years have seen further refinement in sickle cell disease; restriction enzymes were identified which cut, or failed to cut, at the site of the point mutation in the globin gene which causes this disease. More recently, a very short (oligonucleotide) probe for the specific piece of the gene in question has been produced which fails to adhere to the defective gene.

On a more general front, RFLP tracking of disease genes opened the way to carrier detection and prenatal diagnosis in diseases which hitherto could not be identified. All that is needed is a gene probe specific for a section of DNA very close to a disease gene, or for the gene itself, and for which an RFLP could be identified. Table 1 contains a few of the more important diseases which can now be tracked in this way.
Fig. 11. Diagrammatic summary of the DNA probe techniques. (a) The restriction endonuclease (‘scissors which can read’) cut the DNA extracted from the person’s blood cells into thousands of fragments, two of which contain the beta-globin gene. In this individual there is a variation in the cutting sites on the two number 11 chromosomes (a restriction fragment length polymorphism or RFLP) which means that the two copies of the beta-globin gene are on fragments of differing size. (b) The fragments are spread according to molecular weight by electrophoresis on an agarose gel. (c) The DNA is denatured to single stranded form and fixed by ‘Southern blotting’ to a cellulose nitrate sheet. (d) The fixed DNA is stable but invisible. (e) The gene probe; using the enzyme reverse transcriptase, RNA from red cell precursors has been used to generate cDNA which is ‘complementary’ to the coding sequences of the original gene. The tiny quantities of cDNA have been inserted into a vector (such as the DNA of a plasmid) which has then been cultured to produce millions of copies of this human DNA sequence. These DNA fragments, made single stranded and labelled with radioactive phosphorus, form the gene probe. (f) Addition of the gene probe to the sheet carrying the fixed DNA allows some of the radioactive fragment to hybridise firmly with their matching sequence on the DNA spread. (g) An autoradiograph reveals the location of the fragments carrying the beta-globin gene. (h) The family study reveals that the affected child has only the 13.0 kb band and the brother with normal haemoglobin has only the 7.6 kb band. Both parents and the two carrier children have both bands. In this family the beta-globin gene with the ‘sickle cell’ defect is travelling on a chromosome 11 with Hpa I cutting sites which generate a 13 kb fragment. The normal gene is flanked by Hpa I cutting sites 7.6 kb apart.

Using an RFLP in this way as a marker to track the disease gene, prediction of whether a subsequent child is affected may be made on the basis of DNA analysis early in pregnancy without the knowledge of the actual gene defect.
Table 1. About 1,200 autosomal genes have now been localised. Some important disorders for which the chromosomal location of the gene is known are listed. Those for which clinically useful gene specific or closely linked probes are available are shown in roman type.

| Disorder                                      | Chromosome |
|----------------------------------------------|------------|
| Huntington’s chorea                          | 4          |
| Familial adenomatous polyposis               | 5          |
| 21-Hydroxylase deficiency                    | 6          |
| Haemochromatosis                             | 6          |
| Cystic fibrosis                              | 7          |
| Tuberose sclerosis                           | 9          |
| Friedreich’s ataxia                          | 9          |
| Acute intermittent porphyria                 | 11         |
| Sickle cell disease and beta thalassaemia    | 11         |
| Phenyl ketonuria                             | 12         |
| Retinoblastoma                               | 13         |
| Wilson’s disease                             | 13         |
| Alpha 1 antitrypsin deficiency               | 14         |
| Variegate porphyria                          | 14         |
| Adult type polycystic kidney disease*        | 16         |
| Alpha thalassaemia                           | 16         |
| Peripheral neurofibromatosis (NFI)           | 17         |
| Familial amyloidosis                         | 18         |
| Myotonic dystrophy                           | 19         |
| Familial hypercholesterolaemia               | 19         |
| Familial Alzheimer’s disease                 | 21         |
| Central neurofibromatosis (NF2)              | 22         |

*One family in which the APKD gene is not on chromosome 16 has been described.

About 150 X linked genes have been mapped. Important diseases include:

- Haemophilia A and B
- Duchenne/Becker muscular dystrophy
- Fragile X mental retardation
- Steroid sulphatase deficiency

- Hunter’s syndrome
- Lesch Nyhan syndrome
- Ornithine carbamyl transferase deficiency
- Retinitis pigmentosa

Crossover of chromosomes

The genetic analysis of Huntington’s chorea (HC) illustrates this particularly well. In 1982, Gusella and his colleagues discovered that in three large pedigrees the disease marker travelled with the probe G8. This probe was localised to the short arm of chromosome 4, thus localising HC to that site. RFLP analysis of this probe identified four band patterns in the population labelled A, B, C and D. If an affected individual had inherited, let us say, an AC pattern with the C coming from the affected parent, there would be a 96 per cent probability that affected offspring had inherited the type C marker. It is not 100 per cent, because the G8 marker is a short distance away from the HC gene. During gamete formation, matching pairs of chromosomes lie side by side. Breaks in the chromosomes allow sections to be exchanged. Such a crossing over between the marker and the gene would cause the G8 marker to ‘swap sides’. Such crossovers are common events, first demonstrated by Thomas Hunt Morgan in Drosophila. By counting the number of such crossovers, the distance between genes, or in this case between disease genes and the sites of adherence of gene probes, provides an estimate of their distance apart. Traditionally, a 1 per cent crossover rate is called one map unit or one centiMorgan. Using this approach a ‘map’ of the whole human genome is now being constructed which will allow, in the not too distant future, all major genetic diseases to be localised and ultimately to be characterised.

Duchenne muscular dystrophy

A case history will illustrate the clinical importance of such knowledge.

The DMD gene is now known to be unusual in that the coding sequence of 14 kb is scattered across a section of the short arm of the X chromosome 2 million bases (megabases) in length. In some affected boys, large segments of the gene were found to be deleted. The DNA from one such child was obtained and, employing a technique called phenol enhanced reassociation (PERT), DNA which matched the missing section was isolated. Gene probes from this DNA, known as PERT probes, are now used to track DMD as described above.

In the family shown in Fig. 12a, Diane requested counselling; her mother was an obligate carrier of DMD on the basis of a high CK and had had one son who died in adolescence. Diane’s sister Carol had two normal sons and her other brother was unaffected.

At the time of presentation Diane was, unknown to her, already pregnant. Her CK results were unhelpful, so she started with a one in two chance of being a carrier and intended to request termination of all male pregnancies. DNA was obtained from her mother, sister and nephews but her brother had left home and could not be traced. Figure 12b shows the first DNA study; she and her sister had inherited the lower band (2) from father and, therefore, had received the upper band (1) from Eileen. Carol had passed this band to one of her healthy sons. Unfortunately, the 5 per cent crossover rate in family studies of probe PERT 87.15 meant that a probability calculation was needed to calculate the chance that Diane was a carrier, despite sharing the same marker as her normal nephew. Figure 12c shows the complexity of this calculation. It was found that her risk was still one in 8 which she and her husband felt was too high. At this stage an intensive search had located her normal brother and Fig. 12d shows that he, too, had inherited the number 1 allele from his mother. This provided a second line of evidence that in Eileen the disease gene was on the X chromosome with the number 2 marker. Chorionic villus sampling showed that Diane’s male fetus had also inherited her number 1 allele. The 5 per cent crossover rate left Diane with a one in 20 chance of being a carrier and the fetus with a close to one in 20 chance of being affected.

The couple felt that this risk was worth taking and their healthy son was born in July 1988.

This case illustrates the complexity of the calculations which the use of such imperfect markers may involve, despite the sophistication of the laboratory techniques. The human dimension is also evident. Careful explanation of the various options and their implications is
Fig. 12. A family affected by Duchenne muscular dystrophy. (a) The family tree. (b) Limited analysis using PERT 87.15 as a linked marker. (c) Calculation of the risk of Diane being a carrier, based on DNA marker studies in Eileen, Carol and Carol’s son. (d) Completion of prenatal diagnosis for Diane with the additional information that the normal brother had inherited the same marker allele from his mother as Carol’s son and Diane’s fetus. The calculated risk of DMD in the fetus was thus reduced to less than 1 in 20. (The small amount of DNA obtained from the chorionic villus biopsy has produced a faint band, only just visible on the reproduction.)
vital but very time consuming. The costs in terms of clinical and laboratory investment are more than offset, however, by the birth of a healthy son whose intrauterine existence would otherwise have been terminated. Moreover, Diane can now proceed to have further children without intervention with the demonstration that this son received grandmother's X chromosome and was unaffected.

The recent identification of dystrophin, the defective gene product in DMD, and the availability of cDNA probes which can identify deletions in over half of affected males, will further improve the diagnostic accuracy in DMD. Similar progress may be foreseen in other diseases as the newer methods of pulse field gel electrophoresis, which facilitate analysis of larger segments of DNA, together with gene sequencing techniques allow more precise localisation and characterisation of the major gene defects.

Polymerase chain reaction (PCR)

One recent technical advance deserves special mention. Current techniques of DNA extraction are costly and cumbersome, and require relatively large amounts of blood or tissue. This limits the application of DNA analysis in prenatal diagnosis. PCR may overcome this problem. When a section of DNA has been analysed in detail it is possible to produce oligonucleotides specific to base sequences at either end. Using the enzyme DNA polymerase, the intervening length of DNA can then be amplified in large quantities quickly and cheaply from only a few cells. Figure 13 illustrates the use of PCR to carry out rapid diagnosis of cystic fibrosis, using a 330 base pair sequence which spans a polymorphic site very close to the cystic fibrosis gene on chromosome 7. Oligonucleotide primers of known sequence flank this polymorphic site and when added to the reaction mixture anneal to their complementary DNA sequences to initiate DNA synthesis in the presence of Taq Polymerase enzyme. The resulting PCR product is run out on an agarose gel, stained with ethidium bromide and visualised directly under UV, thus avoiding the need for radioactive probes and autoradiography. Figure 13 illustrates a recent use of this new technique.

Coupled with obstetric advances, such as transabdominal chorionic villus sampling, earlier amniocentesis and preimplantation analysis, these molecular genetic techniques hold great promise; a rapid expansion of prenatal diagnostic techniques is in prospect which may even include the analysis of fertilised ova to ensure that only normal pregnancies are initiated.

At a population level, cheaper non-radioactive probes applicable to tiny samples such as blood spots or buccal cells obtained by mouth washes may allow screening for carriers of common disorders. The cystic fibrosis gene alone is carried by about one in 20 Europeans. It is not beyond the bounds of possibility that in a few years it will be possible to screen couples for genetic disease at routine preconception counselling clinics.

Ethical and moral dimensions: the need for compassion

In genetic counselling there is an evident need for effective communication and for compassionate handling of the issues which the advances in the field of clinical genetics lay before us. One area in which these issues are most obvious is the development of presymptomatic testing for Huntington’s chorea. The potential benefits of this facility for families and the community are obvious and many younger members of HC families wish to be informed. Provided they are made fully aware of the implications and the current uncertainties, this is their right. It is equally clear that those who do not wish to be informed have a right to remain in ignorance. Law courts are beginning to grapple with the deeper question of whether, in years to come, affected offspring will argue that their parents failed in their responsibility to undergo such investigations. As yet the phenomenon of ‘wrongful life’ litigation is theoretical in Britain, but this may not remain so. It is essential that the medical profession
should retain a balanced view of the developments in clinical genetics. Wisdom will be needed to realise the enormous benefits in prospect in terms of preventive medicine, while defending the rights of the individual before, as well as after, birth.

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Royal Colleges of Physicians
MRCP (UK)
Part 1
The next MRCP (UK) Part 1 Examination will take place on Thursday 9th February 1989.
Application forms accompanied by the necessary certificates and fee of £125.00 must reach the College of entry by Friday 16th December 1988.
Prospective candidates should have been qualified for 18 months and may enter through any one of the Colleges listed below.

Part 2
The next MRCP (UK) Part 2 Examination will begin on Tuesday 10th January 1989.
Application forms accompanied by the necessary documentation and fees must reach the College of entry by Friday 2nd December 1988.
Prospective candidates should have been qualified for 2½ years and must comply with the regulations concerning training in acute medicine.
The Examination fees: Written Section £120.00 Oral and Clinical Section £130.00. The London College will require separate cheques. The Scottish Colleges will require a single cheque for £250.00.

Royal College of Physicians of Edinburgh,
9 Queen Street, Edinburgh, EH2 1JQ
Royal College of Physicians & Surgeons of Glasgow,
242 St Vincent Street, Glasgow G2 5RJ
Royal College of Physicians of London,
11 St Andrew's Place,
Regent's Park, London NW1 4LE

Reference books
Emery, A. E. H. and Rimoin, D. L. (1983) Principles and practice of medical genetics. Edinburgh: Churchill Livingstone
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DIPLOMA IN CHILD HEALTH
The Diploma in Child Health is designed to give recognition of competence in the primary care of children and is particularly suitable for general practitioners and clinical medical officers.
The next examination will be held on Tuesday 31st January 1989. Application forms and the necessary documentation and fees must reach the College by Friday 9th December 1988.
Experience of twelve months in the care of children is recommended before candidates apply to sit the examination.
Further details and an application form may be obtained from:
The Examinations Office,
Royal College of Physicians of London,
11 St Andrew's Place,
Regent's Park, London NW1 4LE

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