Basic Molecular and Cell Biology

Impact of molecular biology on clinical genetics

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The central activity of clinical genetics is genetic counselling, the aim of which is to provide information about the risks to offspring at a time appropriate to considering the options available for modifying the outcome and to put those risks in perspective.

There is much evidence to show that with serious or life threatening disorders genetic counselling causes many couples to modify their reproductive behaviour, even when the options available are far from satisfactory. Our recently acquired ability to use deoxyribonucleic acid (DNA) probes (radiolabelled single stranded DNA fragments that bind only to specific genes or DNA sequences from the subject's total DNA) to analyse mutations or track the inheritance of genes through families has enormous potential as an aid to genetic counselling. Couple this with the development of chorionic villus sampling in the first trimester of pregnancy and the effect for some families is nothing short of revolutionary.

DNA analysis was first used in the prenatal diagnosis of the haemoglobinopathies. Kan and Dozy reported the diagnosis of sickle cell anaemia on amniotic cells taken in the second trimester,¹ and Old *et al* were first to report fetal diagnosis of β thalassaemia in the first trimester based on the analysis of chorionic villus DNA.² Since then so many monogenic disorders have become amenable to this approach that it is not possible to give a comprehensive up to date list. Schmidtke and Cooper provide a regular listing of cloned human genes or DNA sequences that can be used as DNA markers.³ Their list of independently cloned DNA sequences published in April 1987 contains nearly 3000 entries. The table gives those common conditions in which prenatal or carrier tests are already being used and which constitute most of the activity of genetics units providing DNA analysis as a clinical service. There is no correlation between the incidence of a disorder and the chances that the relevant gene has been mapped or even cloned. No matter how rare the genetic disease specific inquiries on behalf of a family at risk should always be made, especially in X linked disorders. The interval between gene localisation and clinical application of this information can be very short.

Within months of the cystic fibrosis mutation being mapped to the 7q22-31 region of chromosome 7 some lucky couples who had not risked a pregnancy since the diagnosis was made in their child were having prenatal diagnosis in the first trimester.⁴ I say lucky couples because gene tracking (see below) with DNA probes usually helps only certain families. These couples were also lucky because the service was offered to them. The National Health Service is not renowned for its quick reaction time, and the rate of recent progress has taken even those concerned by surprise. There is now a serious shortfall in the genetic counselling and laboratory services needed to translate advances in molecular biology into help for families at risk. This may be overcome partly by ensuring that those caring for such

families are aware of what can be offered and of the importance of referral before a pregnancy. In other words, doctors who fail to take a proper family history and make the appropriate referral do so at their peril.

There are two fundamentally different approaches to genetic prediction by DNA analysis: detection of the mutation and gene tracking.

Conditions for which genetic prediction (prenatal diagnosis, exclusion, or carrier testing) is possible in selected families using DNA analysis

Autosomal dominant:	Autosomal recessive:
Huntington's chorea	β Thalassaemia
Myotonic dystrophy	α Thalassaemia
Adult polycystic kidney disease	Sickle cell anaemia
Tuberose sclerosis*	Cystic fibrosis
Von Recklinghausen's neurofibromatosis*	α_1 Antitrypsin deficiency
X linked:	Phenylketonuria
Duchenne and Becker muscular dystrophy	Congenital adrenal hyperplasia
Haemophilia A	(21 hydroxylase deficiency)
Haemophilia B	

* These two important conditions became amenable to gene tracking during the writing of this article.

Detection of the mutation

The principle of detecting the mutation is the same as that of the traditional diagnostic test—namely, to detect an important difference from normal in the DNA sequence of the gene in question. The first requirement is a DNA probe capable of selectively binding (hybridising) to that part of the gene in which the mutation occurs. This immediately highlights the main limitation of this approach. In most of the conditions listed in the table the site (and type) of mutation within the gene varies between families, and searching the whole gene for anything other than large deletions is currently impracticable. Haemophilia A can result from mutations more or less anywhere along the factor VIII gene, which consists of 186 000 base pairs. The gene implicated in Duchenne and Becker muscular dystrophy is probably two million base pairs long. The opportunities for direct detection of the mutation in clinical practice may therefore be limited.

There are, however, some important exceptions. Firstly, where the mutation is a deletion of most or all of the gene sequence simple restriction fragment analysis will detect it. As explained in the first article of this molecular biology series, the initial step after extracting the DNA from white cells or other tissue is to cut it into a million or so specific fragments with a restriction enzyme. If the fragment of DNA to which the DNA probe binds is missing altogether there will be no radioactive signal on the autoradiograph at the end of the analysis. Alternatively, the gene deletion may result in the DNA fragment detected by the probe being smaller than usual, and this will produce a band on the autoradiograph in an unusual position. In the Far East α thalassaemia is due to deletions,

and mutation detection forms the basis of genetic diagnosis in this condition. In most other disorders investigated deletions account for only a small proportion of the mutations. Nevertheless, every effort should be made to test an affected family for a deletion when a gene specific probe is available.

Secondly, sickle cell anaemia and α_1 antitrypsin deficiency (ZZ variant) are important examples of conditions in which one can expect the mutation to be the same in every affected family. Huntington's chorea may turn out to be another example because the fact that affected individuals generally reproduce before the clinical onset of the disease means that many families will have a common ancestor. Although there are about 40 different ways in which the β globin gene can mutate to produce β thalassaemia, within a given population one particular mutation often predominates, as has been clearly shown for subpopulations in the Mediterranean.^{5,6} The same is likely to be true of cystic fibrosis, making population screening for the carrier state based on DNA analysis a distinct possibility.

Certain point mutations (which entail a change in a single nucleotide base in the DNA) disrupt a restriction enzyme recognition site, and this produces a characteristic change in the band pattern on the autoradiograph. The large number of restriction enzymes each have a specific recognition sequence of four to eight nucleotide bases and will cut double stranded DNA at these sites only. If the mutation removes such a site digestion of the patient's DNA with the appropriate restriction enzyme will lead to a larger DNA fragment in the vicinity of the mutation. A probe for this region of DNA can be used to show this change in DNA fragment size. Figure 1 illustrates how the sickle mutation removes a Mst II cutting site so that the chromosome 11 that carries the sickle mutation produces a larger restriction fragment than normal.⁷

FIG 1—Part of the β globin gene pair on chromosomes 11 with an expanded view of the nucleotide sequence at codons 5, 6, and 7. The amino acids encoded by these codons are indicated alongside. β Globin contributes to adult haemoglobin. A point mutation in this gene at position 6 causes sickle haemoglobin (β^s), in which the normal glutamic acid (glu) is changed to valine (val). This change in amino acid is a reflection of the nucleotide change in the DNA (GAG to GTG). The change in nucleotide base (the sickle mutation) eliminates a cutting site for the restriction enzyme Mst II, which recognises the sequence CCTGAGG but not CCTGTGG. The middle base in the Mst II site (N in the diagram) can be any nucleotide. The loss of the Mst II site on the chromosome carrying the sickle mutation of the β globin gene results in a larger (1.35 kb) restriction fragment than normal (1.15 kb), which will show up as a band in a different position on the autoradiograph if the appropriate DNA probe is used. (Reproduced from Roberts JF, Pembrey M. *An introduction to medical genetics*. 8th ed. Oxford: Oxford University Press, 1985:fig 103.)

Another approach that may be adopted when the precise point mutation is known is a refined hybridisation study using tailor made oligonucleotide probes which can detect a single nucleotide mismatch between the probe and the patient's DNA. This technique (described in an earlier paper in this series) was first used to detect the sickle mutation and has been used, for example, in the prenatal diagnosis of α_1 antitrypsin deficiency.⁸

Although it is satisfying to detect the mutation itself, in most genetic disorders this is not practicable. Either a great variety of mutations can occur within the relevant gene to give the disorder or the gene concerned, though localised to a chromosomal region, has yet to be cloned. In these cases gene tracking comes into its own.

Gene tracking

Gene tracking asks the question: "Has this family member or fetus inherited the same relevant chromosome region(s) as a previously affected member?" There are variations to this question, but all depend on formulating a simple study with the family members available to establish the band on the autoradiograph with which the disease is coinheriting (fig 2). It is an approach that is

FIG 2—Autoradiograph of DNA tracks from four family members digested with restriction enzyme Bgl II and hybridised with probe DX13, which is closely linked to haemophilia A. The restriction fragment length polymorphism is represented by the polymorphic bands 5.8 kb and 2.8 kb. The 5.8 kb band appears to be coinheriting with the haemophilia mutation, and the sister has inherited this band from her mother and the 2.8 kb band from her father. Barring an error due to recombination, she is therefore a carrier.

independent of the particular type of mutation within the gene that is responsible for the disease, and it requires either a gene specific probe or a chromosome region specific probe for a sequence known to be closely linked to the disease locus.

In gene tracking there has to be some way of distinguishing each chromosome of the homologous pair in key family members. This is done by exploiting naturally occurring variations in DNA sequence known as restriction fragment length polymorphisms.

RESTRICTION FRAGMENT LENGTH POLYMORPHISMS

Only a small percentage of total genomic DNA is actually a coding sequence for proteins. The non-coding regions that flank genes, the intergenic DNA, and to some extent the intervening

sequences, are less conserved during evolution, and point mutations are tolerated and become established in populations. Experience so far suggests that on average 1 in 100 to 1 in 200 nucleotide bases differ between the chromosome pair. A number of these DNA sequence polymorphisms concern the recognition sequence of four to eight bases of a particular restriction enzyme, and this results, on digestion, in different size restriction fragments from each chromosome of the homologous chromosome pair. Thus a restriction fragment length polymorphism, is a relatively common change in DNA sequence that either destroys or creates a restriction enzyme recognition site or alters the distance between two sites. In anyone who is heterozygous for a restriction fragment length polymorphism one restriction band pattern corresponds to one chromosome and the other band pattern to the other chromosome of the pair. This allows us to track the transmission of a single chromosome region through a family and to see if a particular monogenic disease coherits with the polymorphic site; in other words, we can perform classical linkage studies.

Figure 2 shows a restriction fragment length polymorphism that is detected by the probe DX13 after the DNA has been digested with the restriction enzyme Bgl II. DX13 detects a DNA sequence (by international agreement called locus DXS15), the function of which, if any, is not known but which is closely linked to the factor VIII gene locus. It can therefore be used for gene tracking in haemophilia A.⁹ Figure 2 also shows how the sister of the haemophiliac has inherited the same band (X chromosome) from her mother as her affected brother and is therefore almost certainly a carrier. In this case, however, her X chromosomes each give a different band on the autoradiograph and so she is "informative" for diagnosis during the first trimester should she want it.

GENE TRACKING IN PRACTICE

Several factors may limit the application of gene tracking. Firstly, the relevant family members must be available. For prenatal diagnosis of cystic fibrosis a sample from a previously affected child is necessary to determine with which chromosome 7 restriction fragment length polymorphism pattern the cystic fibrosis mutation is coheriting. Similarly, it may be important to have samples from a relative with Huntington's chorea or a boy with Duchenne muscular dystrophy. In X linked disorders, however, normal boys or the maternal grandfather may often allow the linkage phase with the DNA marker to be established.

Secondly, key family members must be heterozygous for a restriction fragment length polymorphism—that is, the two homologous chromosomes have to be distinguishable. Most families may be helped if service provision allows multiple probes or restriction fragment length polymorphisms to be used, but there will always be a few "uninformative" families. For this reason referral before a pregnancy at risk is essential because nothing can be promised until after the initial family study. It is also clear that other established

methods for prenatal diagnosis and carrier testing will remain important back ups for these families.

Thirdly, in addition to human errors common to other tests, such as switched or mislabelled samples, gene tracking has an inherent error rate due to recombination or crossing over. One or other parental chromosome is not transmitted to a child intact. Between each generation there is reciprocal exchange of varying sections of the chromosome pair so that, although the sperm or egg contains a single chromosome, it is actually a composite of the parental pair. This normal exchange between homologous chromosomes during gamete formation may lead to "separation" of the mutation from the restriction fragment length polymorphism pattern (allele) with which it had been coheriting and consequently a wrong genetic prediction. To date, with the exception of the Duchenne muscular dystrophy locus, which is huge and probably a recombination "hot spot," errors due to recombination occur in fewer than 1% of cases with the use of gene specific probes. When linked DNA probes are used, as in Huntington's disease, myotonic dystrophy, or cystic fibrosis, the error rate in genetic prediction can be calculated from the observation of how consistently the DNA marker coherited with the disease in studies of large families. Linked probes with a recombination frequency of more than 5% are not usually used. It is important to realise that the recombination frequency may not equate directly with the error rate, and often complicated calculations are required before the reliability of the test is explained to a particular family.

Finally, prediction by gene tracking may sometimes be critically dependent on the stated father being the biological father (especially in X linked diseases as in fig 2). Non-paternity is one of the several difficult clinical, ethical, or technical problems that may arise in applying these new techniques in clinical genetics. The potential to release relatives from the fear of being carriers or help couples have healthy children is enormous, but it is a clinical service that cannot be undertaken lightly. Close coordination between clinicians and scientists in the regional genetics centres, obstetricians, and those caring for the families at risk is essential.

References

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In the 1940s and early 1950s any erosion of the cervix was immediately cauterised as an outpatient procedure. Later it was decided that this risked causing delay in the first stage of labour. Cauterisation was therefore carried out only after dilatation under anaesthetic. Nowadays the procedure appears to have been entirely abandoned. Could this have contributed to the increase in cervical cancer for, whatever its disadvantages, cauterisation presumably destroyed dyskaryotic cells?

Although the term "cervical erosion" has been used for over a century, it is now recognised as a misnomer.¹ The "eroded" area is covered by columnar epithelium and appears red because there is only one layer of cells between the underlying capillaries and the surface.¹ Various terms such as "erythroplakia"¹ or "mucosal ectopy"² have been suggested to replace the word "erosion" but unfortunately it is still in common use. During the 1970s

it was recognised that "erosions" can be found in 10% of normal women and should not be regarded as pathological if the woman is asymptomatic: indeed, even when symptoms are present the erosion is not necessarily the cause.³ Nevertheless, if vaginal discharge is a problem cryosurgery is often effective.¹ In the 1970s cervical erosion accounted for 35 000 hospital admissions a year in Britain,³ but I doubt whether reducing this figure would affect the incidence of cervical cancer, because women who formerly would have undergone cautery now have smears taken: if dyskaryosis is detected they are referred for colposcopy and an ablative procedure if necessary.—JAMES OWEN DRIFE, senior lecturer in obstetrics and gynaecology, Leicester.

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