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The Role of Cloned Genes in the Prevention of Genetic Disease [and Discussion]

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The role of cloned genes in the prevention of genetic disease

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The application of recombinant DNA technology to the study of human genetic disease promises to increase the scope for carrier detection and prenatal diagnosis. Here we summarize current experience with prenatal diagnosis of single-gene disorders by DNA analysis and highlight some of the technical and organizational problems that remain to be solved.

Introduction

The prevention of genetic disease, apart from measures to reduce exposure to mutagens, is based mainly on carrier detection, genetic counselling, and prenatal diagnosis. Currently, prenatal diagnosis of single-gene disorders is restricted to conditions in which the carrier state can be identified in parents. Recent advances in recombinant DNA technology have led to the development of new methods that are being applied with increasing frequency for the detection of genetic disease. Fetal tissue for the extraction of DNA can be obtained either from amniotic fluid cells in the second trimester of pregnancy or by chorion villus sampling (CVS) in the first trimester. Technical details and discussion of the safety of these different approaches have been reported in detail elsewhere (Rodeck & Morsman 1983) and will not be discussed further here.

The development of a prenatal diagnosis programme by fetal DNA analysis involves several steps. First, the particular gene must be isolated, the underlying mutation(s) characterized, and restriction fragment length polymorphisms (RFLPs) defined, either within the gene or adjacent to it. If the underlying biochemical defect is not known, RFLPs sufficiently close to the gene must be found so that it can be 'tracked' reliably by individual pedigree analysis (Botstein *et al.* 1980; Weatherall 1985*a*; Nakamura *et al.* 1987).

Once we have isolated the gene or established an RFLP linkage, the next step is to define the degree of genetic heterogeneity of the disorder, i.e. how many different mutations can give rise to the disease phenotype. In addition an attempt must be made to relate the severity of the clinical phenotype to the particular molecular lesion; it is important in developing a prenatal diagnosis programme to be able to predict the precise clinical outcome associated with a defined molecular lesion.

The next step is to determine whether any of the mutations can be identified directly by gene mapping. These include deletions or major gene rearrangements, or point mutations that alter a restriction enzyme site. Once one or more mutations have been defined it should be possible to construct oligonucleotide probes for their direct detection in parental and fetal DNA. Finally, it is important, once the molecular defect has been determined, to work out simple biochemical screening tests for carrier detection and for prenatal diagnosis in those cases in which fetal DNA analysis is not possible.

Although some of these criteria have been met for many single-gene disorders, the only group of conditions for which it has been possible to obtain an adequate picture of the spectrum of molecular heterogeneity, and for which there is large experience of fetal DNA analysis for prenatal diagnosis, is the inherited disorders of haemoglobin.

The haemoglobin disorders

Globally, the haemoglobinopathies are the commonest single gene disorders; the most important are sickle cell anaemia, α thalassaemia and β thalassaemia (Weatherall & Clegg 1981; Bunn & Forget 1986). In addition, conditions that result from the interaction of structural haemoglobin variants and thalassaemia, notably sickle-cell or haemoglobin E thalassaemia, also cause an important public health problem in many parts of the world (Weatherall & Clegg 1981). These diseases are ideal models for developing prenatal diagnosis programmes. Carrier detection is relatively simple and more is known about their molecular pathology than any other single-gene disorder. Their prenatal diagnosis by fetal blood sampling and globin-chain synthesis is well established (Alter 1984; Weatherall 1985*b*). Hence it has been possible to incorporate DNA analysis into several well-established prenatal diagnosis programmes.

Molecular pathology

The structural haemoglobin variants S and E result from point mutations, both of which can be identified directly in genomic DNA by restriction enzymes (Chang & Kan 1982; Orkin *et al.* 1982*b*; Wilson *et al.* 1982; Thein *et al.* 1987*a*).

There are two main classes of α thalassaemia (Higgs & Weatherall 1983). Normal individuals have two α globin genes per haploid genome, αα/αα. The α^0 thalassaemias are due to large deletions of the α globin complex that remove both α globin genes. The homozygous state for α^0 thalassaemia causes intrauterine death and the clinical picture of the haemoglobin Bart's hydrops syndrome. The α^+ thalassaemias are caused by deletion or inactivation of one of the pair of linked α globin genes and are very heterogeneous. Several different sized deletions have been defined and a number of point mutations that interfere with initiation, splicing or chain termination can also inactivate the affected α globin gene. The compound heterozygous state for α^+ and α^0 thalassaemia produces haemoglobin H disease, a moderately severe haemolytic anaemia.

Recent studies of the molecular pathology of the α^0 thalassaemias have shown that they can result from many different-sized deletions (Nicholls *et al.* 1987). Although the majority of them remove both α globin genes, one deletion has been described recently that, although it inactivates both α genes, ends about 11 kilobases (kb) upstream from the α genes and leaves them both intact. This observation has important implications for the prenatal diagnosis of this condition.

Over 45 mutations have been described as the cause of β thalassaemia (Orkin *et al.* 1982*a*; Weatherall & Wainscoat 1985; Bunn & Forget 1986). Deletions are rare; the only common one, which removes the 3′ end of the β globin gene, is found in northern Indian populations. Most of the other β thalassaemia mutations are single base changes that cause either premature chain termination, defective processing of messenger RNA, or a reduced rate of transcription.

The different mutations that cause α or β thalassaemia that can be diagnosed directly by restriction enzymes are summarized in table 1. Most of the important α thalassaemias can be

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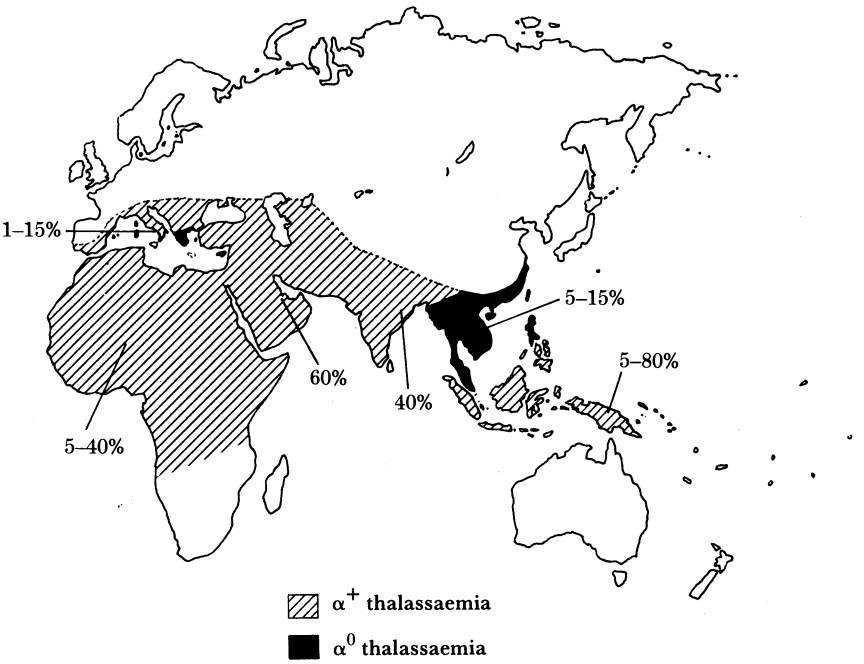

FIGURE 1. World distribution of α thalassaemia.

identified in this way but less than half of the β thalassaemias are due to mutations that alter restriction enzyme sites.

The world distribution of the different mutations that cause α and β thalassaemia is of considerable importance for developing prenatal diagnosis programmes. The distribution of the α thalassaemias is summarized in figure 1. It is clear that α^0 thalassaemia only occurs commonly in the Mediterranean region and southeast Asia, and hence these are the populations in which the severe forms of the condition (Hb Bart's hydrops fetalis and Hb H

TABLE 1. β THALASSAEMIA MUTATIONS DIRECTLY DETECTABLE BY RESTRICTION ANALYSIS

mutation	restriction enzyme
β^0 thalassaemia	
1. Indian deletion	Various enzymes, −619 bp
2. American Black deletion	Various enzymes, −1.35 kb
3. Dutch deletion	Various enzymes, −10 kb
4. −1 codon 6	*Mst*II
5. IVS 2 splice junction	*Hph*I
6. IVS 1 (−25 bp)	*Fnu*4H, *Mst*II
7. IVS 1 (−17 bp)	*Fnu*4H, *Mst*II
8. IVS 1 position 6	*Sfa*N1
9. IVS 2 3′ end AG→GG	*Alu*I
10. β^0 39	*Mae*I
11. β^0 17	*Mae*I
12. β^0 37	*Ava*II
13. β^0 121	*Eco*RI
β^+ thalassaemia	
14. IVS 2 position 745	*Rsa*I
15. −87 C→G	*Avr*II
16. Hb E	*Mnl*I

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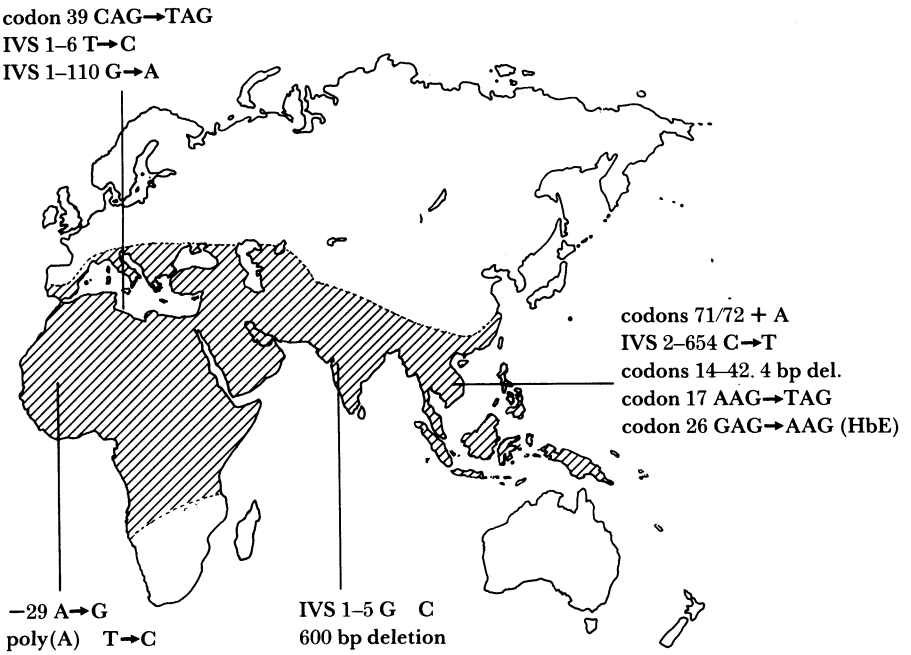

FIGURE 2. World distribution of β thalassaemia.

disease) are found. The distribution of the β thalassaemia mutations is shown in figure 2. It turns out that in each population studied so far there are only a few common mutations that account for the bulk of cases. However, many seriously affected children in all populations studied so far are compound heterozygotes; *ca.* 20% of cases have both a common and a rare mutation (Wainscoat *et al.* 1983; Thein *et al.* 1985*a*).

Phenotype–genotype relations

The clinically important forms of β thalassaemia, and sickle-cell anaemia, show marked clinical heterogeneity (Weatherall & Clegg 1981). This variation may be due to variable severity of the β thalassaemia mutation, the coexistence of α thalassaemia, or the inheritance of a genetic determinant that produces more fetal haemoglobin than usually occurs in severe β thalassaemia or sickle-cell anaemia. A number of 'mild' β thalassaemia mutations have been defined, including point mutations in or near the CAT and TATA boxes, or at the intron–exon junctions. Studies in Mediterranean populations have defined the different phenotypes resulting from the co-inheritance of α and β thalassaemia (Wainscoat *et al.* 1983), and there is increasing evidence that point mutations upstream from the γ globin genes may be involved in the amelioration of β thalassaemia and sickle-cell anaemia by causing unusually high levels of Hb F production in these conditions (Thein *et al.* 1987*b*).

These observations can now be applied to prenatal diagnosis programmes. In many cases it is possible to offer parents precise advice about the likely clinical consequences of the interaction of the different thalassaemia genes.

Restriction fragment length polymorphisms

The arrangement of RFLPs along the β globin gene cluster is not random; they exist in a series of haplotypes that vary between particular populations (Antonarakis *et al.* 1982). Furthermore,

the haplotypes can be subdivided into 5′ and 3′ groups, the relation of which, one to another, appears to be random. This suggests that there is a 'hot spot' for recombination between the two haplotype groups. Within any racial group it is usual to find individual structural haemoglobin variants or β thalassaemia mutations associated with a particular haplotype (Orkin *et al.* 1982*a*). Unfortunately, this phenomenon cannot be used for prenatal diagnosis because, by and large, the same haplotypes occur in normal persons in the same populations. There are a few cases in which individual RFLPs are in strong linkage disequilibrium with particular mutations. These include an *Hpa*I polymorphism and β^S mutation in West Africans (Kan & Dozy 1978), a β^0 thalassaemia mutation and a *Bam*H1 site in Sardinians (Kan *et al.* 1980), and the common form of β^+ thalassaemia in Mediterranean populations (IVS 110) and an *Ava*II site; the latter is absent in about 50% of β thalassaemic chromosomes but only very rarely (4 out of 120) in normal chromosomes in the same populations (Wainscoat *et al.* 1985).

The human α globin gene clusters are highly polymorphic with many RFLPs and two hypervariable regions; recent studies have suggested that heterozygosity approaches 0.95 (Higgs *et al.* 1986). So far no recombination 'hot spots' have been observed. There is a strong association between particular haplotypes and α^0 thalassaemia deletions, although some but not all of the α^+ thalassaemia deletions occur on different haplotypes.

Feasibility studies

Several studies have been carried out, to assess the feasibility of prenatal diagnosis, with DNA obtained by amniocentesis or cvs. Boehm *et al.* (1983) examined amniotic-fluid cell DNA from 78 pregnancies at risk for fetuses with homozygous β thalassaemia; most of the cases were of Mediterranean background and the success rate was about 80%. This study utilized RFLP analysis. Old *et al.* (1984) determined the feasibility of prenatal diagnosis in UK-resident Cypriot and Asian Indian populations by using seven RFLPs in the β globin gene cluster. It was found that 76% of the Asian but only 35% of the Cypriot families had DNA polymorphisms that would permit prenatal diagnosis of a homozygous or compound heterozygous β thalassaemic fetus; in the majority of the remaining families there was a 50% chance of a successful diagnosis of either a normal or a heterozygous fetus. The higher success rate in the Asian population reflected a much greater diversity of RFLP haplotypes than in the Mediterraneans and the presence of a deletion of the β globin gene in north Indian populations. In Cyprus the common β^+ thalassaemia mutation is usually found on an RFLP haplotype that is also the most common normal (β^A) haplotype; thus it is often impossible to distinguish the normal from the β thalassaemia chromosome in heterozygous parents.

This problem has been largely overcome by the discovery of strong linkage disequilibrium between an *Ava*II pseudo β gene polymorphism and the IVS 110 mutation that is particularly common in Cypriots and other Mediterranean populations (Thein *et al.* 1985*b*; Wainscoat *et al.* 1985). Inclusion of this polymorphism in the analysis described above would have increased the feasibility of prenatal diagnosis to between 80 and 90% in the Cypriot population. Similarly, recent studies of a northern Italian population suggest that the same polymorphism increases the prenatal diagnosis success rate from about 70 to 90% (Wainscoat *et al.* 1986).

So far there have only been a limited number of studies of the feasibility of prenatal diagnosis programmes, with the use of oligonucleotide probes to identify specific β thalassaemia mutations. In the study of two Mediterranean populations, with oligonucleotide probes that identify the commonest mutations in these groups (the IVS 1 110 and β 39 nonsense mutations)

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it was found that prenatal diagnosis of β thalassaemia would have been feasible in 65% of Cypriots with the probe for the IVS 110 mutation, and in 75% of Italians by using probes for both mutations (Thein *et al.* 1985*a*). Concurrent haplotype analysis indicated that the relatively low success rate in both populations was due to the presence of several rare β thalassaemia mutations; a number of potentially affected children would have been compound heterozygotes for the common mutation in the population, together with a rare one. Further studies of the Cypriot population have shown that even if the second commonest mutation, a T → C change at position 6 of IVS 1 in the β globin gene, had been analysed by oligonucleotide probe hybridization the success rate would only have increased to 75%; there would still have been a significant number of cases who, because they had at least one rare β thalassaemia mutation, could not have been identified by using a limited number of probes (authors' unpublished observations).

We have now analysed nearly 2000 DNA samples from different families in the U.K. for the purposes of prenatal diagnosis (Old *et al.* 1986*a*). These were from individuals of many different backgrounds but with a predominance of Cypriot and other Mediterranean populations. The overall feasibility of prenatal diagnosis was approximately 80%. Thus, with either RFLP linkage analysis or a limited number of oligonucleotide probes, it does not seem likely that this figure can be improved on, except in rare populations in which the disease is much more homogeneous.

Current results

Boehm *et al.* (1983) have examined amniotic-fluid cell DNA from 78 pregnancies at risk for a fetus homozygous for β thalassaemia; the diagnosis rate was about 80%. The same group have used amniotic-fluid cell DNA to study 138 pregnancies at risk for a fetus with sickle-cell disease. One error was made; a fetus with sickle-cell anaemia was diagnosed as having sickle-cell trait, owing to contamination of the fetal DNA with plasmid DNA. Among 311 prenatal diagnoses carried out in this way at the Johns Hopkins Hospital, this was the only error.

The first successful prenatal diagnoses of β thalassaemia and sickle cell anaemia, by means of CVS-derived DNA, was reported by Old *et al.* (1982). Goossens *et al.* (1983) have also described the successful application of CVS for prenatal diagnosis of sickle-cell anaemia. Experience of over 200 prenatal diagnoses of haemoglobin disorders, with the use of fetal DNA obtained by CVS, was reported recently (Old *et al.* 1986*a*). This included 133 cases at risk for β thalassaemia and 55 for sickle-cell anaemia; there were a few cases at risk for Hb Lepore thalassaemia or homozygous α^0 thalassaemia. Because of the heterogeneous population that comprised this study most of the β thalassaemias were diagnosed by RFLP linkage analysis. A successful diagnosis was made in all but one case, an error due to plasmid contamination. There were two failures due to insufficient material but a diagnosis was made later by amniocentesis. Although there was one example of recombination involving the 'hot spot' in the β globin gene cluster this was noted prenatally and a correct diagnosis was achieved. This study included two sets of twins and a successful diagnosis was made on each of the pair.

The value of having a relatively homogeneous population with respect to β thalassaemia mutations is well shown by the results of Piratsu *et al.* (1983) and Rosatelli *et al.* (1985). Because the majority of cases of β thalassaemia in Sardinia result from the nonsense mutation at position 39 in the β globin gene, by using one oligonucleotide probe they were able to identify 94 couples out of 103 pregnancies at risk for carrying a fetus homozygous for β^0 thalassaemia who had this mutation. DNA was obtained by amniocentesis in 61 cases and by CVS in 33 cases. There were four failures.

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The results of Old *et al.* (1986*a*) indicate that DNA obtained by CVS is much more reliable for prenatal diagnosis than that isolated from uncultured amniotic fluid cells. With the latter there is always the risk of contamination with maternal DNA and the yield is often poor.

The overall results of first-trimester prenatal diagnosis for the haemoglobin disorders, up to July 1986, are summarized in table 2.

TABLE 2. WORLD EXPERIENCE IN FIRST-TRIMESTER PRENATAL DIAGNOSIS OF THE HAEMOGLOBIN DISORDERS, UP TO JULY 1986

(By permission of Dr Hans Galjaard. Figures in parentheses indicate numbers affected.)

		analytical method		
disease	pregnancies tested	direct	oligonucleotide	RFLP
sickle cell	190 (61)	189	1	1
α thalassaemia	48 (10)	42	—	6
β thalassaemia	480 (142)	31	252	197

A comprehensive prenatal diagnosis programme for the haemoglobin disorders

These preliminary studies provide a relatively clear picture of the value of fetal DNA analysis for the prenatal diagnosis of the haemoglobin disorders and allow us to draw some tentative conclusions about how these programmes might be organized in the future. Because these diseases occur at their highest frequencies in parts of the world that are still relatively underdeveloped, it is clear that the simplest and most economical approaches must be taken.

The first requirement for a programme for prenatal diagnosis for the haemoglobinopathies, particularly in the developing countries, is an adequate diagnostic service for accurately defining heterozygotes. This is relatively easy; the methods and technical problems are reviewed in detail elsewhere (Weatherall 1985*b*). The second stage involves a major educational programme to determine whether the population wants prenatal diagnosis and to ensure that they really understand the significance of genetic diseases in their society.

Once we have embarked on a major public health measure of this type, the next step is to determine the common β thalassaemia mutations and the structural haemoglobin variants in the particular population. Because so many thalassaemia mutations have now been described, and because there is at least some information about their world distribution (see figures 1 and 2), the most economical approach to working out the molecular pathology of β thalassaemia is a population oligonucleotide probe survey. This can be combined with an assessment of the RFLP haplotypes, but this approach may not be necessary if sufficient oligonucleotide probes are available. If there are common mutations that are not identified by oligonucleotide probes it may be necessary to clone and sequence the β globin genes. It should be possible to facilitate this procedure by the use of methods for the rapid detection of single base substitutions in genomic DNA (Myers *et al.* 1985). Once the common mutations have been identified, an effort should be made to see if any of them can be identified by using restriction enzymes, or whether they are in linkage disequilibrium with particular RFLP haplotypes. Finally, it is important to determine whether any of the mutations are associated with particularly mild clinical phenotypes. Given this background information it should be possible to set up a prenatal diagnosis programme.

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The pilot studies that have been described in the previous sections leave little doubt that in every population there will be 10–20% of 'at risk' fetuses in which one of the potential β thalassaemia genes will represent a rare mutation. It seems unlikely that it will ever be cost-effective to produce enough oligonucleotide probes to cover all eventualities. Thus families of this type will have to be studied by RFLP linkage analysis and, if this fails, prenatal diagnosis will have to be carried out by fetal blood sampling.

It is therefore apparent that it is not yet feasible to set up a prenatal diagnosis programme for the haemoglobin disorders by using a single method; a few oligonucleotide probes will only cover about 80% of 'at risk' cases and some form of back-up facility for the more difficult families, including RFLP analysis and *in vitro* globin chain synthesis, will be required if the programme is to be completely comprehensive (Weatherall 1985*b*).

Other single-gene disorders

As other single-gene disorders are explored it is becoming clear that we already have a good idea of the repertoire of mutations that result in these conditions. So far, the majority of diseases result from simple *cis*-acting mutations of structural genes. However, as mentioned earlier, at least one form of α thalassaemia results from a deletion that does not involve the structural genes. Clearly, mutations of this type will pose important problems for prenatal diagnosis with genomic DNA.

The degree of heterogeneity of molecular defects is variable. For example, it appears that the mutations of the LDL receptor gene that underlie hereditary hypercholesterolaemia are almost as diverse as the thalassaemias (Brown & Goldstein 1986). On the other hand, some diseases appear to be more restricted in their molecular repertoire. For example, it appears that a particular splice mutation is responsible for the bulk of cases of phenylketonuria in north European populations (DiLella *et al.* 1986). Clearly this will make the prenatal diagnosis of this condition with oligonucleotide probes quite feasible. The same applies to the severe form of α_1 antitrypsin deficiency (Kidd *et al.* 1984). But there is increasing evidence for heterogeneity as evidenced by studies of the molecular pathology of growth hormone deficiency, antithrombin III deficiency and several other autosomal conditions (see Gusella 1986; Cooper & Schmidtke 1986; Davies & Robson 1987).

The X-linked disorders are of particular importance. In many cases these conditions are due to new mutations. The molecular basis of haemophilia and Christmas disease is turning out to be extremely heterogeneous (Lawn 1985; Bentley *et al.* 1986). The muscular dystrophies are of particular interest in this respect (Lindlof *et al.* 1986). The gene for Duchenne muscular dystrophy (DMD) lies in a 137 kb stretch of DNA, DXS164. Eighty-eight of 1346 males with DMD tested in 25 laboratories throughout the world showed a deletion of part or all of DXS164. By using a variety of probes related to this sequence it has been found that, of 52 obligate or possible carriers under the age of 45, prenatal diagnosis would be possible in 49. This has been achieved in a number of 'at risk' pregnancies (Old& Davies 1986; Darras *et al.* 1987); it is currently estimated, from these and other studies, that there is a 5% error rate for carrier detection or prenatal diagnosis of DMD by using this set of probes.

Thus because of the molecular heterogeneity of the X-linked diseases it seems unlikely that oligonucleotide probes will ever have a major role in carrier detection or prenatal diagnosis for these conditions.

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TABLE 3. WORLD EXPERIENCE OF FIRST-TRIMESTER PRENATAL DIAGNOSIS FOR AUTOSOMAL DISORDERS, OTHER THAN HAEMOGLOBINOPATHIES, UP TO JULY 1986

(By permission of Dr Hans Galjaard. Parentheses indicate number of affected fetuses detected.)

dominant		recessive	
disease	no. of analyses	disease	no. of analyses
osteogenesis imperfecta Type IV	1	cystic fibrosis	many
adult polycystic kidney disease	1	phenylketonuria	2 (1)
Huntington's chorea	1	α-1-antitrypsin deficiency	5 (2)
retinoblastoma[a]		adrenal hyperplasia (21-hydroxylase deficiency)	16 (6)

[a] Five DNA analyses in amniotic fluid cells have been performed.

TABLE 4. WORLD EXPERIENCE OF FIRST-TRIMESTER PRENATAL DIAGNOSIS OF X-LINKED DISORDERS, UP TO JULY 1986

(By permission of Dr Hans Galjaard. Parentheses indicate number of probes used.)

disease	no. of pregnancies	no. affected	type of analysis
Duchenne muscular dystrophy	92	31	RFLP (1–9)
Becker variant	7	3	RFLP (1–9)
myotonic dystrophy	1	—	?
haemophilia:			
factor VIII deficiency	70	21	RFLP (2–4)
factor IX deficiency	6	1	RFLP or direct
ornithine transcarbamylase deficiency	8	4	RFLP (1)
Norrie disease	1	—	RFLP (1)
adrenoleucodystrophy	1	1	RFLP (1)
chronic granulomatous disease	1	—	direct
retinitis pigmentosum	3	1	RFLP (1)
total	190	62 (33%)	

A partial list of some of the worldwide results of prenatal diagnosis with fetal DNA is shown in tables 2–4. From experience in the haemoglobinopathies it is clear that CVS is more effective than amniocentesis as a source of DNA. The yield of DNA from uncultured amniotic-fluid cells is low and there is a serious risk of contamination with maternal DNA. Apart from technical problems, such as partial DNA digestion and plasmid contamination, the major difficulties encountered to date have been non-paternity and recombination (Old *et al.* 1986*b*). However, if we consider that these are new techniques, the results are extremely encouraging.

THE FUTURE

What improvements in technology might be applicable to prenatal diagnosis programmes over the next few years? The recently developed methods for *in vitro* enzymic amplification of genomic sequences, the polymerase chain reaction (PCR), increase the sensitivity of restriction enzyme mapping by more than two orders of magnitude and hence reduce the laboratory time required for DNA analysis to less than one day (Saiki *et al.* 1985; Embury *et al.* 1987). By combining this approach with 'dot blot' analysis, it is possible to greatly simplify the prenatal diagnosis of conditions in which the mutation is known (Saiki *et al.* 1986). If these methods

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could be combined with non-radioactive labelling of probes, the procedure would be even simpler.

It is clear, therefore, that fetal DNA analysis is now feasible and that as genes involved in other single gene disorders are isolated prenatal diagnosis will follow naturally. However, many organizational problems remain in applying this technology in both developed and underdeveloped countries. It seems likely that screening with genomic DNA probes will be restricted to particularly common disorders within populations, the haemoglobinopathies in tropical countries and cystic fibrosis in north Europe for example. It is unlikely that it will be feasible to provide a 'blanket' screen for a wide range of genetic diseases by using this technology. Thus its major value will be for families in which there has already been an affected child.

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Discussion

H. Sharma (71 *Barrack Road, Hounslow, U.K.*). Thalassaemia trait is thought to have an advantage in malarious regions. Malaria is now on the increase in some regions. What is the role of the geneticist in preventing thalassaemia and affecting host–parasite relations?

D. J. Weatherall, F.R.S. There is reasonably good epidemiological evidence that both α and β thalassaemia carriers show increased resistance to *P. falciparum* malaria. The cellular mechanisms remain to be determined. If prenatal diagnosis programmes tend to encourage thalassaemia families to have children the overall effect will be to increase the carrier rate rather than to reduce it; thus it will not have any major effect on the numbers of susceptible people in malarious regions.

H. Sharma. Is there any advantage in expression of fetal haemoglobin in adults?

D. J. Weatherall, F.R.S. This is an interesting problem. *In vitro* invasion experiments have suggested that relatively high levels of fetal haemoglobin protect red cells against *P. falciparum* malaria. However, the only condition in which there is persistent fetal haemoglobin production in the absence of a serious haemoglobinopathy, like sickle-cell anaemia or β thalassaemia, is hereditary persistence of fetal haemoglobin (HPFH). Interestingly, high gene frequencies of HPFH have never been found in malarious areas or in any other populations. This would suggest that either these are very recent mutations or that the effect of fetal haemoglobin in protection against malaria is very small. Of course there is some evidence that newborn infants are relatively resistant to *P. falciparum* malaria but there are good immunological reasons for this; it probably does not reflect their high levels of fetal haemoglobin. Perhaps the only possible relation between fetal haemoglobin and malaria is the fact that β thalassaemia heterozygotes have higher levels of fetal haemoglobin during the first six months of life than normal individuals; malaria usually affects infants from the second to third month onwards and it is just possible that this might explain some of the protection afforded to β thalassaemia heterozygotes in malarious areas.

A. E. H. Emery (*The Medical School, University of Edinburgh, U.K.*). In those disorders where major gene rearrangements are the exception and new mutations are frequent, and therefore isolated cases are common, how likely is it that we will be able to use a battery of oligonucleotide probes for carrier detection and prenatal diagnosis in the future?

D. J. Weatherall, F.R.S. This is a very important problem. It seems to me that it is very unlikely that oligonucleotide probe diagnosis will play a major role in carrier detection or prenatal diagnosis of the common X-linked disorders, so many of which are due to new mutations. It would require the development of an enormous battery of probes and I do not think this is likely to be feasible. As I said earlier, even the thalassaemias, which seem to result from a few mutations that are common in particular populations, cannot all be covered with oligonucleotide probes. Their main use may be in more homogeneous genetic disorders like phenylketonuria in north European populations.

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B. Modell (*University College Hospital, London, U.K.*). Is the *Ava*II polymorphism that is in linkage disequilibrium with the Cypriot β^+ thalassaemia gene proximal or distal to the 'hot spot' for recombination? If distal, can one use it to calculate the likely frequency of crossing over? The extent of linkage could be relevant.

D. J. Weatherall, F.R.S. The *Ava*II polymorphic site is on the other side of the 'hot spot' from the β globin gene. The polymorphism is found in about one half of patients with the common Mediterranean β thalassaemia mutation but very rarely on normal chromosomes. The problem is that this particular chromosome must have come under strong selection and this would cloud any numerical analysis between the ratio of chromosomes in which these two genes are linked compared with cases where the mutation is found on a chromosome without the polymorphism. Furthermore, it is always possible that the mutation has occurred on more than one occasion. For these reasons I doubt if we could calculate the crossover frequency at the putative hot spot by looking at distributions of this kind.

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