Prenatal diagnosis of the common haemoglobin disorders

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SUMMARY New techniques of DNA analysis have been applied to the prenatal diagnosis of the common haemoglobin disorders. Currently, it is possible to provide a comprehensive programme for the prevention of these conditions, although this entails the use of several different techniques including globin chain synthesis analysis, direct identification of mutations with restriction enzymes, linkage analysis of restriction fragment length polymorphisms, and the use of oligonucleotide probes. At present, the best combination of these approaches has to be worked out for individual populations, but as the techniques of chorion villus sampling and DNA analysis improve it should be possible to rationalise these prenatal diagnosis programmes and thus make them simpler and less expensive.

Between 1965 and 1974, two of us (JBC and DJW) worked in the Nuffield Institute of Medical Genetics, Department of Medicine, University of Liverpool. Cyril Clarke, then its director, allowed us to continue our studies of haemoglobin synthesis in thalassaemia which we had started together in the USA. At the time he, like many of our colleagues, may well have wondered about our sanity in pursuing research into what must have seemed a rather exotic group of anaemias of little obvious relevance to clinical practice in the UK. If he did he certainly never let us know, and he encouraged our efforts in every way possible. Because one of the first practical applications of this work was for the prenatal diagnosis of the common haemoglobin disorders we thought that an assessment of the current state of this important clinical problem might be an appropriate way of expressing our thanks for Cyril's scientific support and friendship over many years.

Background

The inherited disorders of haemoglobin are the commonest single gene diseases and cause a major public health problem in many parts of the world.¹ As the high neonatal and childhood death rates due to infection and malnutrition in the developing countries are controlled, these conditions will create an even greater drain on health resources. For this

reason many countries are, or are considering, developing prenatal diagnosis programmes for the prevention of the important haemoglobin disorders, particularly sickle cell anaemia, β thalassaemia, some forms of α thalassaemia, and disorders which result from the interaction of structural haemoglobin variants and thalassaemia, notably sickle cell or haemoglobin E- β thalassaemia.

The first attempts at prenatal diagnosis of the haemoglobin disorders made use of fetal blood sampling and in vitro globin chain synthesis to determine directly the products of the mutant gene loci. More recently, techniques have been developed for isolating fetal DNA, either by amniocentesis or chorion villus sampling. Several methods have been used to analyse the DNA including direct identification of the molecular lesion by restriction endonuclease mapping, determination of the chromosome carrying the abnormal globin gene by linkage using restriction fragment length polymorphisms (RFLPs), and, most recently, detecting specific mutations directly with oligonucleotide probes.

Even though this field is still in a state of flux, enough experience has been gained for us to begin to consider the most reliable and economic approach to setting up comprehensive services for the prevention of these common genetic disorders, particularly in the developing countries. To this end we shall summarise current experience in the application of the different techniques of fetal DNA analysis for the prenatal diagnosis of the important

inherited disorders of haemoglobin. Details of the various methods employed have been reviewed recently^{2,3} and will not be described here.

Carrier detection

Carrier detection for the important haemoglobin disorders can be carried out by population screening or in the antenatal clinic and, in the majority of cases, presents no problems. The blood of all women of appropriate racial backgrounds should be screened for sickle cell haemoglobin, and, using an electronic cell counter, the size of their red cells should be determined. The haematological changes that should raise the suspicion of thalassaemia, and their distinction from iron deficiency, are described in detail by Weatherall.⁴ The diagnosis of heterozygous β thalassaemia should be confirmed by estimating the haemoglobin A₂ level. The main practical difficulties that are encountered in thalassaemia screening are distinguishing the heterozygous state for α^0 thalassaemia from the homozygous state for α^+ thalassaemia, the identification of carriers of the rare form of β thalassaemia associated with a normal haemoglobin A₂ level, and the masking of the diagnosis of heterozygous β thalassaemia by the coexistence of one or more α thalassaemia determinants. A practical guide to distinguishing between these different forms of thalassaemia is described by Weatherall.⁴

Fetal blood sampling

The prenatal diagnosis of sickle cell anaemia and different forms of thalassaemia by fetal blood sampling and globin chain synthesis studies is now well established. An analysis of over 4000 diagnoses carried out in this way has been reported recently.² A breakdown of the results of this survey, including the failure rate and complications, is shown in table 1. It is apparent that in experienced hands this approach is now relatively safe and reliable. Its major disadvantage is that fetal blood sampling

FIG 1 The relationship between the DNA content and wet weight of chorion biopsy material. The yield of DNA in a CVS series is shown in the right hand column.

cannot be carried out until late in the second trimester which means a long period of uncertainty for the mother and, if indicated, an often difficult therapeutic abortion at 20 weeks' gestation or later. Although many analytical approaches have been used to try to bypass the well tried haemoglobin synthesis method (see Alter²), none has proved entirely reliable.

Many women find the thought of waiting until the end of the second trimester before a decision can be made about termination of pregnancy quite intolerable. However, the overall results of prenatal diagnosis by fetal blood sampling have been remarkably successful and this approach has led to a major decline in the incidence of new cases of β thalassaemia in several populations.¹ A further advantage of this method is that it can be used for the prenatal diagnosis of a variety of other haematological disorders.^{2,4}

DNA analysis

SOURCES OF FETAL DNA

Fetal DNA can be obtained either from amniotic fluid cells or by chorion villus sampling (CVS). The yield of DNA from amniotic fluid cells is usually low, and if it is prepared directly great care has to be taken to ensure that there is no contamination with maternal blood or tissue. Unless the diagnosis can be made with a single enzyme it is usually necessary to grow amniotic fluid cells in culture to obtain sufficient DNA. In practice this means that it is not possible to obtain a diagnosis until late in the second trimester.

There is now considerable experience of prenatal

TABLE 1 Results of prenatal diagnosis of haemoglobin disorders by fetal blood sampling, June 1974 to December 1984. (Data from Alter.²)

	Thalassaemia	Sickle cell anaemia	Total (%)
No of cases	3636	323	3959 (100)
Errors	24	4	29 (0.8)
Losses	230	14	244 (6.2)
Maternal complications	62	2	64 (1.6)
Premature labour	169	13	182 (6.5)

diagnosis of the haemoglobin disorders using DNA obtained by CVS.^{5,6} The yield of DNA obtained by this technique in the authors' laboratory and the linear relationship of the amount of DNA to the weight of chorion biopsy tissue is shown in fig 1. On average it is possible to obtain about 20 µg of DNA from CVS. The most recent information for fetal loss following this procedure gives a figure of about 4% (B Modell, 1985, personal communication). So far there have been no follow up data on the patterns of development of infants or children following pregnancies in which CVS has been carried out; this information will not be available until the results of current trials comparing amniocentesis and CVS for prenatal diagnosis have been evaluated.

HAEMOGLOBIN DISORDERS AMENABLE TO DIRECT DETECTION BY GENE MAPPING

In working out a strategy for prenatal diagnosis programmes using fetal DNA it is important to appreciate the molecular heterogeneity of the haemoglobin disorders. Most of the structural haemoglobin variants result from single base mutations. The α^0 thalassaemias are due to large deletions of the α globin gene complex which can be identified directly by gene mapping.⁷ The α^+ thalassaemias consist of both deletion and non-deletion variants. However, of the 40 or so different mutations which underline β thalassaemia⁸⁻¹⁰ there is only one large gene deletion; the remainder are single base changes or deletions or insertions of one, two, or four bases.

TABLE 2 *Haemoglobin disorders that can be identified directly by fetal DNA analysis. (Appropriate restriction enzymes shown in brackets.)*

Structural haemoglobin variants*	
Haemoglobin S	(DdeI, MstII, CvnI)
Haemoglobin O Arab	(EcoRI)
Haemoglobin Lepore	
Thalassaemias†	
α^0 thalassaemia	
α^+ thalassaemia (deletion)	
α^+ thalassaemia (non-deletion)	
5 bp deletion IVS-1	(HphI)
T→C initiation codon	(NcoI)
β^0 thalassaemia (deletion)	
β^0 thalassaemia	
IVS-2 splice junction	(HphI)
IVS-1 (-25 bp)	(Fnu4H, MstII)
IVS-1 consensus	(SfaNI)
Codon 6 (-1 bp)	(MstII)
Codon 39, chain termination	(MaeI)
β^+ thalassaemia	
IVS-2 745	(RsaI, KpnI)
-87 C→G	(AvrII)

* Rare variants which can also be identified directly with restriction enzymes are described by Trent *et al.*¹¹

† For a description of the different thalassaemia mutations see Orkin *et al.*⁹ and Weatherall and Wainscoat.¹⁰

The haemoglobin disorders that can be identified directly by restriction endonuclease mapping are summarised in table 2. Of the several enzymes capable of identifying the A→T change that characterises the sickle cell mutation, *MstII* is the most reliable.¹² Trent *et al.*¹¹ have reviewed the less common structural haemoglobin variants which can be identified directly in this way.

Of the β thalassaemia mutations that can be identified directly by restriction enzymes (table 2), the $\beta 39$ nonsense mutation, which can be diagnosed by the enzyme *MaeI*,¹³ is the commonest. Apart from these conditions, the α^0 thalassaemias, two types of non-deletion α^+ thalassaemia, the form of β thalassaemia due to a partial gene deletion which is found in Indian populations, and the haemoglobin Lepore disorders, direct identification of individual mutations with restriction enzymes is not yet possible for the prenatal diagnosis of the major haemoglobin disorders.

RFLP LINKAGE ANALYSIS

Scattered throughout the globin gene clusters every few hundred bases or so, there are single base changes that produce either new restriction enzyme sites or remove previously existing ones.^{8,9,14,15} These harmless polymorphisms provide useful linkage markers for the globin gene complexes.

The arrangement of RFLPs along the β globin gene cluster is not random; they exist in a series of patterns, or haplotypes, in particular populations. Within any racial group it is usual to find individual β thalassaemia mutations associated with a specific RFLP haplotype.^{8,9,15} In other words, these mutations are in linkage disequilibrium with groups of RFLPs in the β gene cluster. However, this phenomenon cannot be used for prenatal diagnosis since 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 a *HpaI* polymorphism and the β^S mutation in West Africans,¹⁶ a β^0 thalassaemia mutation and a *BamHI* site in Sardinians,¹⁷ and the common form of β^+ thalassaemia in Mediterranean populations and an *AvaII* site.¹⁸ The latter site is absent in about 50% of β thalassaemic chromosomes but only very rarely (four out of 120) from normal chromosomes in the same population. More recently it has been established that the particular form of thalassaemia which is in such strong linkage disequilibrium with this RFLP is the IVS-1 position 110 mutation (S L Thein *et al.*, unpublished observations). The latter polymorphism promises to be extremely useful for the prenatal diagnosis of β thalassaemia in this region (see later section).

Since so few of these so-called allele linked polymorphisms have been found to date, and since their linkage to the different thalassaemia mutations is not absolute, in order to identify whether a fetus has inherited two β thalassaemia chromosomes it is usually necessary to look for an RFLP linkage by a family study. This means analysing previously born children or lateral relatives. Thus it is not often possible to use this approach for prenatal diagnosis in first pregnancies.

OLIGONUCLEOTIDE PROBES

The DNA probes that are used for standard gene mapping are too long to detect single base changes of the kind which cause many forms of thalassaemia. However, under appropriate conditions short synthetic DNA fragments (oligonucleotides or oligomers) can be constructed that will hybridise to homologous but not to heterologous sequences, that is, those with any mismatch, even a single base difference.^{19 20} Wallace's group found that short probes consisting of 19 nucleotides (19-mers), which contain the sequence of the normal β globin gene in the region of the β^S mutation, hybridise efficiently with the normal gene but not with DNA which

contains the sickle cell mutation. Using high specific activity labelling of these synthetic probes, normal and sickle sequences can be distinguished after hybridisation of restricted total human DNA. Two 19-mers are made, one directed against the normal sequence, the other against the sequence with the altered base. DNA from normal persons will hybridise only to the normal oligoprobe and DNA from sickle cell homozygotes will hybridise only to the sickle cell oligoprobe; both probes will hybridise to DNA from haemoglobin S carriers.

As well as specific oligonucleotide probes against the β^S mutation, probes have also been made to detect several common β thalassaemia mutations^{3 21-23} (fig 2).

Results of fetal DNA analysis to date

Several centres have described their preliminary experiences with the use of fetal DNA obtained from amniotic fluid cells for prenatal diagnosis of β thalassaemia or sickle cell anaemia. Boehm *et al*²⁴ examined amniotic fluid cell DNA from 78 pregnancies at risk for fetuses with homozygous β thalassaemia; the diagnosis rate was about 80%. The same

FIG 2 Autoradiogram of a family study using the β^{39} oligonucleotide probes. Hybridisation was with the β^A and β^{Th} (β^{th}) probes. Lane β^N =normal β plasmid DNA; β^O = β^{39} plasmid DNA; Ch=child; F=father; M=mother; $\beta^O\beta^O$ =normal genomic DNA; $\beta^A\beta^A$ =homozygous β^{39} genomic DNA.

group have used amniotic fluid cell DNA to analyse 138 pregnancies at risk for a fetus with sickle cell disease.²⁵ One error was made; a fetus with sickle cell anaemia was diagnosed as having sickle cell trait due to exogenous contamination of the fetal DNA with plasmid DNA from the laboratory. Among 311 prenatal analyses carried out in this way at the Johns Hopkins Hospital, Baltimore, this was the only error.²⁵

The first successful prenatal diagnoses of β thalassaemia and sickle cell anaemia using CVS were reported by Old *et al.*⁵ Goossens *et al.*⁶ have also described the successful application of CVS for the prenatal diagnosis of sickle cell anaemia. In Oxford we have now carried out nearly 100 prenatal diagnoses for β thalassaemia, haemoglobin Lepore, sickle cell anaemia, and homozygous α^0 thalassaemia using CVS (table 3). The majority of the cases of β thalassaemia have been identified by RFLP linkage analysis; DNA from pregnancies at risk for homozygous α^0 thalassaemia, haemoglobin Lepore thalassaemia, and the deletion form of β^0 thalassaemia were analysed directly. The only difficulties encountered were a bizarre RFLP analysis which turned out to be due to non-paternity, although it was still possible to determine the genotype of the fetus, one family in which there appeared to be a crossover within the β globin gene complex,²⁶ and one instance of plasmid contamination.

To date, except for a few individual case reports, there has been little published experience of the use of oligonucleotide probes for prenatal diagnosis of the haemoglobin disorders. In a recent study Rosatelli *et al.*²² described 103 pregnancies at risk for carrying a fetus homozygous for β^0 thalassaemia in Sardinia. Using oligonucleotide probes for the β^0 mutation at position 39, 94 couples were identified in which both parents were heterozygous for this mutation. DNA was obtained by amniocentesis (61

cases) or CVS (33 cases). Prenatal diagnosis in those monitored with amniocentesis was carried out by DNA analysis from uncultivated amniotic fluid cells (19) or cultivated cells (38). In four pregnancies the results were uncertain, CVS was unsuccessful in one pregnancy and gave an incorrect diagnosis in another because of maternal contamination. We have used oligonucleotide probes for the common IVS-1 110 and the IVS-1 6 (T→C) mutations for prenatal diagnosis in first pregnancies in UK resident Cypriots (S L Thein *et al.*, unpublished data) (table 4).

Feasibility studies

While these results are encouraging, the critical question that needs to be answered before prenatal diagnosis programmes can be developed is, given the heterogeneity of β thalassaemia, what is the feasibility of using these different approaches for prenatal diagnosis in different high risk populations? Over the last few years we have systematically studied this problem by analysing families with one or more homozygous β thalassaemic children, using the techniques outlined in the previous sections.

Old *et al.*²⁷ have determined the feasibility of prenatal diagnosis in UK resident Cypriot and Asian Indian populations using seven RFLPs in the β globin gene cluster. A total of 20 Cypriot and 42 Asian patients and their families was studied. It was found that 76% of the Asian and 35% of the Cypriot families had DNA polymorphisms which would have permitted prenatal diagnosis of a homozygous or compound heterozygous β thalassaemic fetus; in the majority of the remaining families there would have been a 50% chance of a successful diagnosis of either a normal or a heterozygous fetus. The higher success rate in the Asian population reflects a greater diversity of RFLP haplotypes than in Mediterraneans. In Cyprus, for example, the common β^+ thalassaemia mutation is most often found on an RFLP haplotype which 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 the strong linkage disequilibrium between the *Ava*II β gene polymorphism and the IVS-1 110 mutation which is particularly common in Cypriots and in other Mediterranean populations.²⁸ 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.²⁹ Similarly, recent studies of a northern Italian population suggest that including the *Ava*II polymorphism would increase the pre-

TABLE 3 Summary of first trimester diagnoses for haemoglobinopathies carried out at Oxford*.

Disorder	No	Outcome		
		Normal	Heterozygous	Homozygous
Sickle cell anaemia	30	4	17	11
β thalassaemia	57	6	24	16
α^0 thalassaemia	5	0	4	1
Hb Lepore	1	1	0	0
Hb S/Hb C	1	0	1	0
Hb S/ β thalassaemia	1	0	0	1

* In 11 of the 57 cases of β thalassaemia, there was only a 50% chance of a successful diagnosis. The results for these cases are: normal or heterozygous, 7; heterozygous or homozygous, 4.

TABLE 4 Prenatal diagnosis by oligoprobe analysis.

Family	Results				Diagnosis
	IVS-1 110 β^+		IVS-1 6 β^+		
	β^A	β^{thal}	β^A	β^{thal}	
(1) Cypriot β thal					
F	+	+	ND	ND	β^A/β^{th}
M	+	+	ND	ND	β^A/β^{th}
Fetus	+	—	ND	ND	β^A/β^A (Normal)
(2) Cypriot β thal					
F	+	+	ND	ND	β^A/β^{th}
M	+	+	ND	ND	β^A/β^{th}
Fetus	+	—	ND	ND	β^A/β^A (Normal)
(3) Cypriot β thal					
F	+	—	+	+	β^A/β^{th}
F	+	+	+	—	β^A/β^{th}
Fetus	+	+	+	—	β^A/β^{th} (Carrier)

natal diagnosis success rate from about 70 to 90%²⁸ (table 5).

Because RFLP linkage analysis is only possible in families in which there are appropriate members available for establishing identity of the chromosomes carrying β thalassaemia mutations, this approach is not usually feasible for the first pregnancies, particularly if only the parents are available and there are no lateral relatives.

Recently we have carried out another feasibility study in two Mediterranean populations using oligonucleotide probes that identify the commonest mutations in these countries, that is, the IVS-1 110 and the β 39 nonsense mutations.²³ This study also included an analysis of the RFLP haplotypes associated with the β thalassaemia mutations in each family. Twenty families from the UK resident Cypriot population and 16 families from Milan were studied. The results suggest that prenatal diagnosis of β thalassaemia would have been feasible in 65% of Cypriots using oligonucleotide probes for the IVS-1 110 mutation and in 75% of Italians using

probes for both mutations. 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 affected children were compound heterozygotes for the common mutation in the population and a rare form. Further analysis of the Cypriot population suggested that even if the second commonest mutation, the T→C change at position 6 of IVS-1 in the β globin gene,³⁰ had been analysed by oligonucleotide probe hybridisation the success rate would only have been increased to 75%; there was still a significant number of cases who, because they had at least one rare β thalassaemia mutation, could not have been identified by oligonucleotide probe analysis using a limited number of probes.

Implications for the future development of prenatal diagnosis programmes for the common haemoglobin disorders

There is no doubt from the recent summary of the

TABLE 5 Feasibility of prenatal diagnosis of β thalassaemia in Cypriots and N Italians by RFLP analysis.

Population	Linkage analysis	Total	100% diagnosis	50% diagnosis	No diagnosis
Cypriot	Standard 7 sites	43	11 (25%)	14 (33%)	18 (42%)
	7 sites + <i>Ava</i> II β	43	30 (70%)	12 (28%)	1 (2%)
Italian	Standard 7 sites	25	19 (76%)	6 (24%)	0
	7 sites + <i>Ava</i> II β	25	23 (92%)	2 (8%)	0

use of fetal blood sampling and globin chain synthesis for prenatal diagnosis of the common haemoglobin disorders² that this approach is extremely reliable. It has the advantage that it measures directly the product of the mutant gene, the ideal way of identifying a genetic disorder. However, because it can only be carried out late in the second trimester it carries the major disadvantage of a long wait for the mother and a potentially difficult termination. Thus it cannot be the long term answer to the prenatal diagnosis of these disorders. There is now enough published experience of direct DNA analysis, using either amniotic fluid cells or CVS, to be certain that this approach is an extremely valuable alternative to fetal blood sampling. Until the results of current trials are known, it is still too early to be certain whether CVS will replace amniocentesis in the long term.

The recent feasibility studies using one or two oligonucleotide probes for the common mutations in Mediterranean populations suggest that it will be possible to establish a reliable prenatal diagnosis in only about 60 to 70% of first pregnancies. This is because of the distribution of β thalassaemia mutations, which seem to be similar in most populations. Thus, it appears that in every country in which this disorder is common there are a few very common mutations and a variable number of rare ones.^{8 31 32} This means that in every population a proportion of all seriously affected children will be compound heterozygotes, often for one of the common mutations and a rare one. Thus, in order to cover all eventualities it will be necessary to sequence many β thalassaemia genes and construct large numbers of oligonucleotide probes. Although oligonucleotide probe technology will improve and rapid sequencing methods are already available, it seems unlikely that it will be feasible to mount a massive operation of this kind in every high risk population in the immediate future.

These problems, which reflect mainly the heterogeneity of β thalassaemia, mean that there will be a significant number of families in which a diagnosis by fetal DNA analysis is not possible in the first pregnancy. The feasibility studies using RFLP linkage analysis, particularly if other useful allele linked RFLPs are found, suggest that in many populations it may be possible to establish a useful linkage in 80 to 90% of second or subsequent pregnancies. If oligonucleotide probe analysis were included, the feasibility for diagnosis in second pregnancies might be even higher. It should be remembered, however, that this approach is not without its problems, including non-paternity, the probable existence of at least one 'hot spot' for recombination within the β globin gene cluster,^{15 26} and the tedious and com-

plex analysis which it entails. It is too early to be sure whether it is applicable for widespread use, particularly in developing countries.

Another uncertainty is the degree to which oligonucleotide probe technology can be simplified. Currently, hybridisation conditions are absolutely crucial, and labelling of the probes is difficult and carries the risk of considerable exposure to radioactivity. The development of non-radioactive probes and simpler hybridisation techniques may make this approach much more feasible in the future, although whether it will reach the stage where it is suitable for use in a routine clinical laboratory remains to be seen. Similarly, it is not yet clear how many new RFLPs will be discovered which, like the *Ava*II polymorphism in Mediterranean populations, will be generally useful for prenatal diagnosis, or whether new restriction enzymes will become available for identifying specific mutations.

Given all these uncertainties, and the rapid speed of development and change in the technology of DNA analysis, what is the best current approach to developing a reasonably comprehensive service for prenatal diagnosis of the common haemoglobin disorders? Obviously, in centres in which adequate screening and obstetric facilities exist, and where both haemoglobin synthesis and DNA analysis technology are fully developed, it is now possible to achieve close to 100% success rate for the prenatal diagnosis of these disorders using oligonucleotide probe or RFLP analysis or both, backed up by fetal blood sampling and globin chain synthesis for the relatively few cases in which a diagnosis by direct DNA analysis is impossible.

Thus, for the time being it will be necessary to use a combination of approaches to run a comprehensive prenatal diagnostic service. Since the ultimate aim is simplicity, further attempts should be made to find enzymes which will identify individual β thalassaemia mutations directly or RFLPs which are capable of clearly distinguishing thalassaemic from normal chromosomes in particular populations. The evolution of the prenatal diagnosis programme in Sardinia is a good example of how this field might progress. It started with globin chain synthesis studies and moved to RFLP linkage and oligonucleotide probe analysis; now, since a restriction enzyme has been discovered which is capable of diagnosing the majority of cases of thalassaemia in the population, it should be possible to use this relatively simple approach for most cases. However, there are few populations like Sardinia, in which a single mutation is found in the majority of cases, and in most countries it will be necessary to use a multi-pronged approach, at least for the foreseeable future.

And what of countries which are considering setting up a comprehensive prenatal diagnosis programme de novo? First, a screening programme must be developed and the appropriate obstetric expertise obtained for fetal blood sampling, amniocentesis, and CVS. The necessary skills for DNA analysis should be developed at the same time. The next step will be to determine the common β thalassaemia mutations in the population. While this is being done, a limited programme could be developed using fetal blood sampling and RFLP analysis. The most simple approach to identifying the common β thalassaemia mutations in a particular population is to analyse the RFLP haplotypes; as mentioned earlier, specific mutations are usually associated with a particular haplotype.^{8,9} Current experience suggests that in each population there will be one or two common β thalassaemia mutations. When the haplotypes have been analysed it should be possible to work out the molecular basis for the related β thalassaemias using either oligonucleotide probes or by sequencing the β globin genes. Since the latter is still a sophisticated and time consuming procedure it may be necessary to set up one or more central laboratories, possibly WHO designated, to carry out this work. Once the mutations have been defined it should be feasible to set up a comprehensive prenatal diagnosis service. A limited number of oligonucleotide probes should be synthesised to detect the common mutations. Prenatal diagnosis in first pregnancies should be carried out using these probes or fetal blood sampling; in subsequent pregnancies it should be possible to use oligonucleotide probes or RFLP analysis or both. In each population it will be very important to search for RFLPs which may distinguish thalassaemic from normal chromosomes and, in particular, for restriction enzymes which are capable of identifying the common mutations.

In summary, a country wishing to set up a comprehensive prenatal diagnosis programme for the haemoglobin disorders should develop a competent screening, obstetric, and genetic counselling service, backed up by a well planned approach to educate the public and health administrators about the diseases and why they should be prevented. At the same time laboratory expertise in globin chain synthesis and DNA analysis should be developed, and the common β thalassaemia mutations in the community should be defined. Until prenatal diagnosis by DNA analysis is feasible for every case, it will be necessary to retain the necessary skill for globin chain analysis. The most appropriate diagnostic technique for a particular population should be worked out and form the mainstay of the prevention programme. Future research should

concentrate on simplifying the analytical techniques for DNA analysis, and on looking at ways of obtaining fetal DNA from other sources, either the maternal circulation or, possibly, from fertilised ova.

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