

# Guidelines for the fetal diagnosis of globin gene disorders

Globin Gene Disorder Working Party of the BCSH General Haematology Task Force

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### Working Party Members:

M Bhavnani  
M Brozovic (chair)  
J M Old  
A D Stephens

### Task Force Members:

B J Bain  
M Brozovic (chair)  
D W Dawson  
J M England  
R M Rowan  
A D Stephens  
S M Lewis  
N K Shinton  
M F Murphy  
J K Wood

Correspondence to:  
Dr J M Old, Institute of  
Molecular Medicine, John  
Radcliffe Hospital, Oxford,  
OX3 9DU.

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## Introduction

The inherited disorders of haemoglobin comprise a vast group that include the thalassaemias and sickle cell disease and can involve complex interactions between several different mutant genes. Most of these mutations can now be detected directly by DNA analysis which allows fetal diagnosis to be carried out for couples at risk of an affected fetus without the necessity for linkage studies on any children or relatives. Each mutation is detected by its own specific probes or primers. It is therefore vital to determine accurately the parental genotypes, preferably before fetal diagnosis to avoid mistakes if mutations are missed because of an incorrect diagnosis of the carrier state. It also means that a large number of probes and primers are required, especially for the identification of  $\beta$  thalassaemia mutations, and it is recommended that fetal diagnosis by DNA analysis is only undertaken in referral centres.

The aim of this paper is to: (a) summarise the types of haemoglobin disorders for which fetal diagnosis may be indicated; (b) review the current procedures which are used for fetal diagnosis; (c) provide guidelines for clinicians requesting a fetal diagnosis test; and (d) provide guidelines for clinicians and scientists carrying out a fetal diagnosis test.

## Indications for prenatal diagnosis

The important haemoglobin disorders in the United Kingdom populations at risk are the

thalassaemias, sickle cell disease, and their interactions. These are summarised in the table. All the clinical details, etc, of the thalassaemias and their interactions can be found in the authoritative book by Weatherall and Clegg.<sup>1</sup>

### $\alpha^0$ THALASSAEMIA

The  $\alpha$  thalassaemias are a group of disorders characterised by a reduction in  $\alpha$  globin synthesis. They can be divided into the severe types ( $\alpha^0$  thalassaemias), which have two  $\alpha$  globin genes deleted per chromosome, and the mild types ( $\alpha^+$  thalassaemias), which have one  $\alpha$  globin gene per chromosome either deleted or non-functional. Alpha thalassaemia is most frequently due to deletions, although  $\alpha^+$  thalassaemia can also be caused by point mutations.<sup>2</sup> The homozygous state for  $\alpha^0$  thalassaemia results in haemoglobin Bart's hydrops fetalis syndrome and is lethal, leading to stillbirth or early neonatal death. Prenatal diagnosis for the homozygous state is normally required as the mother of such an infant may have toxemia of pregnancy, obstructed labour, and ante- or post-partum haemorrhage, as well as the psychological burden of carrying a non-viable fetus to term.

Alpha<sup>0</sup> thalassaemia is particularly common in South East Asia (China, Thailand, Vietnam, Malaysia, the Philippines) and also occurs in the eastern Mediterranean countries (Greece, Cyprus). It is extremely rare in Africa, the Caribbean, and India, where  $\alpha^+$  thalassaemia predominates. The compound heterozygous condition of  $\alpha^0/\alpha^+$  thalassaemia results in HbH disease in which three out of the four  $\alpha$  globin genes are deleted or non-functional. Many patients with HbH disease, however, lead a relatively normal life and thus fetal diagnosis is not normally requested. In the United Kingdom there have been 28 prenatal diagnoses carried out for homozygous  $\alpha^0$  thalassaemia up to 1992. These were for couples mainly of Chinese, Vietnamese, and Cypriot extraction.<sup>3</sup>

### $\beta$ THALASSAEMIA

Beta thalassaemia is now known to be caused by more than 100 different mutations,<sup>4</sup> although in each at-risk population, only a limited number of molecular defects are usually prevalent.<sup>5</sup> The mutations differ greatly in their phenotypic effect. At one end of the spectrum are a group of rare mutations involving exon 3 of the  $\beta$  globin gene. These are so severe that they behave in a dominant manner and may produce thalassaemia intermedia in the heterozygous state.<sup>6</sup> At the other end are mild alleles which produce the

## Phenotypes of thalassaemias, sickle cell disease, and various thalassaemia interactions

Type	Phenotype
<b>Homozygous state</b>	
$\alpha^0$ thalassaemia	Hb Bart's hydrops fetalis
$\beta$ thalassaemia:	
$\beta^0$ or severe $\beta^+$ mutation	Thalassaemia major
mild $\beta^+$ mutation	Thalassaemia intermedia
$\delta\beta^0$ thalassaemia	Thalassaemia intermedia
Hb Lepore	Variable: intermedia to major
Hb S	Sickle cell disease
Hb C	No clinical problems
Hb D	No clinical problems
Hb E	No clinical problems
<b>Compound heterozygous state</b>	
$\beta^0$ /severe $\beta^+$	Thalassaemia major
mild $\beta^+$ / $\beta^0$ or severe $\beta^+$	Variable: intermedia to major
$\delta\beta^0$ / $\beta^0$ or severe $\beta^+$	Variable: intermedia to major
$\delta\beta^0$ /mild $\beta^+$	Mild thalassaemia intermedia
$\delta\beta^0$ /Hb Lepore	Thalassaemia intermedia
Hb Lepore/ $\beta^0$ or severe $\beta^+$	Thalassaemia major
Hb C/ $\beta^0$ or severe $\beta^+$	$\beta$ thalassaemia trait $\rightarrow$ intermedia
Hb D/ $\beta^0$ or severe $\beta^+$	Variable: usually similar to $\beta$ thalassaemia trait
Hb E/ $\beta^0$ or severe $\beta^+$	Variable: intermedia to major
O Arab/ $\beta^0$	Severe thalassaemia intermedia
Hb S/ $\beta^0$ or severe $\beta^+$	Sickle cell disease
Hb S/mild $\beta^+$	Usually mild sickle cell disease
Hb S/Hb C	Sickle cell disease, variable severity
Hb S/Hb D Punjab	Sickle cell disease
Hb S/Hb O Arab	Sickle cell disease

phenotype of  $\beta$  thalassaemia intermedia in their homozygous state and, in just a few cases, are so mild in the heterozygous state that they are phenotypically silent with normal mean corpuscular volume concentrations and Hb A<sub>2</sub>.<sup>7</sup> However, most  $\beta$  thalassaemia mutations lie inbetween these two extremes. These are the  $\beta^+$  and  $\beta^0$  alleles which cause  $\beta$  thalassaemia major either in the homozygous state for one allele or in the compound heterozygous state with two different alleles. By defining the mutations it is now possible, in many cases, to predict whether a fetus is at risk for transfusion dependent thalassaemia major or the milder non-transfusion dependent thalassaemia intermedia.

The phenotype of thalassaemia intermedia is itself heterogeneous with both mild and severe forms, because there are many different genetic determinants responsible for producing this phenotype.<sup>8</sup> These include: the homozygous state for a mild  $\beta^+$  allele; the combination of a mild  $\beta^+$  allele with a severe  $\beta^+$  or  $\beta^0$  in some cases (unfortunately the situation is not very clear as this combination sometimes results in  $\beta$  thalassaemia major); two severe  $\beta^+$  or  $\beta^0$  alleles in combination with either  $\alpha$  thalassaemia or a raised HbF determinant, such as the -158  $\alpha\gamma$  globin promoter mutation; one severe  $\beta^+$  or  $\beta^0$  allele in combination with a triplicated  $\alpha$  gene allele; homozygous  $\delta\beta^0$  thalassaemia;  $\delta\beta^0$  thalassaemia in combination with  $\beta^+$  thalassaemia or Hb Lepore; and a  $\beta^0$  or severe  $\beta^+$  allele in combination with Hb Lepore or Hb O Arab.

#### HB S AND ITS INTERACTIONS

The sickle cell gene is caused by an A  $\rightarrow$  T substitution in codon 6 of the  $\beta$  globin gene.  $\beta$  globin haplotype analysis has shown that the sickle cell gene has arisen at least three times in Africa and once in Asia. In patients with sickle cell anaemia bearing homozygous forms of the four most common African haplotypes (the Senegal, Benin, Bantu and Cameroon) the phenotypic expression of this disease varies considerably and it is not possible to predict how severe the disease will be in any particular family because there are several different factors (genetic, cellular, and physiological) which can modify the course of sickle cell disease. Recent evidence suggests, however, that the Bantu (also known as Central African Republic, CAR) is often associated with a clinically severe condition,<sup>9</sup> while homozygotes, for the Asian haplotype from Saudi Arabia and India have high concentrations of Hb F coupled with only a modest level of anaemia and a relatively mild course of the disease, although bone pathology (painful crises, avascular necrosis of the femoral head, and osteomyelitis) remains common.<sup>10</sup> The latter effects seem to be most severe in patients with homozygous  $\alpha^+$  thalassaemia.

The sickle cell gene also interacts with  $\beta$  thalassaemia genes and several structural haemoglobins to produce sickling disorders of variable severity. The clinical course of sickle cell  $\beta$  thalassaemia is very variable, depending

on the particular type of  $\beta$  thalassaemia mutation that is co-inherited. It tends to be particularly mild in African populations because of the likelihood of the co-inheritance of a mild  $\beta^+$  transcription mutation, although even here occasional severe interactions are encountered. A severe course, similar to that found in sickle cell anaemia, results from the interaction of the sickle gene with either a  $\beta^0$  or a severe  $\beta^+$  thalassaemia allele. Haemoglobin sickle cell disease can be mild, though it may be associated with ocular, central nervous system, and bone complications, and thrombotic problems in pregnancy. However, the combination of Hb S/D Punjab, Hb S/O Arab, and Hb S/Lepore result in diseases similar in severity to homozygous sickle cell anaemia.

#### HB E/ $\beta$ THALASSAEMIA

This disorder is restricted mainly to people of Oriental, Indian, or Pakistani background. It shows remarkable clinical variability, ranging from a symptomless disorder to a condition as severe as homozygous  $\beta$  thalassaemia which occurs when Hb E interacts with a  $\beta^0$  thalassaemia allele. It seems likely that interaction with a severe  $\beta^+$  thalassaemia type of allele results in a slightly milder condition but is still a serious disorder in contrast to the more rare interaction of Hb E with a mild  $\beta^+$  thalassaemia allele which results in an extremely mild, almost symptomless, disorder.

#### Globin chain synthesis

This technique may be useful in fetal diagnosis when or if the putative father is not available for tests. Guidelines to the analysis of globin chain biosynthesis in adult blood samples for carrier status determination may be found in the review by Clegg (1983)<sup>11</sup>; those relating to the determination of globin chain synthesis in fetal blood samples for fetal diagnosis can be found in the review by Alter (1989).<sup>12</sup>

#### DNA analysis

Fetal DNA for prenatal diagnostic studies can be isolated from amniotic fluid cells or chorionic villus biopsy specimens.<sup>13</sup> Sufficient DNA (10–70  $\mu$ g) is usually obtained from a chorionic villus sample for both polymerase chain reactions (PCR) and Southern blot techniques. However, 20 ml of amniotic fluid will yield only about 7  $\mu$ g DNA, and sometimes much less than this, or occasionally even none at all. Thus it is an unreliable source of DNA, and although usually sufficient DNA is obtained for PCR, the amount will be borderline for Southern blot analysis; therefore the establishment of a backup cell culture is recommended for amniocyte DNA diagnosis.

#### SOUTHERN BLOT ANALYSIS

This technique is used for identifying  $\alpha$  and  $\beta$  globin gene rearrangements caused by the

various  $\alpha^+$  and  $\alpha^0$  thalassaemia deletion genes,  $\delta\beta^0$  thalassaemia deletions, and the HPFH deletions. Prenatal diagnosis for  $\alpha^0$  thalassaemia is achieved by hybridising Southern blots of *Bam*H I and *Bgl* II digested DNA to  $^{32}$ P-labelled  $\alpha$  gene and  $\zeta$  gene probes. The homozygous state for  $\alpha^0$  thalassaemia is diagnosed by the presence of fragments containing abnormal  $\zeta$  gene in combination with the absence of those containing normal  $\alpha$  gene.<sup>14</sup> The procedure usually takes 10–14 days to achieve a result. Southern blot analysis gives an overall picture of the  $\alpha$  globin gene arrangement and is still very useful for carrier diagnosis of  $\alpha$  thalassaemia and for the confirmation of prenatal diagnosis results obtained by PCR.

#### PCR TECHNIQUES

Techniques based on PCR are now used for the diagnosis of  $\alpha$  thalassaemia,  $\beta$  thalassaemia, some  $\delta\beta$  thalassaemias and HPFH deletions, the analysis of  $\beta$  globin restriction fragment polymorphisms (RFLPs) and the detection of the genes for Hb S, Hb C, Hb D, Hb E and Hb O Arab. A fetal diagnosis result is usually obtained in one to two days if the mutations requiring analysis are known. If the mutations have not been identified beforehand, the parental DNA samples will have to be analysed first before the fetal DNA and the fetal diagnosis will take two to five days.

#### Detection of known mutations

There are three PCR based techniques in general use in fetal diagnosis laboratories: (1) the hybridisation of  $^{32}$ P-labelled allele specific oligonucleotide probes, either to amplified DNA fixed to nylon filters by dot blotting<sup>15</sup> or by reverse dot blotting with the probes fixed to the filter<sup>16</sup>; (2) the use of allele specific primers<sup>17</sup>; and (3) agarose or polyacrylamide gel electrophoresis. The first two techniques detect point mutations and require a battery of specific probes—one for every mutation likely to be encountered. The task of screening parental DNA samples for  $\beta$  thalassaemia mutations is greatly simplified by the knowledge of the ethnic origin of each person. The DNA is then screened first for the common mutations to that particular ethnic group, and then if the tests prove negative, the rarer mutations are analysed for. The mutations carried by both partners can therefore be identified in more than 95% of couples. The remaining few per cent of people with an unidentifiable mutation either have an undescribed one or a rare one from a different ethnic group. In these cases fetal diagnosis can often be carried out by the indirect methods of RFLP linkage or denaturing gradient gel electrophoresis.

The mutations for Hb S, Hb E, Hb D and Hb O-Arab affect a restriction enzyme site in the  $\beta$  globin gene and are detected directly by amplification, followed by restriction enzyme digestion of the product and agarose gel electrophoresis.<sup>14</sup> Similarly, 13  $\beta$  thalassaemia mutations can be detected this way<sup>18</sup> and this method provides an alternative approach to

check prenatal diagnosis results involving these mutations. Gel electrophoresis of amplified product is used for diagnosing the  $\beta$  thalassaemia deletion mutations,<sup>19</sup>  $\alpha^0$  thalassaemia,<sup>20</sup> Hb Lepore<sup>21</sup> and  $\delta\beta$  thalassaemia.<sup>22</sup> Primers which are complementary to sequences flanking the deletion are used to generate a specific product only in the presence of the mutation (with the exception of  $\beta$  thalassaemia, normal DNA is not amplified because the primers are too far apart). Normal DNA is amplified using a flanking primer in combination to one complementary to the deleted sequence.

#### Detection of unknown mutations

There are now several PCR methods for characterising  $\beta$  thalassaemia mutations that are undefined by direct detection methods. These are denaturing gradient gel electrophoresis (DGGE), chemical mismatch cleavage analysis, and single-stranded conformation polymorphism (SSCP) analysis, followed by direct sequencing of amplified single stranded DNA. The method most widely used is DGGE, a polyacrylamide gel system that separates DNA fragments as a function of the melting temperature. It has been found to be particularly useful for the diagnosis of  $\beta$  thalassaemias in geographical areas where a very heterogeneous spectrum of mutations occurs.<sup>23</sup>

#### Indirect detection by RFLPS

Nearly all the  $\beta$  globin RFLPs discovered by Southern blotting can now be analysed by PCR and restriction enzyme digestion.<sup>14</sup> By carrying out a family study with DNA from children or grandparents, or both, the linkage phase of informative RFLPs can be established and  $\beta$  thalassaemia genes can be diagnosed indirectly in fetal DNA without identifying the mutations involved. The technique has a slightly higher risk of error (0.3%) than a direct method because of the chance of recombination between the RFLP and the mutation locus. RFLP linkage analysis provides a useful alternative approach to the diagnosis of  $\beta$  thalassaemia in the 80% of families that are found to be informative, and should be used for confirmation of a diagnosis obtained by direct mutation analysis whenever possible.

#### PCR errors

Misdiagnosis may occur for several technical reasons—that is, failure to amplify one of these two target DNA segments, maternal DNA contamination, contamination with previously amplified target DNA sequences through aerosol contact, mispaternity, sample exchange, incomplete digestion of amplified product by restriction endonucleases, and mispaternity and incorrect phenotype information resulting in a missed allele or incorrect assignment of polymorphic markers. These errors can be encountered by careful laboratory practice in setting up PCR reactions and running appropriate controls,

duplicate analysis of the fetal sample, the use of the minimum number of amplification cycles, the use of a second different diagnostic approach and the amplification of a suitable polymorphic marker, such as a variable number of tandem repeat (VNTR) sequence, to monitor the presence of maternal contamination.<sup>24</sup>

#### Requirements for prenatal diagnosis

When a potentially at-risk couple is detected they will require counselling and if fetal diagnosis is requested it will be necessary to: (a) confirm the parental phenotypes as determined by the first centre; (b) send family or parental blood samples to a specialist DNA diagnostic laboratory; and (c) arrange for fetal sampling and correct despatch of the fetal sample for fetal diagnosis. Ideally confirmation of the parental phenotypes and identification of the DNA mutations involved should be done before arrangements are made for fetal sampling to define the exact nature of risk and to avoid unnecessary invasion of a pregnancy not at risk. To diagnose  $\alpha^+$   $\beta$  thalassaemia or  $\beta$  thalassaemia with normal Hb A<sub>2</sub> and low mean corpuscular haemoglobin may require a combination of careful family studies, globin chain synthesis, and DNA analysis.

#### CARRIER STATE DETERMINATION

This requires a blood count, including red cell indices, haemoglobin electrophoresis, a sickle solubility test and an estimation of Hb A<sub>2</sub> and Hb F. Guidelines for these routine screening tests are detailed in the associated guidelines. These tests should be repeated at the centre that has referred blood and fetal samples for DNA analysis from an at-risk couple. The referral form (see appendix) should be completed and accompany the blood samples to the DNA diagnostic laboratory. Details of the ethnic origin (not place of birth) are particularly important for DNA analysis.

#### $\alpha$ thalassaemia

The typical haematological features for  $\alpha$  thalassaemia carriers are shown in the table of the accompanying guidelines on the investigation of  $\alpha$  and  $\beta$  thalassaemias. Some important points to note are that carriers for  $\alpha^+$  thalassaemia are haematologically normal or have only slightly reduced indices (mean corpuscular haemoglobin of 24–30 pg). The indices for  $\alpha^0$  thalassaemia trait usually fall below the normal range (mean corpuscular haemoglobin of 28–32 pg) in the absence of iron deficiency, but are indistinguishable from those found in people with homozygous  $\alpha^+$  thalassaemia (mean corpuscular volume of 19–24 pg).<sup>25</sup> Therefore, definitive carrier diagnosis can only be done by DNA studies. It is recommended that all pregnant women of Chinese, South East Asian, or eastern Mediterranean origin should be screened for  $\alpha^0$  thalassaemia trait and offered prenatal diagnosis when indicated.

#### $\beta$ thalassaemia

The haematological phenotype of heterozygous  $\beta$  thalassaemia can be modified by interacting genetic or environmental factors. The co-inheritance of  $\alpha$  thalassaemia (usually the deletion of two  $\alpha$  globin genes) may raise the mean corpuscular haemoglobin and volume sufficiently, in some cases, to values that lie within the normal range. Alpha thalassaemia, however, does not affect the Hb A<sub>2</sub> concentration, unlike iron deficiency which may decrease the Hb A<sub>2</sub> value, although it normally remains within the  $\beta$  thalassaemia carrier range. Therefore in any population in which  $\alpha$  and  $\beta$  thalassaemia occur together, the best method of screening for  $\beta$  thalassaemia in pregnancy is to measure the Hb A<sub>2</sub> concentration.

A subgroup of  $\beta$  thalassaemia alleles exists in which heterozygotes have normal or minimally increased Hb A<sub>2</sub> concentrations. The ones with reduced red cell indices may be confused with heterozygous  $\alpha^0$  thalassaemia. Those with normal red cell indices are truly silent (the only abnormality being an imbalance of the  $\beta/\alpha$  globin chain synthesis ratio) and are not detectable by haematological screening techniques. Fortunately, the latter are both rare and very mild alleles, hitherto found only in one or both parents of patients affected by thalassaemia intermedia. These alleles are the Mediterranean – 101 mutation and the Indian CAP + 1 mutation. The triple  $\alpha$  globin gene arrangement is another truly silent allele, which in combination with a  $\beta$  thalassaemia mutation, can result in the phenotype of  $\beta$  thalassaemia intermedia.

Normal Hb A<sub>2</sub>  $\beta$  thalassaemia with reduced red cell indices is usually caused by the double heterozygosity for  $\delta$  and  $\beta$  thalassaemia. The mutation causing defective  $\delta$  gene expression may be in *cis* or *trans* to the  $\beta$  thalassaemia allele, which may be a mild allele such as codon 27 (Hb Knossos) or IVSI-5 (Corfu  $\delta\beta$  thalassaemia), or severe such as IVSI-110, ISII-745, and the  $\beta^0$  codon 39 mutation. However, several  $\beta$  thalassaemia mutations by themselves have been observed in people with borderline to normal Hb A<sub>2</sub> values, the best known example being the Mediterranean mutation IVSI-6. Finally, adults heterozygous for the rare condition of  $(\gamma\delta\beta)^0$  thalassaemia have red cell indices typical of  $\beta$  thalassaemia trait but with normal Hb A<sub>2</sub> and Hb F concentrations.

Adults with normal or low mean corpuscular volume of haemoglobin, normal to reduced Hb A<sub>2</sub> concentration and high Hb F are heterozygous for  $\delta\beta$  thalassaemia or hereditary persistence of fetal haemoglobin. Although the distinction between the two conditions is not always clearcut, in general heterozygotes for  $\delta\beta$  thalassaemia have 5–15% Hb F, hypochromic microcytic red cells, and a heterogenous distribution of Hb F in peripheral red blood cells; those with HPFH have 15–25% Hb F, normal indices, and a homogenous distribution of Hb F. A definitive diagnosis can be made by globin

chain synthesis (normal in HPFH, unbalanced in  $\delta\beta$  thalassaemia), and by DNA analysis.

#### SAMPLE REQUIREMENTS

Adult blood samples in heparin or EDTA for carrier determination by globin chain synthesis have to be fresh—received by the laboratory within a few hours—and are best transported at 4°C. It is essential to contact the referral laboratory about transport arrangements for blood samples for such tests before arranging for the patient to attend. Fetal blood sampling is usually carried out at the prenatal diagnosis centre.

Blood samples in heparin or EDTA for DNA analysis can be sent by overnight delivery or first class post without refrigeration. The maximum delay before deterioration is about three days so blood taken on a Friday is best stored in a fridge until Monday and then posted to arrive Tuesday. Anticoagulated blood (EDTA or heparin) (10 ml) is normally sufficient from each parent. If RFLP linkage analysis is to be performed in addition to direct detection of  $\beta$  thalassaemia mutations then additional blood samples are required from either: (i) a homozygous normal child or an affected child with  $\beta$  thalassaemia major; (ii) a child with  $\beta$  thalassaemia trait plus one set of grandparents; (iii) if no children are available then both sets of future grandparents would be required. The samples should be clearly labelled and be accompanied with a request form detailing the following relevant particulars.

(1) Haematological details: Hb, RBC, MCV, MCH, sickle test, Hb electrophoresis, Hb A<sub>2</sub>, Hb F, H bodies, ferritin, serum iron/total iron binding capacity.

(2) Patient particulars: surname, first name, date of birth, address, ethnic origin, referring consultant, gestational age.

(3) Details of family history and previous children.

(4) Confirmation the family has been counselled as to the nature of the disease and obstetric risks and error rates.

(5) Name and address of contact for arrangement of follow up studies.

Chorionic villus samples must be dissected free of any contaminating maternal decidua by microscopic dissection before sending to the fetal diagnosis referral centre. These samples can be sent by overnight delivery either in tissue culture medium, or preferably, in a special lysis buffer obtainable from the DNA diagnosis laboratory. Amniotic fluid samples (15–20 ml) are best sent to the DNA laboratory as quickly as possible and must be received within 24 hours. If a longer transit time is anticipated the amniocytes should be sent resuspended in tissue culture medium. In many cases it is now possible to arrange for the fetal sample to go to a regional molecular genetics laboratory for DNA extraction. The fetal DNA can then be posted to the haemoglobinopathy diagnosis laboratory without risk of deterioration if delayed in transit.

#### RISKS AND MISDIAGNOSIS RATES

Because both chorionic villus sampling and fetal DNA analysis are still in the stages of development, it is vital that parents are given accurate advice about the current rates of fetal loss and also the likely misdiagnosis rate. Current figures suggest that the fetal loss rate following chorionic villus sampling in the first trimester is in the range of 2–3%. The error in DNA analysis due to such factors as genetic recombination, technical problems in the laboratory, and maternal contamination of chorionic villus sampling specimens has been determined to be 1% for fetal diagnoses carried out using Southern blotting. There are not yet sufficient data to give an absolutely accurate assessment of the misdiagnosis rate for the recently introduced PCR based techniques but experience to date suggests it may be in a similar range of 1–2%.

It is essential that follow up data are obtained on all cases that have undergone fetal diagnosis by chorionic villus sampling DNA analysis; ideally this should include both haematological and developmental assessment, as well as any fetal abnormalities. These data should be available to both the DNA laboratory and the referral centre:

(a) Information on data of birth and birthweight and any information observed in the neonatal period.

(b) Neonatal study of cord blood (electrophoresis or globin biosynthesis), or standard carrier test at six months or later.

(c) Confirmation of genotype by DNA analysis.

#### COUNSELLING

No couple should be offered fetal diagnosis without proper counselling involving properly trained counsellors. Counselling requires an accurate determination of the parental genotypes and a good understanding of clinical outcomes of the haemoglobin disorders and their various interactions. It must include the nature and prognosis of the disorder involved and the treatment available; the genetic risks and possible methods of fetal diagnosis; the obstetric risks and risk of misdiagnosis.

#### Summary of guidelines for centres sending samples for fetal diagnosis

(1) A well run diagnostic service requires close liaison between haematologists, their obstetric colleagues, and the DNA referral laboratory.

(2) Haematological findings determined previously elsewhere should be rechecked for accuracy by the referring hospital.

(3) Counselling of families requires the accurate determination of parental genotypes, a good understanding of the interactions producing the different haemoglobin disorders and the clinical picture of each disorder, and accurate advice on the current rates of fetal loss and the likely fetal DNA analysis error.

(4) All haematology results and patient details should be sent to the DNA referral

laboratory. A referral form to accompany blood samples is provided.

(5) It is essential to contact the DNA referral laboratory to discuss transport arrangements for samples.

(6) Family or parental blood samples for DNA analysis are required for fetal diagnosis of both thalassaemia and sickle cell disease.

Blood samples for DNA analysis ideally should be sent at least one week before a fetal sample to allow time for mutation identification. However, current techniques now allow parental and fetal samples to be sent at the same time, on the understanding that the fetal diagnosis may take a few days longer and there is a small chance of diagnostic failure from not being able to identify one of the  $\beta$  thalassaemia mutations.

(7) It is essential to obtain follow up data for chorionic villus sampling DNA diagnoses. These should always be returned to the DNA laboratory and the referring centre.

#### Summary of guidelines for those carrying out fetal diagnosis

(1) Ensure that adequate haematological data have been provided.

(2) Ensure that the mother (or couple) have been adequately counselled.

(3) Ensure that chorionic villus samples have undergone careful microscopic dissection to remove any contaminating maternal decidua.

(4) Always analyse parental DNA samples and appropriate control DNAs alongside the fetal DNA sample.

(5) Always repeat the fetal DNA analysis test to double check the result.

(6) Use a second alternative DNA analysis test whenever possible to confirm a result.

(7) Use a limited number of amplification cycles to minimise co-amplification of maternal DNA.

(8) Check for maternal DNA contamination by amplification of polymorphic markers.

(9) The fetal diagnosis report should detail the types of DNA analysis used and clearly state the risk of misdiagnosis.

#### Useful addresses

(1) Characterisation of mutations and fetal diagnosis for thalassaemia and sickle cell disease is available as a national service from:

Dr John Old, National Haemoglobinopathy Reference Service, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU Tel: 0865-222 449 Fax: 0865-222 500

(2) Fetal diagnosis for thalassaemia and sickle cell disease is also provided by the following regional centres:

Dr M Petrou, Dr B Modell, Perinatal Centre Department of Obstetrics and

Gynaecology, 88-96 Chenies Mews, London WC1E 6HX Tel: 071-387 9300 extn. 5230 Fax: 071-380 9864

Dr M Layton, Department of Haematological Medicine, King's College Hospital, Denmark Hill, London SE5 9RS Tel: 071-326 3239 Fax: 071-326 3514

For sickle cell disease only:

Dr M Patton, SW Thames Regional Genetics Service, St Georges Hospital Medical School, Cranmer Terrace, London SW17 0RE Tel: 081-672 9944.

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