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Molecular genetics and prenatal diagnosis of the thalassaemias

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INTRODUCTION

The thalassaemias are a heterogenous group of inherited haemoglobin disorders characterized by a reduced output or absence of one or more of the globin chains. The common forms, a and β thalassaemias, are among the most common genetic disorders in the world. There is increasing evidence that heterozygotes for thalassaemias are protected from the severe effects of *falciparum* malaria, this selective advantage has greatly increased the gene frequencies of many thalassaemia alleles throughout the tropical and sub-tropical regions. However, owing to mass population migration, the haemoglobin disorders are increasingly seen in parts of the world where they were not previously recognised. Both a and β thalassaemia show a wide spectrum of clinical phenotypes ranging from severe anaemia and transfusion dependency in the homozygotes and compound heterozygotes to extremely mild forms which are clinically and haematologically silent, the so-called 'silent' carrier states. Furthermore, because the thalassaemias co-exists at a high frequency with the haemoglobin variants like Hb S, E and C in many populations, individuals may inherit more than one type giving rise to an extremely complex spectrum of clinical phenotypes. As the molecular pathology of the thalassaemias are being characterized, it has become possible to relate these heterogeneous clinical phenotypes to the underlying genotypes.

NORMAL HAEMOGLOBIN SYNTHESIS

All human haemoglobins have a tetrameric structure consisting of two identical a-like (a or ζ)and two β-like (ε,γ,δ or β) globin chains, each linked to a heme group. In normal adults the major component is Hb A ($\alpha_2\beta_2$) which constitutes about 97% of the total Hb with a minor component of Hb A, ($\alpha_2\delta_2$) and traces of Hb F ($\alpha_2\gamma_2$). During development two major 'switches' occur in the synthesis of haemoglobin; production of embryonic haemoglobin (Hb Gower 1, $\zeta_2\varepsilon_2$; Hb Gower 2, $\alpha_2\varepsilon_2$ and Hb Portland, $\zeta_2\gamma_2$) switches after the eighth week of gestation to the production of fetal haemoglobin ($\alpha_2\gamma_2$) and then just before birth to the adult haemoglobin (Hb A and Hb A,). At six months after birth fetal haemoglobin (Hb F) comprises less than 5% of the total haemoglobin and continues to fall reaching the adult level of < 1% at 2 years of age. Thus while absence of a globin chain is not compatible with life, mutations affecting the β globin gene only become apparent clinically on completion of the switch from fetal to adult haemoglobin at 2 years of age.

Each of the globin chains is encoded by a structural gene, the a-like genes located in the a globin cluster which spans 30 kb on the tip of chromosome 16p and the P-like genes, in a similar β globin cluster which spans 70 kb on chromosome 11p15.5. In each cluster the genes are arranged 5'→ 3' in the order in which they are expressed during development. While the a-like genes undergo a single development switch (embryonic → fetal/adult) the P-like genes undergo two switches (embryonic → fetal → adult). Immediately upstream of each gene is a promoter region which maintains local control while the entire cluster is controlled by a major regulatory element, referred to as the β locus control region (β LCR) in the β cluster, and the HS-40 in the a cluster. Expression of the structural genes within each cluster is controlled by complex interactions between the local regulatory sequences and the majdr upstream elements of the respective LCR such that the products of the two clusters are expressed in equal amounts maintaining a balance in globin chain production throughout development. A reduced synthesis of a or β globin chains causes chain imbalance and a relative excess of β or a chains, respectively. In both instances the chain excess is harmful and leads to a or β thalassaemias.

The interaction between the local promoter elements, enhancers and the elements of the LCR is mediated by *trans*-acting factors. Much

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interest is focused on how these interact to produce high levels of tissue-specific and developmentally regulated expression of the globin genes. Tissue-specific expression may be explained by the presence of two proteins, GATA-1 and NF-E2, that are specifically expressed in erythroid cells. Binding sites for one or more of these proteins are found in the upstream regulatory elements and some promoters of the globin genes. It seems likely that these two *trans*-acting factors may form part of a network of factors that commit haemopoietic stem cells to erythroid differentiation. The mechanisms by which developmental regulation is controlled are less clear, autonomous control of the embryonic ε and ζ genes and the γ genes implicates negatively acting factors referred to as silencers. So far, the best characterised example of a developmental-stage-specific regulatory factor is the erythroid Krüppel-like factor (EKLF) which appear to be essential for the final steps of definitive erythropoiesis.

β THALASSAEMIAS

The β thalassaemia syndromes result from underproduction of the β globin chains of adult Hb A ($\alpha_2\beta_2$) giving rise to an imbalanced globin chain synthesis and excess of α globin chains. These α chains are extremely unstable and precipitates in red cell precursors, producing an ineffective erythropoiesis and haemolytic anaemia. The severity of disease is directly related to the degree of chain imbalance.

Normal individuals have a single β gene (β/β) on each chromosome 11. The mutations causing β thalassaemia result in a deficit of β globin production that ranges from minimal (mild β^+ thalassaemia) to a complete absence (β° thalassaemia). Molecular analysis of the β thalassaemia genes has demonstrated a striking heterogeneity; the latest repository of the mutations causing β thalassaemia in Hemoglobin shows that > 150 β thalassaemia alleles have now been characterised.

The β thalassaemias are considered to be autosomal recessive disorders since individuals who have inherited one abnormal β gene (carrier) are clinically asymptomatic with minor haematological abnormalities, and the inheritance of two abnormal $ globin genes (homozygotes or compound heterozygotes) is required to produce a clinically detectable phenotype. Due to the vast number of different β thalassaemia mutations many patients with thalassaemia major are compound heterozygotes for two different molecular lesions.

In contrast to α thalassaemia, the vast majority of mutations causing β thalassaemia are non-deletional due to single base substitutions, small insertions or deletions (see Table 1).

Non-deletion forms of β Thalassaemia

These point mutations involve the critical sequences that control the various stages of gene expression and provide a good example of the spectrum of naturally occuring lesions that can' inactivate a mammalian gene. Approximately half of these β thalassaemia alleles completely inactivate the β gene and cause β° thalassaemia. Most are caused by the introduction of a premature termination codon due to frameshift or nonsense mutations. Low levels of nuclear and cytoplasmic mutant β globin mRNA are found in red cell precursors in these mutations. It is not clear how a premature termination codon could lead to a reduction of mRNA.

Studies show that the different in-phase termination mutants exhibit a 'positional' effect. Frameshifts and nonsense mutations that result in premature termination early in the sequence (in exon 1 and 2) are associated with minimal amounts of mutant β mRNA. In heterozygotes for such cases, no β chain is produced from the mutant allele and only half the normal β globin is present, resulting in a typical asymptomatic phenotype. In contrast, mutations that produce in-phase terminations later in the β sequence, in exon 3, are associated with comparable amounts of mutant β-mRNA. Such mutations even when present in a single copy, results in a moderately severe anaemia and are said to be 'dominantly inherited'. Small amounts of truncated $variant chains have been isolated in one case (heterozygous β codon 121). These truncated β chains, however, are non-functional and are not able to form viable tetramers thus resulting in ineffective erythropoiesis and clinical disease even in the heterozygous state.

Other mutations which result in β° thalassaemia include those which affect the initiation codon (ATG) and mutations at the splice junctions (5' and 3') which completely abolish normal splicing.

The β^+ thalassaemia mutations allow the production of some β globin but the output is reduced. The reduction in β globin output ranges from minimal to almost complete absence. The severity of these β^+ thalassaemia alleles can be correlated with the degree of reduction in MCV in heterozygotes. A large

TABLE 1: Mutations causing β Thalassaemia

	No	Type
Deletional	**17**	
Upstream deletions	3	β°
β gene deletions	14	β°
Nondeletional	**131**	
Transcriptional mutants	18	Mild to very mild β⁺
5' untranslated region (5'UTR)	5	Very mild β⁺
RNA processing mutants	38	
Splice junction	15	β°
Consensus site	13	Severe to mild β⁺
Aberrant splicing	10	β°
Introns	6	β⁺,β°
Coding regions	4	β°
RNA translation mutants	56	
Nonsense	12	β°
Frameshift	44	β°
RNA cleavage and polyadenylation	6	mild β⁺
CAP (+1) Site	1	Very mild β⁺
3' untranslated region (3'UTR)	2	?
Initiation codon	5	β°
Unlinked to β Cluster	**3**	**?**
Total	**151**	

number of β⁺ thalassaemia alleles are caused by mutations that affect RNA processing and are located within the consensus sequences flanking the splice junctions or within introns or exons. The latter lead to the creation of new splice sites which can partially or completely eliminate normal splicing. An example of cryptic splice site involved in alternative splicing can be found in exon 1 of the β gene, at codons 24-27. A mutation within this region can make the site resemble more closely a true splice site. An important example is the G → A (Glu → Lys) mutation in codon 26 which leads to the production of Hb E. There is a low level of normally spliced mRNA which contains the exon 1 mutation leading to the production of Hb E as well as abnormal splicing into the codons 24-27 region which does not produce any recognisable β globin. The overall reduction in splicing is the molecular basis for the mild β⁺ thalassaemia phenotype of HbE. The β^E gene is prevalent in SE Asia, reaching a frequency of 75% in north east Thailand. Its interaction with β thalassaemia accounts for a large proportion of the thalassaemia major in SE Asia. Mutations affecting the conserved sequences in the 5'promoter i.e. TATA box, proximal CACCC and distal CACCC box, typically cause a 70-80% reduction in promoter activity and are often very mild. Mutations affecting the polyadenylation signal (AATAAA) at the 3' end, also generally result in a mild β⁺ thalassaemia phenotype.

Among the β⁺ thalassaemia alleles is a subgroup which cause a minimal deficit in $ chain production. Heterozygotes for such mutations have normal Hb A_2 levels and normal red cell indices and are often referred to as 'silent' carriers. This group includes the C-T mutation at position -101 upstream of the β gene, the A → C mutation at position CAP (+1), and the mutations in the 5' untranslated region (S'UTR). The β thalassaemia is 'silent' when present in a single copy but becomes evident in homozygotes or compound heterozygotes for one of these mutations.

Deletions causing β Thalassaemia

Rarely β thalasassaemia is caused by deletions of the β cluster. Although uncommon, these

deletions provide some insight into the mechanisms responsible for the differential expression of the β-like globin genes. These deletions fall into two classes: upstream deletions and deletions involving the β gene.

Upstream deletions

Three deletions, described in families of Dutch, English and Hispanic origin, are of particular interest because they remove substantial regions of the 5' end of the β gene clusters but leave the β gene itself intact and yet result in a β thalassaemia phenotype. These deletions silence the ρ globin gene because they remove all or a substantial proportion of the regulatory sequences in β LCR. While deletion of the three 5'LCR elements (hypersensitivesites 2-4) inactivates the β gene, a family study showed that deletion of the 3' most LCR element (HS1) does not affect the activity of the β gene.

Deletions involving β globin gene

Twelve different deletions affecting only the β globin gene have been described. These deletions range from 290 bp to > 45 bp. Of these, only the 619 bp deletion at the 3' end of the β gene is common, but even that is restricted to the Sind populations of India and Pakistan where it constitutes –20% of the β thalassaemia alleles. The other deletions, although rare, are of particular phenotypic interest because they are associated with an unusually high level of HbA_2 in heterozygotes. The mechanism underlying the markedly elevated levels of HbA_2 and the variable increases in Hb F in heterozygotes for these deletions is related to the removal of the 5' promoter region of the β globin gene which removes competition for the upstream β-LCR leading to an increased interaction of the LCR with the γ- and 6- genes in *cis*, thus enhancing their expression.

Other deletions which lead to β thalassaemia, remove extensive regions of the cluster including the ε, $^{G}\gamma$, $^{A}\gamma$, Ψβ and 6 genes and part or all of the β gene.

PHENOTYPIC VARIANTS OF β THALASSAEMIA

Dominantly inherited β thalassaemias

The dominantly inherited β thalassaemias produce a clinically detectable phenotype when present in a single copy whereas individuals heterozygous for ρ thalassaemias an typically clinically asymptomatic. This group of variant β thalassaemias includes the β thalassaemic haemoglobinopathies which can be defined as structural haemoglobin mutants presenting with a phenotype of thalassaemia. The underlying molecular abnormalities are remarkably heterogenous and includes amino acid substitutions, amino acid deletions, elongated or truncated β globin chains (Table 2). Although nucleotide sequence analysis predicts the synthesis of a β variant it is unusual to demonstrate an abnormal β globin from these mutations. Globin biosynthesis studies including short term incubation and pulse chase experiments showed a rapid decline of some of the variants, suggesting that these variants are highly unstable and rapidly catabolised.

The phenotype of this class of β thalassaemia appears to be dependent on the extent of ineffective erythropoiesis and intravascular haemolysis which in turn depends on the stability of the β chain variant, itsability to form α/β dimers and tetramers, and the stability of the tetramers.

Unlike typical β thalassaemia which is prevalent in malaria-endemic regions, dominantly inherited β thalassaemias are rare, occurring in dispersed geographical regions including Northern and Eastern Europe, Japan and Korea where the gene frequency for β thalassaemia is very low. Except for the codon 121 GAA → TAA mutation, all the dominant β thalassaemia alleles have been described in single families, many as *de novo* events (Table 2). It is postulated that the low frequency of the dominant β thalassaemia mutations is due to the lack of positive selection as in the case for the recessive forms.

Clinically, since spontaneous mutations are common in dominant β thalassaemia, it is important that the disorder should be suspected in any patient with a thalassaemia intermedia phenotype even if both parents are haematologically normal and the patient is from an ethnic background where β thalassaemia is rare.

β Thalassaemia due to a trans-acting determinant

It has been estimated that –1% of the β thalassaemia genes in the world remain uncharacterised. In such cases, it has been postulated that mutations may be found in the upstream β-LCR, or in the 3' enhancer region from 700-1100 nucleotides 3' of the β gene. Three families have been described in which the genetic determinant responsible for the β-thalassaemia phenotype segregates indepen-

TABLE 2: Dominantly inherited β Thalassaemia (including hyperunstable Hbs)

		β variant	Ethnic Group
I	**Single base substitutions**		
i)	Cod 28 (CTG → CGG) Leu → Arg	Hb Chesterfield*	English
ii)	Cod 30 (AGG → ACG) Arg → Thr	Hb Monroe	Black American
iii)	Cod 32 (CTG → CAG) Leu → Glu in *cis* with Cod 98 (GTG → ATG) Val to Met	Hb Medicine Lake*	Caucasian American
iv)	Cod 60 (GTG → GAG) Val to Glu	Hb Cagliari*	Italian
v)	Cod 106 (CTG → CGG) Leu → Arg	Hb Terra Haute	N European
vi)	Cod 110 (CTG → CCG) Leu to Pro	Hb Showa-Yakushiji	Japanese
vii)	Cod 114 (CTG → CCG) Leu to Pro	Hb Durham	Irish American
viii)	Cod 115 (GCC → GAC) Ala to Asp	Hb Hradec Kralove (Hb HK)	Czech
ix)	Cod 127 (CAG → CCG) Gln to Pro	Hb Houston	N Italian/French
II	**Deletion of intact codons → Destabilisation**		
i)	Cod 32/34 (-GGT) Val-Val to Val	Hb Korea*	Korean
ii)	Cod 1271128 (-AAG) Glu-Ala to Pro	Hb Gunma	Japanese
iii)	Cod 134-137 (-12, +6) Val-Ala-Gly-Val to Gly-Arg		Portuguese
III	**Premature termination → Truncated β variant**		
i)	Cod 121 (GAA → TAA) Glu to Term	Several families**	Caucasian, N. Europeans
ii)	Cod 127 (CAG → TAG) Gln to Term		English
IV	**Frameshifts → Elongated β variants**		
i)	Cod 30/31 (+CGG/Arg) → 147		Spanish
ii)	Cod 94 (+TG) → 156aa	Hb Agnana*	S. Italian
iii)	Cod 109 (-G) → 156aa	Hb Manhattan	Askenazi Jew
iv)	Cod 114 (-CT, +G) → 156aa	Hb Geneva	Swiss-French
v)	Cod 123 (-A) → 156aa	Hb Makabe	Japanese
vi)	Cod 123-125 (-ACCCCACC)	Hb Khon Kaen	Thai
vii)	Cod 124 (-A)		Russian
viii)	Cod 125 (-A)		Japanese
ix)	Cod 126 (-T) — 156aa	Hb Vercelli*	N. Itallian
x)	Cod 1281129 (-4, +5, -11) → 153aa		Irish

* Spontaneous mutations
** Several families reported including one spontaneous mutation

dently of the β globin gene cluster implicating the presence of a trans-acting determinant.

INTERMEDIATE FORMS OF β THALASSAEMIA

Thalassaemia intermedia is an ill-defined clinical term used to describe patients with disease phenotypes that range from transfusion-dependent disorders almost as severe as thalassaemia major to mild anaemias compatible with normal life. The criteria on which the diagnosis is based is that patients present later in life relative to thalassaemia major and that they are capable of maintaining a reasonable level of haemoglobin (≥ 6 gm/dl) without transfusion. At the severe end of the spectrum, patients present between the ages of 2 and 6 years, and although they are just capable of surviving without blood transfusion, it is clear that growth and development are retarded. Many will show the skeletal and facial changes and progressive splenomegaly as seen in untreated thalassaemia major. As they become older they develop iron-overload because of increased gastrointestinal absorption of iron.

At the other end of the spectrum, patients are completely asymptomatic until adult life and are transfusion independent with haemoglobin levels of 10-12 gm/dl. Such patients are diagnosed either during episodes of infection when they become anaemic or by a chance haematological examination. There is usually some degree of splenomegaly.

Table 3 lists some of the molecular interactions associated with the phenotype of thalassemia intermedia.

Because of the extreme variability of these disorders, these patients should be regularly followed from early childhood and the disease carefully monitored in terms of the growth charts and iron accumulation.

a THALASSAEMIAS

The geographical distribution of a thalassaemia is very similar to that of β thalassaemia. Although the a thalassaemias are more common, they pose less of a public health problem since the severe homozygous states cause death in utero and the milder forms that survive into adulthood do not cause a major disability.

The a thalassaemia result from under production of the a chains of fetal (Hb F α2γ2) and adult (Hb A α2β2) haemoglobin giving rise to an excess of γ or β chains which form the tetrarners, Hb Bart's (γ4) and Hb H (β4).

As in β thalassaemia, the critical factor in determining the severity of disease in a thalassaemia is the degree of chain imbalance. The pathophysiology of a thalassaemia, however, differs fundamentally from that of β thalassaemia in that the anamia is related to haemolysis due to intracellular precipitation of Hb Bart's and Hb H, rather than to ineffective erythropoiesis. Ultimately, the severity of anaemia and the amount of abnormal haemoglobin produced is directly related to the degree of a chain deficiency.

Normal individuals have two a globin genes (αα/αα) on each chromosome 16. Defects can affect one or both of the a genes resulting in a reduced output (a+thalassaemia) or absence (a^o thalassaemia) of a globin from that particular chromosome. In contrast to β thalassaemia, the majority of a thalassaemia (>95%) are caused by deletions; the phenotype of a+thalassaemia may result from deletion of one of the linked a globin genes ($-\alpha^{3.7}/\alpha\alpha$ or $-\alpha^{4.2}/\alpha\alpha$) or from a point mutation inactivating one of the a genes, usually a 2 ($\alpha^T\alpha/\alpha\alpha$). aothalassaemia results from extensive deletions that remove both the linked a 2 and α1 genes (––/m). Unlike the a+ thalassaemia deletions, aothalassaemia dele-

TABLE 3: Molecular Basis of β Thalassaemia Intermedia

I	Homozygous or compound heterozygous state for β thalassaemia 1. inheritance fo mild β^+ thalassaemia alleles e.g. βIVS1-6 T-C, β promoter mutations 2. co-inheritance of a thalassaemia • effect more evident in β^+ thalassaemia 3. β thalassaemia with elevated y chain production • polyrnorphism at position -158 $^G\gamma$ gene (Xmn 1-$^G\gamma$ site) • y promoter mutations • heterocellular HPFH X linked, 6q-linked
II	Compound heterozygotes for β thalassaemia and deletion forms of HPFH or δβ thalassaemia
III	Compound heterozygotes for β thalassaemia and β chain variants, e.g. Hb E/β thalassaemia
IV	Inheritance of deletion forms of β thalassaemia which remove the 5' β promoter region
V	Heterozygotes for β thalassaemia 1. co-inheritance of extra a globin genes (a a d a a or αααα/αααα) 2. dominantly inherited forms of β thalassaemia (including some thalassaemichaemoglobinopathies)

tions are limited in their geographical distribution hence often referred to by their geographical origin, for example, $--^{SEA}/\alpha\alpha$. More rarely, several deletions which remove the upstream control region (HS -40) leaving the a genes intact, have been described. These completely inactivate the a globin gene complex. A minority of a thalassaemia results from point mutations which affect the sequences which control the different stages of a globin expression. With the exception of one phenotypically mild a thalassaemia mutant, all of these affect the dominant a 2 gene. In general, the non-deletion α^+ thalassaemia determinants ($\alpha^T\alpha/\alpha\alpha$) give rise to a more severe reduction in a chain synthesis than deletion ($-\alpha/\alpha\alpha$) α^+ thalassaemia, the homozygous state ($\alpha^T\alpha/\alpha^T\alpha$) for such variants often result in Hb H disease.

Interactions between the different a thalassaemia give rise to a spectrum of clinical phenotypes which fall into three broad groups: carriers for a thalassaemia with three or two functional a genes ($-\alpha/\alpha\alpha$, $\alpha^T\alpha/\alpha\alpha$, -4-a, or $--/\alpha\alpha$) who are clinically asymptomatic with mild hypochromic microcytic anaemia, Hb H disease and Hb Bart's hydrops fetalis syndrome. Hb H disease results from the interactions of α^+- and α^0- thalassaemia determinants and gives rise to a moderately severe form of haemolytic anaemia which is commonly seen in the Mediterranean, Middle East and South East Asia. The absence of a genes ($--/--$) gives rise to lethal intrauterine haemolytic anaemia (Hb Bart's hydrops fetalis syndrome) which again occurs commonly in southeast Asia and the Mediterranean region.

A common non-deletion α thalassaemia variant in Southeast Asia is Hb Constant Spring (Hb CS) which is due to a single base substitution (TAA → CAA) in the $\alpha 2$ globin termination codon. This results in readthrough of the 3' untranslated sequence until another in-phase termination codon is encountered 31 codons later. Homozygotes ($\alpha^{CS}\alpha/\alpha^{CS}\alpha$) or compound heterozygotes ($\alpha^{CS}\alpha/--$) for Hb CS have a less severe form of Hb H disease. Very low levels of this elongated a globin chain (5-8% of the total haemoglobin in homozygotes) are found; the defective α^{CS} chain production is a consequence of the instability of the α^{CS} mRNA.

These other variants (Hb Icaria, Hb Seal Rock and Hb Koya Dora) involving different base substitutions in the a 2 termination codon have been identified.

ACQUIRED HB H DISEASE

Although Hb H disease is almost always inherited, it is occasionally seen in individuals with a variety of haematological disorders within the myelodysplastic syndromes. These individuals are predominantly elderly males and haematologically normal before the onset of the disease. The α globin gene cluster is intact and the acquired a thalassaemia appears to be a clonal disorder involving the neoplastic cell line. It is likely to be due to reduced α-gene transcription but the nature of the specific defect remains completely unknown.

a THALASSAEMIA WITH MENTAL RETARDATION (ATR) SYNDROMES

A rare form of a thalassaemia associated with mental retardation has also been described. There are two distinct syndromes of a thalassaemia and mental retardation. One group, ATR-16, results from extensive deletions of 1-2 Mb of the tip of chromosome 16 which remove the a globin gene cluster. The patients have relatively mild handicap and variable skeletal and facial dysmorphisms. The syndrome is thought to result from loss of a variable number of genes at the tip of chromosome 16p. The second group, ATR-X is characterised by severe mental retardation and a striking homogeneous pattern of dysmorphic facies and genital abnormalities. In these cases the a globin gene cluster is intact. The retarded patients are all males, the underlying mutation is a *trans*-acting abnormality encoded in the *XH2* gene on the X-chromosome.

PRENATAL DIAGNOSIS

The thalasaemias are a major health problem in many populations and because there is no definitive treatment, major efforts are concentrated on prevention.

Preventative programmes in the past were based on education, population screening, heterozygote detection and genetic counselling but were not entirely effective. Most countries now combine this approach with screening programmes at antenatal clinics. When heterozygous mothers are detected, their partners are tested and if they are also carriers, the couples are offered prenatal diagnosis and selective termination of pregnancy.

Although molecular analysis of the β thalassaemia genes has demonstrated a striking heterogeneity, population studies indicate that

probably only 20 β thalassaemia alleles account for >80% of the β thalassaemia mutations in the whole world. This is because each population group has a few (4-6) common mutations together with a varying number of rare ones. Furthermore, each ethnic group also tends to have its own combination of structural haemoglobin variants and a thalassaemia mutations. Hence the strategy for prenatal diagnosis depends on prior characterisation of the prevalent mutations likely to be encountered in the ethnic group of the individual being studied. Identification of the mutation is achieved by molecular analysis of fetal DNA enzymatically amplified by the polymerase chain reaction (PCR). Chorionic villus sampling (CVS) between 10 and 12 weeks of gestation is the method of choice for obtaining fetal material. Amniocentesis is an alternative, usually carried out between 15 and 20 weeks gestation. Approaches that are currently being explored include pre-implantation diagnosis and isolation of fetal normoblasts from maternal blood by flow cytometry.

DNA is first screened for the expected known mutations using one or more of the PCR-based techniques such as restriction analysis, allele-specific hybridisation or amplication, alterations in electrophoretic mobility, GAP or deletion specific PCR and DNA sequence analysis. When applying these techniques, one has to bear in mind the spectrum and nature of mutations one is likely to encounter. The reverse dot blot method which is capable of screening multiple alleles simultaneously, is probably the method of choice for molecular diagnosis of β thalassaemia.

Southern blot hybridisation remains the method of choice for detection of the common deletion a+and α° thalassaemia mutations as one hybridisation is capable of screening for a number of genotypes. Although GAP PCR has been applied for the identification of the common a+thalassaemia alleles, $-\alpha^{3.7}$ and $-\alpha^{4.2}$ as well as the α° thalassaemia alleles, $--^{MED}$, $--^{SEA}$ and $(\alpha)^{20.5}$, amplication can be technically difficult and not always reproducible due to the unusually high GC content of the a globin gene cluster. For detection of the nondeletion α^{+} thalassaemia variant, the α1 or α2 genes can be selectively amplified followed by restriction analysis or allele-specific hybridisation.

CONCLUSION

The mutations underlying the α and β thalassaemias are many and remarkably diverse. Interaction between these different genotypes have provided a picture of molecular mechanisms for the phenotypic diversity of monogenic diseases. Apart from providing a rationale for genetic counselling and prenatal diagnosis, analysis of these mutations has given much insight into the mechanisms regulating globin gene expression.

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