Review Article

GENOTYPE PHENOTYPE RELATIONSHIPS IN β THALASSAEMIAS

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ABSTRACT

The homozygous state for β-thalassaemia usually results in thalassaemia major, which requires monthy blood transfusions and regular infusions of the iron chelating agent desferrioxamine, for life. Some patients are less severely affected and survive either with no blood transfusion or without regular blood transfusion. This milder syndrome is termed thalassaemia intermedia. A significant amount of genetic information is now available in order to predict the thalassaemia intermedia phenotype from the genotype. Important ameliorating genetic factors are mild β-thalassaemia mutations, co-inheritance of α-thalassaemia, and presence of polymorphisms adjacent to the β-globin gene complex or mutations that increase HbF production by enhancing gamma-globin chain production. Accurate and precise prediction of phenotype from genotype will have important implications for prenatal diagnosis. Early diagnosis of thalassaemia intermedia is also important in order to avoid treatment with regular blood transfusions as for thalassaemia major, since a significant part of the morbidity and mortality arises from iron overload due to regular transfusion.

Key Words: Thalassaemia major, Thalassaemia intermedia, genotype, phenotype

INTRODUCTION

The β-thalassaemias are autosomal recessively inherited chronic anaemias. Their global distribution coincides with that of falciparum malaria, and they are common in the Mediterranean, Middle East, Africa and South East Asia. Migration of populations has extended the thalassaemias to Northern European countries, North and South American countries and Australia. Thalassaemia at the present time is a global disease that is increasingly common because of improved patient survival.

The homozygous state for β-thalassaemia usually results in thalassaemia major, which requires monthly blood transfusions and regular infusions of the iron chelating agent desferrioxamine, for life. Some patients are less severely affected and survive either with no blood transfusion or without regular blood transfusion. This milder syndrome is termed thalassaemia intermedia (1). Its relative frequency in different populations ranges from about 2-10% of cases of homozygous β-thalassaemia. Early diagnosis of thalassaemia intermedia is important in order to avoid treatment with regular blood transfusions as for thalassaemia major, since a significant part of the morbidity and mortality in homozygous β-thalassaemia arises from iron overload due to regular transfusion (2). This prediction of mild phenotype from genotype could decrease morbidity and mortality, and increase length and quality of life (3).

The β-thalassaemias are very heterogeneous at the molecular level. The large majority of molecular defects, which result in thalassaemia, are single nucleotide substitutions affecting the critical areas for the function of the β-globin gene; only a minority are produced by the mechanism of gene deletion. Globin chain imbalance is the major factor determining the severity of β-thalassaemia. Homozygous β-thalassaemia is characterised by a marked deficit (β^+) or complete absence (β^0) of globin chains, leading to a great excess of α-globin chains, which precipitate in the red cell precursors causing ineffective erythropoiesis. However, if the degree of β-globin chain imbalance is reduced, the less severe condition, thalassaemia intermedia, will result. There are three major mechanisms, which can ameliorate the clinical picture of homozygous β-thalassaemia:

1. Inheritance of mild β^+-thalassaemia, genes.
2. Co-inheritance of α-thalassaemia, which reduces the excess γ-globin chains.
3. Enhanced γ-globin chain production, which partially compensates for the lack of β-globin chains.

In addition other molecular mechanisms can cause thalassaemia intermedia. These are rare in clinical practice and include inheritance of deletion forms of β-thalassaemia which remove 5'β promoter region, heterozygous β-thalassaemia and co-inheritance of extra α-globin genes (ααα/αα or ααα/ααα), and heterozygosity for hyperunstable haemoglobin variants (dominantly inherited β-thalassaemia).

Mild β Thalassaemia Mutations

The majority of β-thalassaemia mutations result in a total absence or severe reduction of β-globin chain synthesis. However, there are several mild β-thalassaemia mutations, which result in a less severe reduction in the synthesis of normal β-globin chains (Fig.1). Homozygotes for these mutations tend to have a mild form of thalassaemia intermedia, usually not requiring regular transfusions. Compound heterozygotes for one of these mutations and a severe defect of β-globin chain production results in a markedly heterogeneous clinical picture ranging from thalassaemia intermedia to late presenting thalassaemia major (4).

A common mutation responsible for thalassaemia intermedia in the Mediterranean region is a T→C substitution at position 6 in the first intervening sequence of the β-globin gene (IVS1 nt.6) (5,6). It results in a reduction in the normal splicing of the primary RNA transcript, and reduces HbA synthesis to about 30% of normal. Patients homozygous for this mutation run haemoglobin levels in the 7-10 g/dl range, and consistently tend to have a mild disease. IVS 1 nt. 6 is the commonest of the mild β-thalassaemia mutations in Turkey, with a frequency of nearly 20% among Turkish thalassaemics as reported by Atalay et al, and Başak et al (7,8). It is therefore frequently encountered in Turkish patients with thalassaemia intermedia.

Another common set of mild mutations involved in thalassaemia intermedia are the mutations within the promoter sequences 5' to the β-globin gene. They include -28 in Chinese and Kurdish Jews, -29 in American blacks, and -31 in Japanese (9-11). No β-thalassaemia mutations have yet been described in the second of the conserved sequences (CCAAT box). The third conserved sequence is the CACCC box, and mutations at -87 position in Mediterraneans (5, 12) and -88 position in Blacks and Asian Indians (13) are mild β-thalassaemia mutations. The mutation at position -101 is a common cause of mild silent β-thalassaemia in Italians (14).

Cap site mutations are a less common but an important cause of mild β-thalassaemia. In one study of Asian Indian thalassaemics Cap+1 mutation was found to be present exclusively in thalassaemia intermedia patients (15). Homozygosity for the Cap site mutation tends to result in a mild, transfusion independent thalassaemia intermedia phenotype (4).

Another group of mild β-thalassaemia mutations involves single base substitutions in the first exon of the β-globin gene. There are two structurally abnormal haemoglobins:

1. Haemoglobin E, caused by a mutation at codon 26, resulting in substitution of Glu to Lys. This causes alternative splicing in exon 1 at the cryptic splice site generated by the codon 26 mutation (16). This site has sufficiently enhanced affinity with the splicing apparatus to allow it to compete with the normal donor site and retard IVS 1 excision. Therefore, the nucleotide substitution in the exon has a deleterious effect on both the pattern and the rate of processing of the globin RNA. Compound heterozygosity for β-thalassaemia and HbE produce a wide spectrum of clinical severity with a number of patients developing

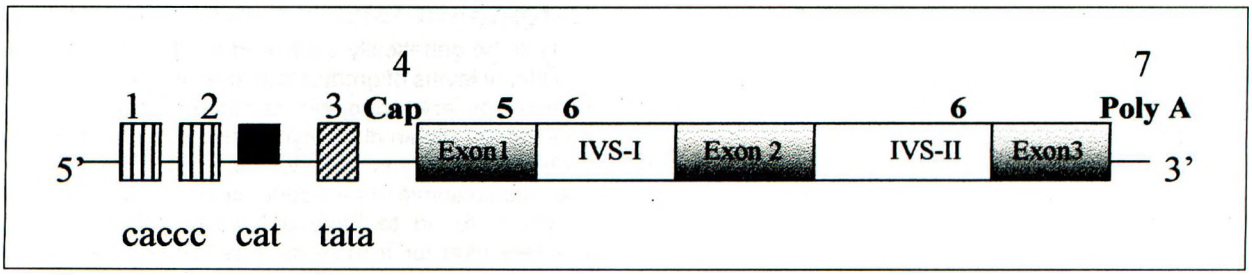

Fig.1. Mild β-thalassaemia mutations
Promoter site mutations (1-3):
CACCC box mutations are located at:
1. -101
2. -86, -87, -88
TATA box mutations are located at:
3. -29, -30, -31
4. Cap site mutations
5. Mutations activating alternate splice sites: e.g codon 26 G→A (H→E), codon 27 G→T (Hb Knossos).
6. Consensus sequence mutations: IVS 1 nt 6 T→C, IVS II nt 844C→G
7. Polyadenylation site mutations

thalassaemia major (17, 18). Homozygotes for HbE are clinically asymptomatic.

2. Haemoglobin Knossos, caused by codon 27 mutation, resulting in substitution of Ala to Ser. This leads to abnormal RNA processing and reduced synthesis of this variant in a similar mechanism as in the case of HbE. Unlike HbE/β-thalassaemia, compound heterozygosity of β-thalassaemia and Hb Knossos consistently leads to the clinical picture of thalassaemia intermedia (19). Compound heterozygous state for Hb Knossos and β-thalassaemia, even though rare, is also a recognised cause of thalassaemia intermedia in Turkey (20).

Co-Inheritance of α Thalassaemia

In homozygous β-thalassaemia there is a great excess of α chains that precipitate in the red cell precursors causing ineffective erythropoiesis. Therefore, as expected, co-inheritance of α-thalassaemia leading to a reduction in α-globin production is an ameliorating factor (21).

The effects of coincidental α-thalassaemia on the clinical phenotype of homozygous β-thalassaemia has been investigated in Greek Cypriots, Asian Indian, and Sardinian populations (13,22,23). The conclusions from these studies are that co-inheritance of the deletion of two α-globin genes (-α/-α), or a non-deletional mutation silencing the function of one of the α_2- globin genes, leads more frequently to thalassaemia intermedia than to a thalassaemia major phenotype in homozygous β0- thalassaemia. On the other hand, β^o homozygotes with a single α-globin deletion (-α/αα), usually develop thalassaemia major. In homozygous β^+ thalassaemia, even a single α-globin deletion seems to be able to produce a mild phenotype in some cases. Co-inheritence of three α-globin gene deletions (-α/--) frequently ameliorates the severe clinical picture of homozygous β-thalassaemia to thalassaemia intermedia (4). Alpha globin gene deletions, the most common of which is the 3.7kb α-globin gene deletion, are a frequent cause of thalassaemia intermedia in Turkey (24).

Enhancement of γ Chain Synthesis

The marked chain imbalance in homozygous β-thalassaemia can also be reduced by inheritance of a determinant for enhanced production of γ chains, which will then combine with excess α-chains to produce HbF (25). Increase in HbF production can occur either by mutations that enhance γ-globin chain production, or by the presence of polymorphisms adjacent to the β-globin gene complex.

Mutations That Enhance HbF Production

1. δβ-thalassaemia

Many of the δβ-thalassaemias result from deletions of the δ and β globin genes. These are associated with persistent γ-chain synthesis at a much higher level than is observed in β-thalassaemia. This seems to result from an enhancer effect on γ-chain production caused by remote sequences being brought into the vicinity of the γ gene by the deletion. Patients homozygous for δβ-thalassaemia have 100% HbF and are generally mildly affected. Similarly patients, who are compound heterozygotes for δβ-thalassaemia and β-thalassaemia usually, but less consistently, present with the clinical picture of thalassaemia intermedia (4, 26). δβ-thalassaemia, though uncommon, can be encountered in Turkey, and include a novel Turkish form as described by Öner et al (27).

2. Haemoglobin Lepore

Hb Lepore disorders are forms of δβ-thalassaemia, and they result from production of δβ-fusion genes, which direct the synthesis of δβ fusion chains. They result from non-homologous crossing over between the δ and β-globin genes (28), and there are several different forms of Hb Lepore depending on the exact position of the abnormal crossing over. The δβ chains of Hb Lepore are synthesized inefficiently, and there is less output of γ chains to compensate for the deficiency of δ and β chains than in the δβ-thalassaemias described above. Hence, the Hb Lepore disorders usually produce a more severe clinical phenotype. Homozygous Hb Lepore usually results in thalassaemia intermedia, whereas the compound heterozyopus state for Hb Lepore and severe β-thalassaemia more commonly results in thalassaemia major (26). Hb Lepore is rare, but nevertheless, encountered in Turkey (29).

3. Hereditary Persistence of Fetal Haemoglobin (HPFH)

HPFH is the genetically controlled synthesis of HbF in adult life, at levels of greater than the normal 1%, in the absence of erythropoietic stress or thalassaemic imbalance of globin chain synthesis. Inheritance of an HPFH determinant is usually suspected to be present in a thalassaemia intermedia patient if one of the parents is found to have a level of HbF which is unusually high for a thalassaemia heterozygote. The mutations, which cause HPFH fall into two classes:

a) Deletional, with large deletions of δ and β-globin genes, but leaving embryonic and fetal globin genes intact and causing high HbF production. Co-inheritance of deletion HPFH with heterozygous β^+ thalassaemia usually results in a clinically

asymptomatic condition, the red cell indices being very similar to those of heterozygous β-thalassaemia, but the HbF hevel greatly increased (60-80%) and HbA_2 normal or elevated (4). Deletional HPFH is rarely encountered in Turkey (30).

b) Non-deletional, in which the β-globin gene remains intact. Some forms of non-deletional HPFH mutations are due to single base changes upstream of either Gγ or Aγ genes. For instance Greek HPFH is due to mutation at -117 position 5' to cap site of the Aγ gene (31), and mutations at -196, -198, and -202, which also result in HPFH, are situated 5' to the Gγ gene (32). These HPFH determinants are associated with HbF levels of 5-25% in heterozygotes.

Haplotype Associated γ Chain Synthesis

Some patients have unusually high levels of HbF despite the absence of an obvious HPFH determinant in either parent. One explanation for such findings is that some β-thalassaemia mutations are present on chromosomal backgrounds which enhance the expression of γ genes. It has been shown that in some populations one common haplotype (- + - + +) is over-represented in patients with thalassaemia intermedia and is associated with raised HbF levels (33, 34). This particular haplotype is associated wtih nucleotide C→T change at -158 position relative to the Gγ gene, and is detectable by the restriction enzyme Xmn 1. In contrast to the non-deletional HPFH mutations described above, the increase in HbF that is associated with the Xmn 1 Gγ polymorphic site is dependent on erythropoietic stress, as occurs in homozygous β-thalassaemia (35, 36). Xmn 1 Gγ polymorphic site is present in the Turkish population as reported by Altay et al, and should be searched for in patients with thalassaemia intermedia phenotype (37).

Implications for Prenatal Diagnosis

Accurate and precise prediction of phenotype from genotype would also have important implications for prenatal diagnosis. Diagnosis of homozygous β-thalassaemia in the fetus usually leads to selective abortion, and it has not been possible to discriminate between fetuses with either thalassaemia major or intermedia. This is a source of discomfort for clinicians as some terminations result in the loss of fetuses who would otherwise have developed into mildly affected children (3, 4, 38).

Genotypes which can be expected to give a mild phenotype include homozygosity for mild β^+thalassaemia mutants, and homozygosity for β^+thalassaemia with co-inheritance of types of α-thalassaemia equivalent to the loss of 2 alpha-globin genes (-α/-α, or --/αα). Similarly, patients homozygous for δβ-thalassaemia will have a mild disorder. In a recent study, the total number of alleviating mutations present in a thalassaemic patient was also found to have a significant influence on the phenotype of homozygous β-thalassaemia (38).

However, some molecular interactions seem to be associated with a range of clinical outcome. These include homozygous β-thalassaemia with co-inheritance of heterozygous α^+ thalassaemia (-α/αα), or co-inheritance of the homozygous (+/+) or heterozygous (-/+) state for the Xmn 1 Gγ site polymorphism. Current genetic information does not account for the full range of phenotypic variation, but such information is of potential value both in the clinical management and the prenatal diagnosis of homozygous β-thalassaemia.

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