Phenotype-genotype correlation in $\beta$-thalassemia

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Abstract

The clinical manifestations of $\beta$-thalassemia are extremely heterogeneous, ranging from severe transfusion-dependent anemia, to the mild non transfusion dependent thalassemia intermedia and to the asymptomatic carrier state. The remarkable phenotypic variability is primary due to variations in the different globin genes (primary gene modifiers). The main pathophysiological determinant of the severity of $\beta$-thalassemia syndromes is the extent of $\alpha$/non-$\alpha$ globin chain imbalance. Therefore, any factor capable of reducing the globin chain imbalance may have an ameliorating effect on the clinical picture. The most common mechanisms responsible of the amelioration of the phenotype are mild or silent $\beta$ thalassemia alleles, coinheritance of $\alpha$ thalassemia, or of genetic determinants associated with increased $\gamma$ globin chain production. Rarely, other complex mechanisms including dominantly inherited $\beta$ thalassemia, somatic deletion of $\beta$ globin gene and coinheritance of extra $\alpha$ globin genes with heterozygous $\beta$ thalassemia have been reported. In addition to the variability of the phenotype resulting from primary gene modifiers, other genetic factors (secondary gene modifiers), mapping outside the $\beta$ and $\alpha$ globin cluster, may influence the disease complications. Among these factors the ones best so far defined are those affecting bilirubin, iron and bone metabolism. However, the new methods of DNA analysis (i.e. GWAS and related methods) are expect expand the number of genes or gene variants involved in the phenotypic variability and in the response to treatment of $\beta$ thalassemia.

Introduction

$\beta$-thalassemias are among the commonest monogenic disorders. More than 200 $\beta$-thalassemia defects have been so far identified (list available at http://globin.cse.psu.edu/globin/Thalvar), but population studies indicate that about 40 account for 90% of the $\beta$-thalassemias world wide.$^1$ There are two main varieties of $\beta$-thalassemia alleles $\beta^+$-thalassemia in which the $\beta$-globin chains are absent and $\beta^+$/non-$\beta$-thalassemia in which $\beta$-globin chain production is variably reduced. The clinical manifestations of $\beta$-thalassemia are extremely heterogeneous ranging from very severe anemia, to mild thalassemia intermedia, to the asymptomatic state of thalassemia trait. This phenotypic variability is mainly due to genetic factors, which have been largely, but not completely, defined in recent years. This article reviews the molecular basis of this phenotypic diversity with an overview of the modifier genes. Modifier genes can be divided in primary, which modulate the clinical severity of the disease and secondary which affect some of the disease complications.

Primary genetic modifiers

Homozygous $\beta$-thalassemia

Homozygosity or compound heterozygosity for $\beta$-thalassemia most commonly result in the clinical phenotype of transfusion-dependent thalassemia major, but a consistent proportion of homozygotes may develop milder forms (thalassemia intermedia), which range in severity from thalassemia major, to the $\beta$-thalassemia carrier state.$^{2,4}$ Since the main pathophysiological determinant of the severity of the $\beta$-thalassemia syndromes is the extent of $\alpha$/non-$\alpha$ globin chain imbalance, any factor capable of reducing the $\alpha$/non-$\alpha$-chain imbalance result in a lesser degree of $\alpha$ globin chain precipitation and may have an ameliorating effect on the clinical picture (Figure 1).

The most common mechanism consistently resulting in thalassemia intermedia is the coinheritance of homozygosity or compound heterozygosity for a mild $\beta$-thalassemia allele, namely a $\beta$-thalassemia defect associated with a consistent residual output of $\beta$-chains from the affected $\beta$-globin locus. Most mild $\beta$-thalassemia mutations result from mutations in the promoter of the $\beta$-globin gene, the poly-A cleavage site, cryptic splice sites in exons or consensus sequences in introns. The most common mild mutations are reported in table 1. Hemoglobin E (HbE), which is a thalassemic structural variant, prevailing in Southeast Asia and characterized by the presence of an abnormal structure as well as biosynthetic defect, should be included in this group. The nucleotide substitution at codon 26, producing the HbE variant ($\alpha^2 \beta^2 \gamma^{26} E^>$K), activates a potential cryptic RNA splice region, resulting in alternative splicing at this position. The homozygous state for HbE results in a mild hemolytic microcytic anemia. Compound heterozygosity for $\beta$-thalassemia and HbE results in a wide
range of often severe, but sometimes mild or even asymptomatic clinical phenotypes. (Table 1) Compound heterozygotes for a mild and a severe defect have variable phenotypes, ranging from severe to mild forms. Therefore from the clinical point of view, the presence of a mild and a severe β-thalassemia allele, does not predict a mild clinical picture.

The silent alleles are the mildest β-thalassemia alleles they show normal hematological features and can be identified solely by a slight unbalance of α/non α-globin chain synthesis ratio.7,8 Homozygosity for silent alleles produces a very mild form of thalassemia intermedia and the β-silent/β-mild or β-silent/β-severe genotypes also result in mild β-thalassemia. A list of the most common silent and mild β-thalassemia alleles is reported in Table 1.

The homozygous β° or severe β⁺ thalassemia, despite the severity of the mutation at the β⁺-locus, may develop an attenuated form resulting from coinherited modifying ameliorating genetic factors.

One mechanism leading to mild β-thalassemia is the coinheritance of homozygous β-thalassemia, with α-thalassemia determinants that reducing the α-globin chain product decrease the α/non α chain imbalance.1,10,11 Another mechanism is the coinheritance of genetic determinants able to sustain a continuous production of γ chains in adult life, thereby reducing the extent of the α/non α chain imbalance. This occurs in δ β°-thalassemia, which is due to deletions of variable extent within the β⁺-globin cluster, and in those deletions involving only the 5' region of the β⁺-globin promoter. Other conditions associated with high γ-chain output are the cotransmission of the non-deletion forms of HPFH (Hereditary Persistence of Fetal Hb), due to point mutations at Gγ or Aγ promoters (-196 C→T Aγ→-158 C→T Gγ). A C→T mutation at position -196 Aγ has been found to be associated in cis with the codon 39 nonsense mutation in some Sardinian β-thalassemia chromosomes (Sardinian δβ°-thalassemia).12 Compound heterozygosity for this determinant and for typical β⁺-thalassemia thus develop thalassemia intermedia since the increase in γ-chain production from the -196 Aγ gene, compensates for the absence of β⁺-chain production from the affected β⁺ locus. Homozygous state for the Sardinian δβ°-thalassemia is clinically normal and can be detected solely by hematological analysis.13 The C→T substitution at -158 Gγ is silent both in normal subjects and β-thalassemia heterozygotes, but leads to a high HbF production rate during hematopoietic stress, as occurs in homozygous β-thalassemia or sickle cell anemia.14 The -158 Gγ mutation is associated with IVS II n 1 (G→A), frameshift 8 (AA), frameshift 6 (A-A) and sometime with codon 39 nonsense mutations, thereby explaining the mild phenotype possibly associated with these mutations.

Genetic determinants capable to sustain a continuous production of HbF in adult life and mapping outside the β⁺-globin cluster have been mapped on chromosome 2p16 and chromosome 6q23.15-17 The locus on chromosome 2p16 has been located by GWAS on the BCL11A gene and the SNP rs11886868 in its intron 2 was found strongly associated with HbF levels. The BCL11A variants were shown to influence HbF levels in Sardinians with elevated HbF levels and in non-anemic Caucasians from a European twin study.18 The same variant was significantly higher in β⁰ thalassemia heterozygotes for the codon 39 nonsense mutation with a mild phenotype (thalassemia intermedia) and in patients with mild sickle cell disease.15,18 Accoding to these results the determination of BCL11A polymorphism in young homozygous β-thalassemia and sickle cell anemia patients may serve as a prognostic indication for the severity of the disease. Furthermore targeted downregulation of BCL11A in patients could elevate HbF levels and thereby ameliorate the severity of these inherited anemia.

Other recent studies have shown another genetic variant responsible for HbF variation mapping in the HBS1L-MYB region on chromosome 6.19 Recent studies have shown that the HBS1L-MYB intergenic polymorphisms contain regulatory sequences controlling MYB expression.20 Further studies however are necessary to elucidate the biological role of these two genes in the modulation of HbF. Coinheritance of these HPFH determinants and α-thalassemia contribute in the amelioration of the phenotype of homozygous β-thalassemia accounting for 75% of difference in clinical severity.21

However, despite the significant progress made in this field of thalassemia disorders, in a significant number of cases of thalassemia intermedia, the responsible that molecular mechanism has not been so far elucidated.

Heterozygous β⁺-thalassemia

Heterozygous β⁺-thalassemia, whether β⁰ or β⁺, is completely asymptomatic and characterized by microcytosis, hypochromia, increased HbA₂ levels and unbalanced α/non α globin chain synthesis. However, several environmental or genetic factors may modify this phenotype, leading either to thalassemia intermedia, despite the presence of a single β⁺-globin gene affected, or to hematologically atypical carrier states.

To date, two main mechanisms have been identified which may increase the clinical severity of β⁺-thalassemia heterozygotes. The first is the coinheritance with heterozygous β⁺-thalassemia of triple or quadruple α⁺-globin gene arrangement, which, byworsening of the imbalance of α/non α⁺-globin chain synthesis, may cause an excess of unassembled α⁺-chains, thereby resulting in premature destruction of red blood cell precursors. β⁺-thalassemia carriers who are heterozygous or homozygous for the triplicated α⁺-globin gene arrangement, develop the clinical phenotype of thalassemia intermedia.22-24 This effect has been consistently detected in homoyzogosity for these arrangements but inconstantly in heterozygotes.

The same thalassemia intermedia phenotype has also been reported in β⁺-thalassemia carriers who coinherited an α⁺-globin gene quadruplication.26-28

The other mechanism increasing the severity of the β⁺-thalassemia carrier state is heterozygosity for mutations in β⁺-globin gene that result in hyper-unstable hemoglobins, which precipitate in the red cell membrane together with unassembled α⁺-globin chains, resulting in markedly ineffective erythropoiesis. Most of these mutations lie in the third exon and lead to the production of a markedly unstable Hb variant often not detectable in peripheral blood.30,31 β⁺-thalassemia resulting from hyperunstable β⁺-chains is transmitted in a dominant fashion or may result from a de novo mutation.

The diagnosis of dominant β⁺-thalassemia is difficult because the unstable β⁺-globin chains in peripheral blood are not easily detected. Such conditions should be suspected from the presence of β⁺-tha-
lassemia-like disorders of intermediate severity arising de novo or transmitted according to a dominant pattern.

Very rarely β-thalassaemia intermedia may develop in subjects heterozygous for β-thalassaemia because of a mosaic somatic deletion of the in trans β-globin gene in a subpopulation of hematopoietic cells.32, 33

Compound heterozygosity for β-thalassaemia and some β-chain structural variant (HbD-Los Angeles β 121 Glu→Gln; HbC β 6 Glu→Lys; HbO-Arab β 121 Glu→Lys unstable variants) may produce thalassaemia intermedia, as a result of globin chain imbalance in combination with the modified structural and functional characteristic of the variants.34

Finally the proposed role, if any, of the α-Hb stabilizing protein (AHSP) as a modulating factor of the phenotype has not yet clarified.35 AHSP forms a stable complex with free α-Hb to protect them from precipitation, thereby acting as a specific molecular chaperone. In the mouse system, knockout of AHSP led to reduced lifespan of circulating red blood cells, caused increased apoptosis of erythroid precursors, and exacerbated the severity of heterozygous β-thalassaemia, which usually displays a thalassaemia intermedia phenotype.35 The studies of ASHP in humans led to inconstant results. It seems, however, that variation of the ASHP level may be able to aggravate the phenotype of simple heterozygotes for β-thalassaemia.36, 37

### Secondary genetic modifiers

The clinical phenotype of homozygous β-thalassaemia may also be modified by the coinheritance of other genetic factors mapping outside the β-globin gene cluster and affecting some disease complications. Among these factors the ones best delineated so far are those affecting bilirubin, iron, bone metabolisms.38

Because of the rapid turnover of red cell precursors and the resulting breakdown of the heme products, both homozygotes and heterozygotes for β-thalassaemia may develop mild jaundice and have the propensity to gallstone formation. In transfused thalassaemia major patients a further increase in bilirubin production results from the breakdown of transfused red blood cells. It has been shown that in these subjects the level of indirect bilirubin as well as gallstone formation, are related to a polymorphic motif in the promoter of the gene involved in the hepatic glucuronidation of bilirubin, namely bilirubin UDP-glucuronosyltransferase (UGT1A1). In normal individuals, the UGT1A1 promoter has six TA (TA6) repeat in the TATA box. Homozygotes for an additional repeat (TA7) develop mild unhydrated hyperbilirubinemia (Gilbert’s syndrome) because of less efficient activity of UGT1A1. Studies in thalassaemia major, thalassaemia intermedia and the β-thalassaemia carrier state have shown that patients developing hyperbilirubinemia, jaundice and gallstone usually have more commonly the less efficient TA7 motif.39-41 It is clear from these findings that the Gilbert syndrome associated mutation, acts as a modifying gene in β-thalassaemia by determining or promoting the development of jaundice or gallstones.

Patients with β-thalassaemia, especially when not adequately chelated, develop iron overload in many organs including liver, heart and endocrine glands, in part because of the destruction of transfused red blood cells and in part, particularly in thalassaemia intermedia, because of increased iron absorption. Some studies seem to indicate that the common mutation of the HFE gene (C282Y), which causes the common type of hereditary hemochromatosis (HH), might be involved in determining the variability of iron overload in patients with thalassaemia intermedia.42 However, in thalassaemia major the presence of a single mutation in HFE gene (C282Y and H63D) does not influence the severity of iron loading, assessed by serum ferritin and liver iron concentration, likely because the effect of the mutations on iron overload is hidden by the treatment (i.e. post-transfusional iron overload and iron chelation).43 Furthermore, homozygosity for the H63D mutation whose functional significance in HH is still being evaluated, when coinherited with heterozygous β-thalassaemia, seems to determine an increase in iron overload.44 Conversely, coinherited homozygosity for β-thalassaemia appears to increase the rate of iron accumulation in C282Y homozygotes.45

Another common complication in adults with β-thalassaemia is the development of marked and progressive osteoporosis which depends on many factors including hypogonadism and extent of iron chelation. However, recent evidence seems to indicate that the development of this complication may be also related to polymorphisms of the genetic loci involved in bone metabolism, namely vitamin D receptor and the COLIA1 gene, even though these results have not been confirmed in other studies.49-54

### Conclusions

We presented an overview of the molecular basis of β-thalassaemias correlating the genetic heterogeneity with the wide clinical spectrum of the disease.

Several primary and secondary gene modifiers have been identified, but in a number of cases and for some disease-related complications, the responsible molecular mechanism has not been so far elucidated.

Even though phenotype prediction from genotype is not always accurate, the information obtained from extended genetic analysis may be used for planning appropriate management and providing adequate genetic counselling, and may reveal potential new targets for therapeutic intervention.
References


