

# Hemoglobinopathy

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## Pathophysiology of $\beta$ Thalassemia— A Guide to Molecular Therapies

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The central mechanism underlying the pathophysiology of the  $\beta$  thalassemias can be related to the deleterious effects of imbalanced globin chain synthesis on erythroid maturation and survival. An imbalance of the  $\alpha$ /non- $\alpha$  globin chains leads to an excess of unmatched  $\alpha$  globin which precipitates out, damaging membrane structures leading to accelerated apoptosis and premature destruction of the erythroid precursors in the bone marrow (ineffective erythropoiesis). Close observation of the genotype/phenotype relationships confirms the pathophysiological mecha-

nism and provides clues to molecular therapies, all of which aim to reduce the  $\alpha$ /non- $\alpha$  chain imbalance. They include inheritance of the milder forms of  $\beta$  thalassemia, co-inheritance of  $\alpha$  thalassemia, or genetic factors (quantitative trait loci, QTLs) for increasing  $\gamma$  globin expression. Currently, the most promising molecular therapeutic approaches include increasing  $\beta$  globin gene expression by stem cell gene therapy and increasing  $\gamma$  globin expression using pharmacological agents or by transduction of the  $\gamma$  globin genes.

The pathophysiology of  $\beta$  thalassemia has been the subject of several extensive reviews;<sup>1</sup> this article concentrates on its relationship to the development of two approaches to molecular therapy: induction of fetal hemoglobin, and stem cell gene therapy.

### Pathophysiology

$\beta$  thalassemia occurs when there is a quantitative reduction of  $\beta$  globin chains that are usually structurally normal.<sup>2</sup> They are caused by mutations that nearly all affect the  $\beta$  globin locus and are extremely heterogeneous. Almost every possible defect affecting gene expression at transcription or post-transcriptional level, including translation, have been identified in  $\beta$  thalassemia.<sup>3</sup> These genetic defects lead to a variable reduction in  $\beta$  globin output ranging from a minimal deficit (mild  $\beta^+$  thalassemia alleles) to complete absence ( $\beta^0$  thalassemia).

In  $\beta$  thalassemia, the synthesis of normal  $\alpha$  globin chains from the unaffected  $\alpha$  globin genes continues as normal, resulting in the accumulation within the erythroid precursors of excess unmatched  $\alpha$  globin. The free  $\alpha$  globin chains are not able to form viable tetramers and instead precipitate in the red cell precursors in the bone marrow forming inclusion bodies. These  $\alpha$  chain inclusions can be demonstrated by both light and electron microscopy in the erythroid precursors in the bone marrow as well as in the peripheral red cells following splenectomy. They are responsible for the extensive intramedullary destruction of the erythroid precursors and hence the ineffective erythropoiesis that underlies all  $\beta$  thalassemias.

Anemia in  $\beta$  thalassemia thus results from a combination of ineffective erythropoiesis, peripheral hemolysis, and an overall reduction in hemoglobin synthesis. The severity of disease in  $\beta$  thalassemia correlates well with the degree of imbalance between  $\alpha$  and non- $\alpha$  globin chains and the size of the free  $\alpha$  chain pool. Thus, factors that reduce the degree of chain imbalance and the magnitude of  $\alpha$  chain excess in the red cell precursors will have an impact on the phenotype.

### Clinical Phenotypes of $\beta$ Thalassemia

Typically,  $\beta$  thalassemia is inherited as haploinsufficient Mendelian recessives. The most severe end of the clinical

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spectrum,  $\beta^{\circ}$  thalassemia, is characterized by the complete absence of Hb A ( $\alpha_2\beta_2$ ) and results from the inheritance of two  $\beta^{\circ}$  thalassemia alleles (homozygous or compound heterozygous states).<sup>2</sup> This normally results in the transfusion-dependent state of  $\beta$  thalassemia major and, at worst, the patients present within 6 months of life with profound anemia and, if not treated with regular blood transfusions, die within their first 2 years. Individuals who have inherited a single  $\beta$  thalassemia allele, whether  $\beta^{\circ}$  or  $\beta^{+}$ , have thalassemia trait. They are clinically asymptomatic but may have a mild anemia with characteristic hypochromic microcytic red blood cells, elevated levels of Hb A<sub>2</sub> ( $\alpha_2\delta_2$ ) and variable increases of Hb F ( $\alpha_2\gamma_2$ ). Inheritance of two  $\beta$  thalassemia alleles, however, does not always lead to thalassemia major; many patients with two  $\beta$  thalassemia alleles have a milder disease, ranging from a condition that is slightly less severe than transfusion-dependence to one that is asymptomatic and often mistaken for thalassemia trait. The diverse collection of phenotypes between the two extremes of thalassemia major and trait, constitute the clinical syndrome of thalassemia intermedia.

The heterozygous states for  $\beta$  thalassemia also show a tremendous phenotypic diversity, comparable to that for the inheritance of two  $\beta$  thalassemia alleles. In some cases, the  $\beta$  thalassemia allele is so mild that it is phenotypically 'silent,' with no anemia or hematological abnormalities.<sup>4,5</sup> In others, the heterozygous state causes a phenotype almost as severe as the major forms, that is, the  $\beta$  thalassemia allele is dominantly inherited.<sup>6</sup>

The phenotypic diversity of thalassemia intermedia is equaled by its underlying genetic diversity. Close observations of the genotype/phenotype correlation in the intermediate state has provided tremendous insights and clues for the development of molecular therapy for the  $\beta$  thalassemias.<sup>7</sup>

### Genotype/Phenotype Correlation in Thalassemia Intermedia

The main genetic interactions that result in a phenotype of thalassemia intermedia are summarized in **Table 1**. Thalassemia intermedia can result from the inheritance of one or two  $\beta$  thalassemia alleles. In 60%-90% of the cases<sup>8-10</sup> the patients have inherited two  $\beta$  thalassemia alleles and the reduced disease severity can be explained by the inheritance of the milder forms ( $\beta^{++}$  and 'silent'  $\beta$  thalassemia alleles) that allow the production of a significant proportion of  $\beta$  globin chains. Co-inheritance of a single  $\alpha$  globin gene deletion ( $\alpha\alpha/\alpha-$ ) has very little effect on  $\beta^{\circ}$  thalassemia, but individuals with 2  $\alpha$  globin gene deletions ( $\alpha\alpha/\alpha-$  or  $\alpha\alpha/--$ ) and  $\beta^{+}$  thalassemia have a mild disease requiring intermittent transfusions. The role of increased Hb F response as an ameliorating factor becomes evident in the group of thalassemia intermedia patients who are mildly affected despite having minimal amounts or no Hb A ( $\alpha_2\beta_2$ ), and without  $\alpha$  thalassemia. Although the presence of the *cis Xmn1-G $\gamma$*  site promotes some Hb F increase, manifested

by a late presentation, the magnitude of the Hb F response is moderate; many of these patients subsequently become transfusion-dependent. However, there are some  $\beta^{\circ}$  thalassemia patients who have a mild disease and remain transfusion-independent despite being *Xmn1-G $\gamma$* <sup>-/-</sup> and without  $\alpha$  thalassemia.<sup>8,11</sup> In many cases, family studies have shown that this inherent capacity for producing Hb F is due to a genetic determinant(s) that is not linked to the  $\beta$  globin complex. Indeed, analysis of a group of thalassemia intermedia patients revealed seven sib-ships with discordant phenotypes despite having inherited identical  $\alpha$  and  $\beta$  genotypes.<sup>8</sup> Their steady state Hb F values ranged from 1 g/L to as much as 8-9 g/L, and was ascribed to co-inheritance of quantitative trait loci (QTLs) for Hb F that are not linked to the  $\beta$  globin complex (i.e., *trans*-acting).

Other Hb F determinants within the cluster are related to the mutation itself. Small deletions or mutations that involve the promoter sequence of the  $\beta$  globin gene are associated with variable increases in Hb F and unusually high Hb A<sub>2</sub> levels, and reflect the competition between the  $\gamma$  and  $\beta$  globin gene promoters for interaction with the up-

**Table 1. Thalassemia intermedia. The common genetic interactions that underlie the phenotype of thalassemia intermedia**

- I Homozygous or compound heterozygous state for  $\beta$  thalassemia
  - a) Inheritance of mild  $\beta$  thalassemia alleles,  $\beta$  'silent' and  $\beta^{++}$ , in homozygous or compound heterozygous states  
Phenotype depends on the sum total of  $\beta$  globin output
  - b) Co-inheritance of  $\alpha$  thalassemia  
Phenotype depends on severity of imbalance between  $\alpha$ /non- $\alpha$  globin reflecting severity of  $\alpha$  and  $\beta$  globin deficit
  - c) Increased Hb F response
    - *Xmn1-G $\gamma$*  polymorphism
    - $\beta$  globin gene promoter mutations deletions or point mutations
    - *Trans*-acting quantitative trait loci (QTLs) for Hb F on 6q, Xp, and 8q
- II Heterozygous state for  $\beta$  thalassemia
  - a) Co-inheritance of extra  $\alpha$  globin genes ( $\alpha\alpha\alpha/\alpha\alpha$ ,  $\alpha\alpha\alpha/\alpha\alpha\alpha$ ,  $\alpha\alpha\alpha\alpha/\alpha\alpha$ ,  $\alpha\alpha\alpha\alpha/\alpha\alpha\alpha\alpha$ )
  - b) Dominantly inherited  $\beta$  thalassemia (Hyperunstable  $\beta$  globin chain variants)
  - c) Somatic deletion of the other  $\beta$  globin locus—mosaicism
- III Compound heterozygotes for  $\beta$  thalassemia and  $\beta$  chain variants  
e.g., Hb E/ $\beta$  thalassemia
- IV Compound heterozygotes for  $\beta$  thalassaemia and HPFH or  $\delta\beta$  thalassemia

A considerable variation in clinical phenotype of these genetic interactions has been observed.

stream  $\beta$  LCR.<sup>7</sup> Hence, although such deletions cause a complete absence of  $\beta$  globin product, the severity of the phenotype is offset by the concomitant increase in hemoglobin F.

Inheritance of single copies of  $\beta$  thalassemia gene can also lead to thalassemia intermedia. In the majority of cases this is caused by the co-inheritance of extra  $\alpha$  globin genes, triplicated ( $\alpha\alpha\alpha$ ) or quadruplicated ( $\alpha\alpha\alpha\alpha$ ). In a number of cases, the unusually severe heterozygous state is associated with a normal  $\alpha$  globin genotype. In such cases, the  $\beta$  thalassemia mutation itself leads to the synthesis of highly unstable, structurally abnormal  $\beta$  globin chain variants.<sup>6</sup> The hyperunstable  $\beta$  chain variants are rapidly destroyed in the erythroid precursors, giving rise to a functional deficiency of  $\beta$  chains and simulating a phenotype of  $\beta$  thalassemia. The non-functional  $\beta$  chain variants together with the unmatched  $\alpha$  globin chains aggravate the ineffective erythropoiesis causing a disease phenotype even when present in a single copy. Hence, the term “dominantly inherited  $\beta$  thalassemia.”

Recently, somatic mosaicism of the  $\beta$  globin gene have been described causing thalassemia intermedia. In one report, the proband had moderately severe thalassemia intermedia despite being constitutionally heterozygous for a common  $\beta^0$  thalassemia mutation ( $\beta^0$  39 C→T) with a normal  $\alpha$  genotype.<sup>12</sup> Subsequent investigations revealed that he had a somatic deletion of a region of chromosome 11p15 including the  $\beta$  globin complex in trans to the thalassemia gene giving rise to a mosaic of cells, 50% with one normal  $\beta$  globin gene and 50% without any normal  $\beta$  globin gene (but only the  $\beta$  thalassemia gene). The sum total of the  $\beta$  globin product is ~25% less than the normally asymptomatic  $\beta$  thalassemia trait. Subsequently, two unrelated Italian patients were also reported to have thalassemia intermedia caused by somatic deletions of chromosome 11p15 in a subpopulation of hematopoietic cells.<sup>13</sup>

These unusual cases once again illustrate that the severity of anemia of  $\beta$  thalassemia reflects the quantitative deficiency of  $\beta$  globin chain production. Furthermore, with respect to potential gene therapy, expression of a single  $\beta$  globin gene in a proportion of the red blood cells appears to be sufficient to redress the chain imbalance to produce a condition mild enough not to need major medical intervention.

Given the differences in the spectrum of  $\beta$  thalassemia mutations, differences in the frequency of  $\alpha$  thalassemia, and prevalence of the different QTLs for Hb F, the relative importance of the different factors would vary accordingly in different population groups. It is important to note that the genotypic factors are not mutually exclusive.

## Factors Modifying $\beta$ Thalassemia— Clues to Molecular Therapy

### Primary modifiers—magnitude of $\beta$ globin deficit

More than 200 mutations causing  $\beta$  thalassemia have been described and, with the exception of a few deletions, the vast majority of  $\beta$  thalassemia are caused by point mutations within the gene or its immediate flanking sequences.<sup>3</sup> A few  $\beta$  thalassemia mutations that segregate independently of the  $\beta$  globin cluster have been described in several families; in such cases, *trans*-acting regulatory factors have been implicated. Examples of reduced  $\beta$  globin production caused by loci outside the  $\beta$  globin complex include those arising in the general transcription factor TF11H<sup>14</sup> and the erythroid-specific transcription factor GATA-1.<sup>15</sup>

Functionally the  $\beta$  thalassemia alleles can be classified as  $\beta^0$  or  $\beta^+$  reflecting the resulting phenotype:  $\beta^0$  thalassemia, in which there is a complete absence of  $\beta$  globin production, and  $\beta^+$  thalassemia, in which there is some, although reduced,  $\beta$  globin product. Mild  $\beta$  thalassemia, sometimes referred to as  $\beta^{++}$  alleles allow a moderate amount of  $\beta$  globin to be produced, while in ‘silent’  $\beta$  thalassemia, the deficit in  $\beta$  chain production is minimal and carriers have minimally reduced or normal red cell indices and their Hb A<sub>2</sub> levels are normal.

The severity of these  $\beta$  thalassemia alleles are well borne out in numerous clinical studies and close observations of genotype/phenotype correlations. The ‘silent’ mutations are normally identified in the compound heterozygous states with a severe  $\beta$  thalassemia allele which results in thalassemia intermedia, or in homozygotes who have a typical phenotype of  $\beta$  thalassemia trait. The ‘silent’  $\beta$  thalassemia alleles are not common, except for the –101 C-T, which accounts for a large number of the milder forms of  $\beta$  thalassemia in the Mediterranean.<sup>5</sup>

The mild  $\beta^+$  thalassemia ( $\beta^{++}$ ) alleles are associated with clearly defined changes in heterozygotes and result in disorders of intermediate severity in homozygotes. Interactions with the severe alleles are less predictable due to the wider range of  $\beta$  globin output<sup>10,16</sup> and extend from transfusion dependence to intermediate forms of  $\beta$  thalassemia at the mild end of the spectrum.

The variable severity of the different  $\beta$  thalassemia alleles is reflected in their phenotypic effect in heterozygotes, in the degree of hypochromia and microcytosis as indicated by the mean cell hemoglobin (MCH) and mean cell volume (MCV) values, respectively. Rund et al<sup>17</sup> showed that the  $\beta^0$  thalassemia alleles demonstrated lower MCVs that were also within a tighter range (mean 63.1 fL, SD = 3.4) while the  $\beta^+$  alleles were associated with higher MCVs within a wider range (mean 69.3 fL, SD = 5.6). Similarly, there was a wider range of MCH values for  $\beta^+$  mutations (mean MCH 21.8 pg, SD = 2.03) compared to that for the  $\beta^0$  mutations (mean MCH 19.7 pg, SD = 1.26). The broader range of MCV and MCH in  $\beta^+$  thalassemia when compared to  $\beta^0$  thalassemia, is not surprising given the broad range

in the deficit of  $\beta$  globin production, from barely detectable levels at the severe end, to just a little short of normal in the 'silent'  $\beta$  thalassemia alleles.

A more recent study has taken the correlation between the severity of  $\beta$  thalassemia alleles with hematological parameters to a finer level. Skarmoutsou et al<sup>18</sup> measured a series of hematological parameters, including reticulocyte hemoglobin content (CHr), soluble transferrin receptor (sTfR), reticulocytes and Hb A<sub>2</sub> and F levels in 57 iron-replete individuals with heterozygous  $\beta$  thalassemia. sTfR values, a reliable quantitative assessment of the erythropoietic activity, were lowest in the very mild  $\beta$  thalassemia ( $\beta^{\text{silent}}$ ) and highest in  $\beta^{\circ}$  thalassemia heterozygotes. CHr, a product of reticulocyte hemoglobin and volume, was lower in  $\beta^{\text{silent}}$  thalassemia compared to normals but the difference was not statistically significant. However, the CHr values between the  $\beta^{\text{silent}}$  and the severe groups ( $\beta^+$  and  $\beta^{\circ}$  thalassemia alleles) was significant, being much higher (27.0-32.0 pg) in the former, compared to 19.5 to 25.3 pg in the latter group. Furthermore, while sTfR values showed a positive correlation with Hb A<sub>2</sub>, there was a significant negative correlation between CHr and Hb A<sub>2</sub> levels. This study confirms that all heterozygous  $\beta$  thalassemias have some degree of ineffective erythropoiesis that varies with the severity of the  $\beta$  thalassemia mutation.

### Secondary modifiers

The severity of anemia in  $\beta$  thalassemia reflects the degree of globin chain imbalance and the excess of  $\alpha$  globin chains with all their deleterious effects on the red cell precursors. This globin chain imbalance can be genetically modified by two factors: variation in the amount of  $\alpha$  globin production and variation in fetal hemoglobin response

**$\alpha$  globin genotype.** In many populations in which  $\beta$  thalassemia is prevalent,  $\alpha$  thalassemia also occurs at a high frequency and hence it is not uncommon to co-inherit both conditions. Homozygotes or compound heterozygotes for  $\beta$  thalassemia who co-inherit  $\alpha$  thalassemia will have less redundant  $\alpha$  globin and tend to have a less severe condition. As with  $\beta$  thalassemia, the different  $\alpha$  thalassemias that predominate in different racial groups display a wide range of severity.<sup>19</sup> This interaction alone provides the basis for considerable clinical heterogeneity; the degree of amelioration depends on the severity of the  $\beta$  thalassemia alleles and the number of functional  $\alpha$  globin genes. At one extreme, patients who have co-inherited Hb H (equivalent of only one functioning  $\alpha$  gene) with homozygous  $\beta$  thalassemia, have thalassemia intermedia.<sup>20</sup>

Just as co-inheritance of  $\alpha$  thalassemia can reduce the clinical severity of homozygous  $\beta$  thalassemia, the presence of increased  $\alpha$  globin product in  $\beta$  thalassemia heterozygotes, tips the globin chain imbalance further and crosses the threshold, converting a typically clinically asymptomatic state to that of thalassemia intermedia. This is related to the co-inheritance of triplicated or quadruplicated  $\alpha$  globin genes. The co-inheritance of 2 extra  $\alpha$  globin

genes ( $\alpha\alpha\alpha/\alpha\alpha\alpha$ ) or ( $\alpha\alpha\alpha\alpha/\alpha\alpha$ ) with heterozygous  $\beta$  thalassemia results in the thalassemia intermedia. However, the phenotype of a single extra  $\alpha$  gene ( $\alpha\alpha\alpha/\alpha\alpha$ ) with heterozygous  $\beta$  thalassemia is more variable and depends on the severity of the  $\beta$  thalassemia allele.<sup>21,22</sup>

**Variation in fetal hemoglobin production.** The role of increased Hb F response as an ameliorating factor becomes evident in the group of homozygous  $\beta^{\circ}$  thalassemia patients who are not able to produce any hemoglobin A ( $\alpha_2\beta_2$ ) but yet have a mild disease with a reasonable level of hemoglobin, all of which is Hb F.<sup>8,11</sup> Production of fetal hemoglobin after the neonatal period in  $\beta$  thalassemia is an extremely complex process and still poorly understood. There appears to be a genuine increase in  $\gamma$  chain synthesis, presumably reflecting the expansion of the ineffective erythroid mass. The effect is augmented by the selective survival of the erythroid precursors that synthesize relatively more  $\gamma$  chains. Hence all  $\beta$  thalassemias, heterozygous or homozygous, have variable increases in their levels of Hb F. Against this background, there are undoubtedly genetic factors involved.<sup>23</sup> Studies have shown that 89% of the variation in the level of Hb F and F cells (sub-set of erythrocytes that contain Hb F) is genetically controlled.<sup>24</sup> About one-third of the genetic variance is due to a common genetic variant, C-T at position -158 of the G $\gamma$  globin gene, also referred to as the *Xmn1*-G $\gamma$  polymorphism but more than 50% of the genetic variation in F cell levels is due to factors (QTLs) not linked to the  $\beta$  chromosome.<sup>25</sup>

The *Xmn1*-G $\gamma$  site is common in all population groups and is present at a frequency of 0.32 to 0.35.<sup>25</sup> Unlike the rare mutations in the  $\gamma$  globin promoter that are consistently associated with large discrete effects of increased Hb F levels of 10%-35% in heterozygotes, the so-called pancellular hereditary persistence of fetal hemoglobin (HPFH), the change at G $\gamma$ -158 does not always raise the Hb F levels in otherwise normal individuals.<sup>26</sup> The sequence in the -158 region is not a recognized binding motif for any of the known transcription factors. Nonetheless, although it has little effect in normal individuals, clinical studies have shown that, under conditions of hemopoietic stress, for example in homozygous  $\beta$  thalassemia and sickle cell disease, presence of the *Xmn1*-G $\gamma$  site favor a higher Hb F response. This may explain why the same mutations on different  $\beta$  chromosomal backgrounds (some with and others without the *Xmn1*-G $\gamma$  site) are associated with different clinical severity. However, the Hb F response associated with the *Xmn1*-G $\gamma$  site is usually moderate and may not be sufficient to explain the wide difference in phenotype observed in some cases.

Close observation of Hb F levels in sib-ships and families with  $\beta$  thalassemia have implicated the presence of high Hb F genetic determinant(s) that are not linked to the  $\beta$  globin cluster. This is in keeping with our genetic studies, which showed that > 50% of the variation in F cell levels in the general population is accounted for by *trans*-acting factors.<sup>25</sup> Indeed, analysis of probands with mild

$\beta^0$  thalassemia intermedia, and their family pedigrees, leaves little doubt that there is genetic interaction between the unlinked HPFH determinants (usually referred to as heterocellular HPFH) with  $\beta$  thalassemia.<sup>8,11</sup> Interaction of heterocellular HPFH with Hb S has also been noted to result in Hb F levels of up to 30% and a milder phenotype in sickle cell anemia. Indeed, intensive linkage studies have mapped QTLs controlling F cell levels to three regions of the genome: chromosome 6q23, Xp22 and 8q11.

The putative F cell production (FCP) locus on chromosome Xp22.2 was mapped in a group of normal individuals and individuals with sickle cell disease,<sup>27</sup> while the QTL on chromosome 6q23 was mapped in an extensive Asian Indian kindred with  $\beta$  thalassemia and heterocellular HPFH.<sup>28</sup> Further analysis reduced the candidate region to 1.5 Mb<sup>29</sup> and showed that it contains five protein coding genes including a very large uncharacterized gene with 28 exons that spanned 215 kb (*AHII*), and several genes that do not appear to be protein coding.<sup>30</sup> In the same Asian Indian family with  $\beta$  thalassemia and heterocellular HPFH, after adjusting the F cell levels for the effects of *Xmn1*-G $\gamma$  site and 6q QTL, a repeat linkage led to the localization of another QTL on 8q11.<sup>31</sup>

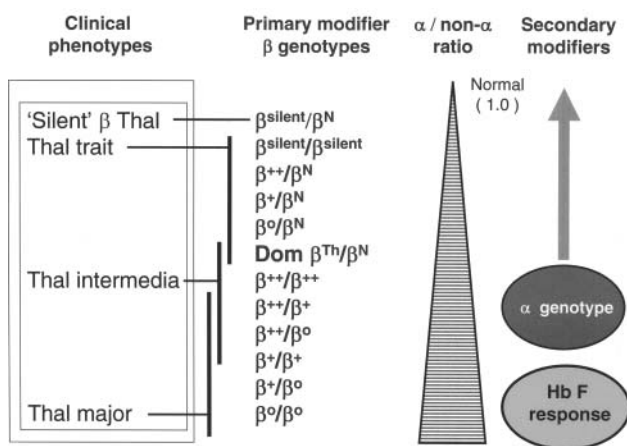
However, unlike the 6q QTL, the effects of the 8q locus are conditional on the *Xmn1*-G $\gamma$  site, suggesting a genetic interaction between the site and the 8q QTL. The 8q QTL defines another class of genetic determinants of F cell levels, one that is conditional on *cis*-acting sequences of the  $\beta$  globin complex, which could explain some of the inconsistent associations of high Hb F levels with the *Xmn1*-G $\gamma$  site that has been observed even within families.<sup>32</sup> Recently, not only have we been able to replicate the linkage

to chromosome 8q in a different ethnic population,<sup>33</sup> we could also show that the linkage was conditional on the *Xmn1* site in the European population.<sup>34</sup> In another recent study, 180 SNPs (single nucleotide polymorphisms) in 38 candidate genes that might modulate Hb F levels, were studied in 280 patients with sickle cell anemia. Strong association of Hb F with SNPs in a region adjacent to the QTL on chromosome 6q22.2–23.2 were revealed.<sup>35</sup> Despite these QTLs, studies indicate that chromosomal locations of other QTLs have yet to be determined<sup>36</sup> and, thus, heterocellular HPFH appears to be genetically heterogeneous.

As the genetic basis for the propensity to produce Hb F becomes unravelled it is becoming clear that the conglomeration of the *Xmn1*-G $\gamma$  polymorphism, the QTLs on 6q, Xp and 8q and others, linked and unlinked to the  $\beta$  globin complex, contribute to the quantitative trait of Hb F that constitute the loosely defined syndrome of heterocellular HPFH. These QTLs presumably play an important role in the fine tuning of  $\gamma$  globin production in normal adults, in response to 'erythropoietic stress' and possibly in one's capacity to respond to pharmacologic inducers of Hb F synthesis. Until the different entities become better defined, detection of an inherent capacity for increased Hb F production is, at present, difficult and usually inferred from family studies.

**Other potential modifiers.** Apart from the number of  $\alpha$  globin genes and an inherent capacity to produce Hb F, the proteolytic capacity of the erythroid precursors in catabolizing the excess  $\alpha$  globin chains has often been suggested as another factor, but this effect is difficult to define. Recently, the newly discovered  $\alpha$  hemoglobin stabilizing protein (AHSP),<sup>37</sup> a chaperone of  $\alpha$  globin, has been suggested as another genetic modifier,<sup>38</sup> but so far clinical studies have been inconclusive. A study of the AHSP gene did not find any mutation or association between AHSP haplotypes and disease severity in Hb E/ $\beta$  thalassemia patients in a Thai population.<sup>39</sup> Other studies indicate that AHSP mRNA levels are abnormally low in some patients with unusually severe  $\beta$  thalassemia.<sup>40</sup>

Interaction of the genetic modifying factors on the phenotype of  $\beta$  thalassemia is summarized in **Figure 1**.



**Figure 1. Primary and secondary modifiers of the  $\beta$  thalassemia phenotype.** They include variable output from the  $\beta$  globin ( $\beta$  genotype); variable output from the  $\alpha$  globin genes ( $\alpha$  genotype) and variable Hb F response (co-inheritance of different QTLs controlling Hb F and F cell levels).

The consequences of these factors is the degree of chain imbalance ( $\alpha$ /non- $\alpha$  globin ratio) and severity of ineffective erythropoiesis.

### Clues to Molecular Therapy

What clues can we derive from the extensive studies of the genotype/phenotype relationship of the different forms of thalassemia?

- i.  $\beta$  thalassemia, a disorder of deficient synthesis of  $\beta$  globin chains, conceptually, should be correctable by gene replacement therapy. But correction of the defect in  $\beta$  globin gene synthesis will require an amount of globin equivalent to approximately half, but preferably more, of the output of a normal globin gene. The desired outcome will be the conversion of a severe transfusion-dependent thalassemia to a thalassemia intermedia or, even better, to thalassemia trait.
- ii. An alternative to the development of effective gene

therapy to increase  $\beta$  globin is to devise strategies to increase Hb F (to reactivate the 'sleeping'  $\gamma$  globin genes). Effective strategies for enhancing  $\gamma$  globin production would have therapeutic implications not just for  $\beta$  thalassemia but also sickle cell disease (SCD), and they include pharmacological approaches and  $\gamma$  globin gene transduction.

- iii. Nature has shown that an effective means of reducing the severity of  $\beta$  thalassemia is to reduce the amount of free  $\alpha$  globin through co-inheritance of  $\alpha$  thalassemia. Perhaps downregulating the endogenous  $\alpha$  globin genes may be more efficacious than increasing  $\beta$  globin expression.
- iv. With the discovery of the *AHSP* gene, a potential molecular therapy may be the upregulation of *AHSP* protein or synthesis of an agent to mimic *AHSP* stabilization of those redundant  $\alpha$  globin chains, thus limiting the formation of  $\alpha$  inclusion bodies and ineffective erythropoiesis.

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