Homozygous β-Thalassemia Without Anemia


A 37-year-old man of Guyanese origin was found to have homozygous β-thalassemia without anemia. There were no physical stigmata of thalassemia. The hematocrit value was 41 to 45.8, the mean corpuscular volume was 61 fL, and the mean corpuscular hemoglobin was 18.9 pg. The HbF was 45% with a β2/γ2 ratio of 3:1. An acid elution preparation of the peripheral blood showed heterogeneous distribution of HbF, but all erythrocytes stained for fetal hemoglobin. The β/α synthesis ratio in the peripheral blood was 0.25; the (β + γ)/α ratio was 0.55. Haplotype analysis revealed homozygosity for the −−−++pattern (Senegal, type IX) at seven polymorphic restriction sites within the β-like gene complex. Digestion of DNA with XmnI indicated that the −158 C-to-T transition was present in both β-globin gene clusters. Oligomer hybridization analysis demonstrated homozygosity for the −29 A-to-G mutation in the β-globin promoter region. Although this form of thalassemia can cause transfusion-requiring anemia, the high-HbF, high−γ2 phenotype associated with the linked −−−+++subhaplotype and −158 C-to-T substitution appears to have ameliorated the disease in this subject.

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RESULTS

The propositus is a 37-year-old black man of Guyanese origin. Physical examination of the subject revealed robust normal stature and no hepatosplenomegaly or other physical stigmata of thalassemia. Bone density was noted to be normal on a chest x-ray study.

Although the subject had normal hemoglobin and hematocrit values, his RBC count was elevated to 6.79 x 10^12/L, and both the mean corpuscular volume (61 fL) and the mean corpuscular hemoglobin (18.9 pg) were markedly reduced (Table 1). His daughter was not anemic but also had hypochromic and microcytic RBCs (Table 1). The subject's peripheral blood film demonstrated moderate anisocytosis, poikilocytosis, target cells, and hypochromia (Fig 1) and was consistent with a thalassemia syndrome. There were no nucleated erythroid cells.

The HbA2 level was elevated to 5.6%, while the HbF level was markedly increased to 40.3% by alkali denaturation and 45% by HPLC (Table 1). The 6γ/α ratio was elevated to 3:1 rather than the expected 2:3 ratio. The acid elution test showed that the HbF in the proband's erythrocytes was heterogeneously distributed. However, every cell stained prominently for HbF (Fig 1). The daughter had an HbA2 level of 5.7% and a slightly elevated HbF concentration of 2.6% (Table 1). The subject's blood showed a deficit in β-globin synthesis with excess α chain production (Table 1); the β/α ratio was 0.25. Chain synthesis was slightly greater than that of β; the (γ + β)/α ratio was 0.55. A similar study of the daughter's blood confirmed the diagnosis of β-thalassemia trait. The β/α ratio was 0.64 (Table 1).

Because specific β-thalassemia mutations in several racial groups have been linked with specific patterns of restriction-site polymorphisms, we studied the DNA of the propositus using endonuclease digestion and hybridization analysis.
Table 1. Selected Hematologic Values and Clinical Chemistry Studies

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Propositus</th>
<th>Daughter</th>
<th>Wife</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>12.6-13.2</td>
<td>13.0</td>
<td>12.6</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>41-45.8</td>
<td>44.4</td>
<td>37.7</td>
</tr>
<tr>
<td>RBC (x 10^6/μL)</td>
<td>6.8</td>
<td>6.5</td>
<td>4.4</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>61</td>
<td>68</td>
<td>86</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>18.9</td>
<td>20.5</td>
<td>28.7</td>
</tr>
<tr>
<td>HbA2 (%)</td>
<td>5.6</td>
<td>5.7</td>
<td>3.3</td>
</tr>
<tr>
<td>Hbf (%)</td>
<td>40.3 ± 45±</td>
<td>2.6</td>
<td>0.6</td>
</tr>
<tr>
<td>G/A</td>
<td>3:1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>β/α synthesis</td>
<td>0.25</td>
<td>0.64</td>
<td>0.93</td>
</tr>
<tr>
<td>(γ + β)/α synthesis</td>
<td>0.55</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>185-315</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>1.4-3.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Serum iron (μg/dL)</td>
<td>90</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>TIBC (μg/dL)</td>
<td>195</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviations: PCV, packed cell volume; LDH, lactic dehydrogenase; TIBC, total iron-binding capacity.
* Alkaline denaturation.  
† HPLC.

Hemoglobin concentrations and clinical chemistry studies are normal in the propositus and his mother. The daughter’s pattern was consistent with heterozygosity for the TATA box region of the β-globin promotor. DNA from the propositus was digested with BamHI, subjected to agarose gel electrophoresis, and hybridized to radioactive 19-mer oligonucleotide probes that selectively recognize either A or G at position -29. DNA from a normal subject showed a 1.9-kb band that hybridized only to the normal probe recognizing A at position -29. The subject’s DNA hybridized only to the mutant probe recognizing G at -29 and not to the normal probe, thereby indicating homozygosity for the TATA box mutation.

Elevation of HbF with increased 0γ production in subjects with thalassemia and sickle cell anemia has been reported in association with a C-to-T transition at position -158 in the promoter region of the 0γ gene, thus creating a new XmnI digestion site. An XmnI digest of normal DNA exhibited only an 8-kb γ-specific fragment (Fig 2). The DNA of the subject showed only a 6.7-kb fragment, which indicated the presence of the additional XmnI-susceptible site and established homozygosity for the C-to-T change at -158 (Fig 2).

A form of 0γ β+ hereditary persistence of HbF has been seen in black subjects heterozygous for the point mutation C-to-G at position -202 of the 0γ gene promoter region, which eliminates an Apal restriction site. Normal DNA exhibits 15.5-kb (0γ), 4.3-kb (0γ and 0γ) fragments when digested with both the restriction enzymes Apal and BamHI. The presence of the C-to-G change at position -202, S' to the 0γ gene, results in the appearance of a new 2.6-kb (0γ) fragment. DNA of the propositus as well as a normal control lacked the 2.6-kb fragment which ruled out the presence of the -202 mutation (Fig 2).

Another possible cause of an increased proportion of 0γ chains in the HbF of an adult is the presence of a second 0γ gene in place of the normal Aγ gene. Since the Aγ gene but not the 0γ gene has a PstI susceptible site in exon III, the presence of a second 0γ gene results in the loss of a 0.8-kb γ-specific fragment and the appearance of 3.5-kb fragment. Such a 3.5-kb fragment was absent from the PstI digest of the DNA of the propositus, which ruled out the presence of two 0γ genes on chromosome 11 (Fig 2).

The S' flanking region of the 0γ gene was examined for
structural changes that might contribute to increased gene expression. A 6.7-kb HindIII fragment extending from IVS2 to a point 5.5 kb 5' to the δγ gene was cloned from the DNA of the propositus (Fig 3). A variety of restriction endonucleases gave normal results except for the absence of an NcoI site normally found approximately 350 base pairs (bp) 3' to the 5' limit of the fragment (Fig 3).25 Five hundred nucleotides extending 5' from codon 24 of the δγ gene into the promoter region were sequenced and confirmed the C-to-T change reported in the previously mentioned NcoI site. The double-headed arrows indicate the regions that were sequenced, H, Hind III; B, BamHI; X, Xbal; A, Apal; Av, Avai; Hpa, Hpal; P, PstI.

**DISCUSSION**

The subject reported here has a remarkably mild variety of homozygous β-thalassemia without anemia. Although the RBCs are very small, the red cell count is elevated so that the total hemoglobin concentration in the blood remains within normal limits. This lack of anemia is almost certainly a reflection of a marked decrease in the exaggerated intramedullary and peripheral red blood destruction usually characteristic of homozygous β-thalassemia. Support for this view is provided by the absence of circulating normoblasts, lack of splenomegaly, the normal plasma lactic dehydrogenase level, and the relatively normal serum bilirubin concentration.

In our present understanding of erythrocyte pathophysiology, three endogenous factors appear clearly capable of producing milder disease: (a) β gene mutations, which only slightly decrease β-globin synthesis relative to α-g increased γ chain production in a large proportion of the RBC population; and (c) concomitant α-thalassemia. Such conditions have the net effect of limiting the amount of deleterious, free, excess α-globin present during the stages of maturation of the erythroid precursors.24

The variability in the deficit in β-globin production in the different β-thalassemia syndromes now can be explained by varying degrees of interference with functional β-mRNA production. Mutations in the promoter region of the β-globin gene, such as the -29 A-to-G change in the TATA box noted in the present case, appear to only partially inhibit β-mRNA synthesis.25-28 The extent of the deficit in β-globin production that is caused by the thalassemia gene under study can be very roughly estimated from the globin chain synthesis experiments performed on the peripheral blood of the subjects. The daughter with heterozygous β-thalassemia
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has a β/α synthesis ratio of 0.64. It thus appears that, although the –29 A-to-G mutant gene is capable of directing the synthesis of significant amounts of β-globin, homozygotes still have a major deficit in β production relative to α. Another black homozygote reported with this same mutation, an adolescent girl, had mild anemia but was not transfusion dependent.\(^{15}\) In blacks this mutation is likely to be the most common form of β-thalassemia; 12 of 27 randomly obtained β-thalassemia genes in black subjects contained this mutation (Antonarakis et al\(^{15}\) and Kazazian HH, unpublished observation). The high frequency of mild β-thalassemia in blacks may be a reflection of the prevalence of this TATA box mutation. However the lesser degree of deficit in β chain production in this type of β-thalassemia as compared with other syndromes is not likely to be the sole cause of the mildness of the disease. This is emphasized by the finding that a Chinese man homozygous for the identical –29 A-to-G mutation was severely anemic and required transfusions.\(^{31}\)

Is efficient compensatory synthesis of HbF responsible for the absence of anemia in our proband with homozygous β-thalassemia? Approximately 45% of the hemoglobin in the peripheral blood was HbF. The acid elution study indicated that, although the HbF was not equally distributed among the RBCs, there was easily demonstrable HbF in all cells. Increased levels of HbF along with a high proportion of \(^{5}\)γ polypeptide chains has been observed in American blacks,\(^{32-34}\) Senegalese,\(^{20}\) Jamaicans,\(^{33}\) Arabs,\(^{36}\) and Asian Indians,\(^{37,38}\) subjects with sickle cell disease. In all these subjects, the high-HbF, high-\(^{5}\)γ phenotype is always associated with the \(+ + +\) subhaplotype at the HindIII sites in the \(^{6}\)γ and \(^{4}\)γ genes and the HincII in the \(\psi\)β gene and \(3\) to it. The C-to-T change at –158 in the \(^{5}\)γ globin promoter is closely linked to this pattern of polymorphic restriction sites.\(^{21,32}\) The milder form of sickle cell anemia noted in the Shiite Arabs of the eastern province of Saudi Arabia has been attributed to the increased HbF.\(^{40}\) The clinical manifestations of sickle disease may also be milder in the Senegalese as compared with other African populations in which HbF levels are lower.\(^{41}\) Relatively mild homozygous β-thalassemia has also been noted to be associated with the high-\(^{5}\)γ phenotype, the –158 C-to-T change, and the \(+ + +\) subhaplotype. Examples of this are the Dutch \(β^{+}\) mutation,\(^{21,32,42}\) thalassemia intermedia in Asians and Italians,\(^{46}\) and a group of ten Turkish subjects with β-thalassemia intermedia recently reported.\(^{44}\) The latter subjects, who were reported to have normal growth and no transfusion requirements, nevertheless were significantly anemic and had splenomegaly, and five required splenectomy. Thus those patients were much more seriously affected than was the propositus of the present report.

The absence of anemia in our subject appears therefore to be a result of high-HbF production most likely due to some property of the chromosome bearing the linked \(+ + +\) subhaplotype and the –158 C-to-T change in association with a β gene promoter mutation causing reduced but not completely suppressed β-globin production. In the absence of the high-HbF, high-\(^{5}\)γ phenotype, homozygosity for the TATA box mutation can cause transfusion-dependent anemia as illustrated by the Chinese subject.\(^{31}\) On the other hand, in the presence of a thalassemia mutation more disruptive of β-globin synthesis, the unknown genetic factor linked to the \(+ + +\) subhaplotype and the –158 C-to-T change that causes increased HbF production cannot completely suppress clinical disease.

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