CONCISE REPORT

The Effect of α-Thalassemia on the Expression of the β-Thalassemia/HPFH Heterozygote in a Black Family

By E. Beutler, E. Turner, and W. Kuhl

A 2-yr-old black girl presented with a thalassemic clinical picture and was found to have nearly 100% fetal hemoglobin in her red cells. Pedigree analysis indicated that she was a heterozygote for the hereditary persistence of fetal hemoglobin gene and for a β-thalassemia gene. A brother, who also had nearly 100% fetal hemoglobin in his red cells, manifested, in contrast to his sister, no anemia and only minimal splenomegaly. Examination of the family’s α-globin loci using the restriction endonuclease Eco RI demonstrated that the brother had a single α-locus deletion that he had inherited from his mother. The mild clinical manifestations of this boy are consistent with the often expressed view that excess α chains may contribute significantly to the hematologic manifestation of β-thalassemia.

MATERIALS AND METHODS

Blood counts were performed with a Coulter model S+ counter. Hemoglobin A2 and F determinations were performed by column chromatography, using microcolumns from Isolab, Inc., Akron, Ohio. Hemoglobin F determinations were also performed by alkali denaturation. Globin chain synthesis was estimated by measuring 35S-methionine incorporation into globin chains that were separated on Cellogel. Restriction mapping of the globin chains was performed on peripheral blood leukocytes. The DNA, purified by phenol extraction and by digestion with protease K and RNase, was incubated with the restriction endonuclease Eco RI, separated by agarose electrophoresis, and transferred to nitrocellulose paper using the Southern blot technique. The α-globin and β-globin gene probes were prepared from E. coli containing recombed PMB9 plasmid DNA, kindly provided by Dr. Bernard Forget.

Clinical Findings

T.H. is a black girl born in 1975 and initially referred to us in 1977 because of anemia and marked hepatosplenomegaly detected in the course of examination for an upper respiratory infection. This had been found to be associated with virtually 100% fetal hemoglobin in the red cells. Her hemoglobin at that time was 9.1 g/dl, RBC 4.42 x 1012/liter, and hematocrit 27.6%. Her mother’s red cells were found to contain 22% hemoglobin F and the father’s, whose hemoglobin was 12.0 g/dl, red cell 6.7 x 1012/liter and hematocrit 38.6%, was found to have 1.17% HbF and 5.6% HbA2. The child was seen again in 1980 because of increasing anemia. At this time, the liver was palpable 6 cm below the right costal margin and the spleen 3.5 cm below the left costal margin. Right ventricular hypertrophy was detected on echocardiography. Skull x-rays were normal. Blood from a clinically normal brother, 2 yr her senior, contained 78% HbF. In spite of the fact that his red cells contained as much fetal hemoglobin as those of his sister, his hemoglobin level

Table 1

<table>
<thead>
<tr>
<th>Age</th>
<th>Hb (g/dl)</th>
<th>RBC (x 10^12/liter)</th>
<th>Retic (%)</th>
<th>MCV (μl)</th>
<th>Plasma Iron (mg/dl)</th>
<th>Fcn (ng/ml)</th>
<th>HbF (%)</th>
<th>HbF + A2 (%)</th>
<th>HbA2 (%)</th>
<th>α/α+α2 x 1000</th>
<th>RI Fragments (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propositor</td>
<td>5</td>
<td>8.9</td>
<td>4.15</td>
<td>8.0</td>
<td>64.8</td>
<td>136/272</td>
<td>61</td>
<td>69</td>
<td>95.2</td>
<td>2.85</td>
<td>1.10</td>
</tr>
<tr>
<td>Mother</td>
<td>33</td>
<td>12.4</td>
<td>4.62</td>
<td>1.2</td>
<td>79.0</td>
<td>80/322</td>
<td>35</td>
<td>22</td>
<td>7.3</td>
<td>2.59</td>
<td>0.98</td>
</tr>
<tr>
<td>Father</td>
<td>36</td>
<td>12.5</td>
<td>6.58</td>
<td>1.8</td>
<td>58.3</td>
<td>78/244</td>
<td>194</td>
<td>1.2</td>
<td>7.9</td>
<td>5.49</td>
<td>1.43</td>
</tr>
<tr>
<td>Brother</td>
<td>7</td>
<td>11.5</td>
<td>5.77</td>
<td>4.6</td>
<td>61.0</td>
<td>69/266</td>
<td>97</td>
<td>75</td>
<td>93.7</td>
<td>3.33</td>
<td>0.87</td>
</tr>
<tr>
<td>Normal range</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14.0–18.0</td>
<td>4.70–5.10</td>
<td>0.5–1.5</td>
<td>80–94</td>
<td>42–135/280–400</td>
<td>43–313</td>
<td>-2</td>
<td>5.5–8.5</td>
<td>1.5–3.0</td>
<td>0.94–1.02</td>
<td>23</td>
</tr>
<tr>
<td>Female</td>
<td>12.0–16.0</td>
<td>4.20–5.40</td>
<td>0.5–1.5</td>
<td>81–99</td>
<td>42–135/280–400</td>
<td>8–151</td>
<td>-2</td>
<td>5.5–8.5</td>
<td>1.5–3.0</td>
<td>0.94–1.02</td>
<td>23</td>
</tr>
</tbody>
</table>

*Alkaline denaturation. © 1981 by Grune & Stratton, Inc.
was essentially normal at 11.5 g/dl, his spleen tip was barely palpable, and his reticulocyte count was only modestly elevated to 4.6%. The boy was active in sports and had been considered to be in excellent health. Some of the pertinent 1980 laboratory findings are summarized in Table I.

RESULTS

The findings in the H. family are summarized in Table 1 and in Fig. 1. The results of restriction mapping of DNA from this family are presented in Fig. 2. It is known that the Eco RI endonuclease restriction sites lie outside the two normal α-globin loci, so that a 23 kb DNA fragment is generated. ² It is apparent from Fig. 2 that this normal pattern, representing the existence of four α-globin loci, was generated when the DNA from the patient’s father and from the patient herself was subjected to restriction mapping. When an α-globin locus is missing, a shorter fragment, consisting of 19 kb, is encountered. This pattern, representing the loss of one α-globin gene, was generated from the DNA of the father’s and from her mother. The Eco RI restriction map of the β-globin locus of all four family members contained the 6.2, 5.7, 3.9, and 2.4 kb fragments, which are normally found.

DISCUSSION

The apparent homozygous expression of HPFH in T.H. indicates that she has inherited a β⁰-thalassemic gene from her father and a HPFH gene from her mother. Since the latter gene is presumably less efficient in directing hemoglobin production than is the normal β-globin locus, she manifested a moderate degree of β-thalassemia. Since her brother must have inherited the same abnormal β-globin loci, his much milder clinical expression was surprising.

The results of restriction mapping of this family's DNA provides a satisfying explanation for this apparent anomaly. The patient has four α-globin loci. Thus, with the limited production of non-α chains imposed by her inheritance of a β⁰-thalassemic gene from the father and a HPFH gene from the mother, free α-chains accumulate. These presumably damage the red cell and result in increased hemolysis. Although the patient’s brother’s hematopoietic cells are similarly impaired in their capacity to produce non-α chains, the effect is ameliorated by the fact that he has only three α-loci. Since much of the clinical effect seems to be due to unbalanced globin chain synthesis, ⁸ a very mild clinical state resulted.

Until now modern DNA mapping technology has been largely a research tool. As these methods become more generally available, they will undoubtedly prove to be useful in solving clinical problems as well.

REFERENCES


The effect of alpha-thalassemia on the expression of the beta-thalassemia/HPFH heterozygote in a black family

E Beutler, E Turner and W Kuhl