Genetic Implications of the Interaction of Two Types of Beta-Thalassemia Genes in a Patient With Thalassemia Major

By Giuseppe Russo, Florindo Mollica, Lorenzo Pavone, Salvatore Musumeci, and Corrado Baglioni

A patient with thalassemia major is described in which two types of thalassemia genes are present, one that reduces $\alpha$-chain synthesis and the other that suppresses it completely. The identification of these two types of $\beta$-thalassemia genes is based on the presence or absence of HbA in other members of the same family who are also HbC carriers. The genetic implications of the interaction of these two thalassemia genes are discussed in view of recent theories on the nature of the molecular defect characteristic of each type of thalassemia.

The term thalassemia defines a heterogeneous group of hematological disorders, each determined by the partial or complete suppression of the synthesis of one of the hemoglobin chains. In particular, two types of $\beta$ thalassemia have been differentiated: one with partial suppression of $\beta$-chain synthesis, and the other with complete suppression. These two types of thalassemia will be here referred to as $\beta$ thalassemia and $\beta^0$ thalassemia, respectively.

The molecular basis of thalassemia has not yet been completely clarified. Several authors have published, however, rather suggestive evidence that the amount of functional messenger RNA (mRNA) coded for by the $\beta$-thalassemia allele is severely reduced; this reduced amount of mRNA seems to be translated at a normal rate and appears thus to function normally. A different interpretation of the molecular defect of $\beta^0$ thalassemia has recently been presented. Conconi et al. have proposed that this mutation affects a gene for a factor specifically involved in the translation of the mRNA for $\beta^A$ globin.

The present paper deals with a family in which both types of $\beta$-thalassemia genes are present and interact in one patient. The identification of the two types of $\beta$-thalassemia genes is based on their interaction with the $\beta^C$ allele, due to the fortuitous presence of HbC in this family. It has thus been possible to distinguish clearly the $\beta$-thalassemia /$\beta^C$ double heterozygotes who have HbA in the range of about $10^a$, from $\beta^0$-thalassemia /$\beta^C$ double heterozygotes in whom...
Fig. 1. Hemoglobin electrophoresis on cellulose acetate (glycine buffer pH 9). N, normal control.
HbA is not detectable at all. The study of the family with the two thalassemia genes suggests that these two types of thalassemia are caused by a similar molecular defect, since there was no influence of the β thalassemia or the β* genes on the production of β chain by the β0-thalassemia gene.

MATERIALS AND METHODS

Routine examinations were performed by standard hematological techniques. Hemoglobin F was determined according to Singer et al. Hemoglobins were analyzed by electrophoresis on cellulose acetate in glycine buffer at pH 9.0. Quantitative analysis was performed by elution of the various hemoglobins from cellulose acetate strips. Agar gel electrophoresis followed the method of Robinson et al. Hemoglobin C was identified by two-dimensional tryptic peptide analysis according to Baglioni. Globin was analyzed by column chromatography according to Clegg et al.

RESULTS

The propositus (III, 4, Fig. 1) was admitted from a small village in Eastern Sicily at 80 days of age because of pallor noted about 2 wk previously. His weight was 4.85 kg and height 51.5 cm. Physical examination revealed pallor and a moderate spleen and liver enlargement (spleen palpable 2 cm, liver 4 cm below the costal margins). A routine hematological examination of the propositus and of his family was carried out (Table I) and the hemoglobin analyzed on cellulose acetate (Fig. 2). The following conclusions could be drawn (Fig. 1): Subject I, 1 (the maternal grandfather) is a HbC carrier; I, 2 (the maternal grandmother) is heterozygote for β-thalassemia (see below) with increased A2; II, 2 (the mother of the propositus) has inherited two abnormal genes, since her electrophoretic pattern shows HbC and HbF only (91°α and 9°α, respectively) without evidence of HbA. The thalassemic gene inherited from her mother is thus of the βα-thalassemia type. This 25-yr-old woman had been pale from puberty and had needed an occasional blood transfusion during her previous pregnancy because of severe anemia. Physical examination showed pallor, jaundiced sclerae, and enlargement of the spleen, which was palpable about 6 cm below the costal margin: II, 1 (the father of the propositus) is heterozygote for β-thalassemia with increased A2.

Fig. 2. Pedigree of C family. The arrow indicates the propositus.
Table 1. Hematological Data

<table>
<thead>
<tr>
<th>Subject*</th>
<th>Age (yrl)</th>
<th>Hemoglobin (g/100 ml)</th>
<th>RBC (× 10⁵/cu mm)</th>
<th>MCHC (g/100 ml)</th>
<th>MCH (fL)</th>
<th>MCV (µl)</th>
<th>Erythrocytic Morphologic Changes</th>
<th>Target Cells</th>
<th>Osmotic Fragility</th>
<th>Hb F (%)</th>
<th>Hb Electrophoresis</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. 1</td>
<td>57</td>
<td>15.0</td>
<td>5.5</td>
<td>32</td>
<td>27</td>
<td>85</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
<td>0.5</td>
<td>AC</td>
</tr>
<tr>
<td>I. 2</td>
<td>54</td>
<td>9.1</td>
<td>4.4</td>
<td>25</td>
<td>19</td>
<td>76</td>
<td>+</td>
<td>-</td>
<td>Increased</td>
<td>1.1</td>
<td>A(A₂ 4.8%)</td>
</tr>
<tr>
<td>II. 1</td>
<td>32</td>
<td>11.6</td>
<td>5.0</td>
<td>29</td>
<td>21</td>
<td>80</td>
<td>++</td>
<td>++</td>
<td>Increased</td>
<td>1.7</td>
<td>A(A₂ 3.7%)</td>
</tr>
<tr>
<td>II. 2</td>
<td>25</td>
<td>8.0</td>
<td>4.2</td>
<td>25</td>
<td>19</td>
<td>76</td>
<td>+</td>
<td>+ +</td>
<td>Increased</td>
<td>6.2</td>
<td>CF</td>
</tr>
<tr>
<td>III. 1</td>
<td>6</td>
<td>9.7</td>
<td>4.6</td>
<td>26</td>
<td>21</td>
<td>80</td>
<td>+ +</td>
<td>+ +</td>
<td>Increased</td>
<td>12.1</td>
<td>CFA</td>
</tr>
<tr>
<td>III. 2</td>
<td>4</td>
<td>10.4</td>
<td>4.9</td>
<td>29</td>
<td>21</td>
<td>73</td>
<td>++</td>
<td>+ +</td>
<td>Increased</td>
<td>8.7</td>
<td>CFA</td>
</tr>
<tr>
<td>III. 3</td>
<td>2½/2</td>
<td>11.2</td>
<td>4.0</td>
<td>30</td>
<td>26</td>
<td>93</td>
<td>-</td>
<td>-</td>
<td>Increased</td>
<td>1.9</td>
<td>AC</td>
</tr>
<tr>
<td>III. 4</td>
<td>3½/12</td>
<td>7.2</td>
<td>3.6</td>
<td>27</td>
<td>20</td>
<td>72</td>
<td>++</td>
<td>++</td>
<td>Increased</td>
<td>87.1</td>
<td>F(+ A7% &amp; A₂)</td>
</tr>
<tr>
<td></td>
<td>5/12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>76.6</td>
<td>F(+ A7% &amp; A₂)</td>
</tr>
</tbody>
</table>

*See Fig. 2.

See Fig. 1: A₃ values determined by elution from cellulose acetate strips: normal values 11 2.3 ± 0.6%.

The family has emigrated into West Germany and it has only been possible to examine a sample of III. 4 hemoglobin obtained at the age indicated. The hemoglobin pattern is substantially unchanged with a relatively small amount of HbA and increased HbA₂.
III, 1 and III, 2 are $\beta^C/\beta$-thalassemia double heterozygotes, having inherited the $\beta^C$ gene from their mother and the $\beta$-thalassemia gene from their father. In contrast with the electrophoretic pattern of their mother, both children show HbA. The hemoglobin pattern was 76\(^\circ\_) C, 15\(^\circ\_) F, and 9\(^\circ\_) A in III, 1, and 79\(^\circ\_) C, 11\(^\circ\_) F, and 10\(^\circ\_) A in III, 2. The presence of HbA in these children indicates clearly that the father is a heterozygote for $\beta$-thalassemia. III, 3 is an HbC carrier and has not inherited either of the $\beta$-thalassemia genes from his parents. The propositus III, 4 has instead inherited both these genes since he shows the hematological picture of a subject with thalassemia major (Table 1). This patient synthesizes a significant though small amount of HbA.

Since it was relevant to demonstrate unequivocally the presence of HbA in this patient, we have analyzed its globin by column chromatography according to Clegg et al. The pattern observed showed clearly the presence of $\beta^A$ globin, though in relatively modest amounts (approximately 10\(^\circ\_) of the non-$\alpha$ chains). The electrophoresis on cellulose acetate also showed the presence of HbA; the amount of HbA present could not be determined in this case, due to the large fraction of HbF migrating close to it.

**DISCUSSION**

There are numerous reports in the literature of families in which $\beta$-thalassemia genes have been observed in combination with abnormal $\beta$ alleles. The combination of a thalassemia gene with an abnormal allele for the structural gene of the corresponding peptide chain has led to the definition of “interacting” thalassemia; in this condition the abnormal hemoglobin is present in larger amount than HbA. In some cases the amount of HbA present can be extremely reduced or HbA can be altogether absent. It has often been assumed then that the thalassemic gene is of the $\beta^0$ type, even though only careful family studies would allow the exclusion of other explanations, like a $\beta^B$-thalassemia gene.

We have limited our survey of the literature to some families in which HbC has been observed in combination with $\beta$ thalassemia. Portier et al. have described a family in which a woman with HbC/thalassemia disease (84\(^\circ\_) HbC and 16\(^\circ\_) HbA) married a subject with thalassemia minor and 7\(^\circ\_) HbF; in their offspring three children showed an electrophoretic pattern of HbC (80\(^\circ\_) HbC and HbF (10\(^\circ\_) HbF). The absence of HbA in these three children could be explained by the inheritance of the $\beta^C$ gene from the mother and of a gene suppressing $\beta^A$-chain synthesis from the father, but the nature of this gene could not be exactly defined.

In another family reported recently by Coquelet et al., the propositus was affected by HbC/thalassemia with 12\(^\circ\_) HbA: two of the maternal uncles showed a pattern of HbC + HbF, without HbA. HbC homozygosity, however, could not be ruled out for these two subjects.

In the family reported by us the presence of two varieties of $\beta$ thalassemia is unquestionable. The mother of the propositus has a genotype $\beta^C/\beta^0$ $\text{thal}$; the father is a $\beta$-thalassemia carrier, as shown by the genotype $\beta^C/\beta^\text{thal}$ of the daughters III, 1 and III, 2. The propositus III, 4 has thus the genotype $\beta^\text{thal}/\beta^0$ $\text{thal}$. 


The presence in the propositus of small amounts of $\beta^A$ chains indicates that the molecular defect of the $\beta$-thalassemia and of the $\beta^0$-thalassemia genes present in this family are of a similar nature. The molecular defect of $\beta$ thalassemia has been analyzed in detail by several authors. They have shown that $\beta^A$ chains of thalassemic subjects are synthesized at a normal rate and on polyribosomes of normal size. These studies have thus ruled out any molecular defect at the level of initiation or elongation of peptide chains. At the same time, the amount of mRNA coding for $\beta^A$ chains has been directly estimated by translation in heterologous cell-free systems, and it has been found that there is a marked reduction of mRNA for $\beta^A$ chain in homozygous patients.

A recent publication by Conconi et al. proposes a different explanation for $\beta^0$ thalassemia. The molecular defect, according to these authors, is in a factor specifically involved in the translation of $\beta^A$ chain mRNA. They have reported that addition of a supernatant fraction from reticulocytes of normal individuals to ribosomes of patients from Ferrara homozygous for $\beta^0$ thalassemia results in the cell-free synthesis of $\beta^A$ chains. This finding could possibly be explained by the presence of mRNA in the supernatant fraction; this has recently been clearly demonstrated. This explanation seems excluded by an experiment of Conconi et al. in which supernatant from reticulocytes of sickle cell homozygotes was used. This supernatant appears to stimulate synthesis of $\beta^A$ chains, and mRNA for these chains cannot be present in reticulocytes of $\beta^0$ homozygotes.

The molecular defect postulated by Conconi et al. does not account for the pattern of hemoglobin synthesis observed in our propositus. If in $\beta$ thalassemia there is a reduction in the amount of functional $\beta^A$ mRNA but a normal amount of the hypothetical factor, and if in $\beta^0$ thalassemia there is some $\beta^A$ mRNA present but the hypothetical factor is absent, we should have observed active synthesis of $\beta^A$ chains in our propositus. It cannot be excluded, however, that the $\beta^0$ thalassemia studied by Conconi et al. differs from the $\beta^0$ thalassemia present in our family in the nature of its molecular defect. The patients studied by Conconi et al. were selected from a rather limited geographical location near Ferrara, whereas the family described here originates from Sicily.

It seems possible that the study of individuals from Ferrara in which both a $\beta^0$ thalassemia gene and an abnormal $\beta$ allele are present may help to corroborate the evidence for the molecular defect proposed by Conconi et al. The complete absence of mRNA for $\beta^A$ chain seems to us the most simple and straightforward explanation for $\beta^0$ thalassemia. The hemoglobin pattern of II, 2 in the family reported here substantiates this point effectively; the failure of this $\beta^0/\beta^0$-thalassemia double heterozygote to produce hemoglobin A shows that it does not synthesize active $\beta^A$ chain mRNA.

ACKNOWLEDGMENT

The authors are indebted to Professor Luciano Tentori, Instituto Superiore di Sanità, Rome, for the fingerprint of the abnormal hemoglobin; to Dr. Giuseppe Incalcaterra, Piazza Armerina, for help in obtaining blood specimens; and to Dr. Bernard G. Forget, Children's Hospital Medical Center, Boston, for discussions and criticism.
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