Heterozygous Beta-Thalassemia in Association with Hereditary Elliptocytosis: A Family Study

By PASQUALE E. PERILLIE AND AMOZ I. CHERNOFF

CONTINUED BROADENING of our knowledge concerning the genetic relationships among the inherited abnormalities of the red blood cell is dependent upon the careful study of certain pedigrees. Descriptions of families in which thalassemia has occurred in association with an abnormal hemoglobin have provided some valuable information regarding the mode of genetic transmission and expressivity of the thalassemia gene and certain abnormal hemoglobins. Such reports have also provided an explanation for some of the clinical variability encountered in the thalassemia states.

Up to the present time, only a few instances of the combination of hereditary elliptocytosis and other inherited traits have been described. This is probably due to the relative rarity of elliptocytosis and the fact that no specific defect in the affected red cell has been discovered which would facilitate the diagnosis of this disorder. At the present time the diagnosis of elliptocytosis depends upon careful study of the morphology of the red blood cells in several members of a family suspected of harboring the gene for elliptocytosis.

The data presented in three previous descriptions of the combination of thalassemia and hereditary elliptocytosis did not permit any conclusions regarding the genetic relationship of these two genes. The following is the report of an Italian family in which a beta-thalassemia gene occurred in combination with a gene for elliptocytosis. Hematologic and genetic studies in this family permit concluding that the two genes are neither allelomorphic nor closely linked. In addition, there is suggestive evidence that the clinical effects of both genes are summated when they are both present in the same individual.

CASE REPORTS

The family A is of Italian extraction and its pedigree is presented in figure 1. Propositus (II-1) a 43-year-old male, was first seen while hospitalized at the West Haven Veterans Administration Hospital in April, 1963. He had been convalescing from a myocardial infarction when anemia was discovered. The physical examination revealed no abnormalities. Examination of the peripheral blood showed marked elliptocytosis with slight hypochromia. Stools were guaiac-positive and the bone marrow demonstrated normoblastic erythroid hyperplasia. Upper gastrointestinal series demonstrated a hiatal hernia. Because the patient had been on continual anticoagulation therapy during the previous 3 weeks of hospitalization, it was felt that his anemia was due to gastrointestinal bleeding, perhaps as...
Fig. 1.—Pedigree of Family A.

A complication of anticoagulant therapy. The anemia responded promptly to oral iron therapy. Two months later, despite complete resolution of the anemia, marked elliptocytosis persisted. Family history revealed that the father (I-1), who had been well all of his life, died of pneumonia in 1938, at the age of 45 years at the Grace-New Haven Community Hospital. Review of the father’s hospital record showed that he was not anemic during his hospitalization. His peripheral blood smear, however, was described as showing marked elliptocytosis. The father’s final diagnoses were death due to bronchopneumonia and an associated diagnosis of familial ovalocytosis.

II-8: F. T., a 38-year-old white female had been known to be anemic since adolescence. She had complained of chronic fatigability and had been excused from gym classes while in high school. She had never required hospitalization except for the delivery of 2 daughters. Both pregnancies were apparently uncomplicated. Because of her anemia, she had been treated unsuccessfully with a variety of hematinsics including iron, vitamin B₁₂ and folic acid. Physical examination revealed pallor of the skin, conjunctiva and mucous membranes. A soft splenic tip was palpable in the left upper abdominal quadrant. There were no other abnormal findings.

II-5: Am. A., a 45-year-old white construction worker considered himself to be in excellent health. He had never required hospitalization and has served in the armed forces uneventfully. Physical examination was entirely normal.

III-6: C. A., the 16-year-old daughter of II-5, was a healthy appearing young female with no complaints. She took an active part in her school physical education program. Physical examination was normal.

III-7: L. T. was a healthy, 18-year-old female with no complaints. Physical examination was normal.

**Materials and Methods**

**Hematologic Methods**

Routine hematologic investigations were carried out following accepted methods. Additional studies included serum iron concentration, red cell osmotic fragility, serum bilirubin, and the brilliant cresyl blue dye test for G-6-PD deficiency. Wright-Giems stained peripheral blood smears from each family member were examined and scored for the number and type of elliptocytes following the method of Motulsky et al. In this method elliptocytes are usually classified as one of three types: Type I – oval; Type II – elliptical; Type III – rod forms.
Table 1.—Hematologic and Biochemical Findings of Family A

<table>
<thead>
<tr>
<th>Pedigree and Designation</th>
<th>Hgb (Gm. %)</th>
<th>Hct (%)</th>
<th>MCV (μl)</th>
<th>MCH (γγ)</th>
<th>Retic (%)</th>
<th>Target Cells (%)</th>
<th>Elliptocytes (%)</th>
<th>Osmotic Fragility</th>
<th>Serum Iron (Normal 65-130 γ γ)</th>
<th>Alk. Hb (Normal &lt;23%)</th>
<th>A1 Hb (Normal 2-8.1%)</th>
<th>Indirect Bilirubin (Normal &lt;0.5 mg. %)</th>
</tr>
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<tbody>
<tr>
<td>I (1)*</td>
<td>14.5</td>
<td>40</td>
<td>81</td>
<td>23</td>
<td>0</td>
<td>80 32 8</td>
<td>Normal</td>
<td>80</td>
<td>&lt;2.0</td>
<td>2.48</td>
<td>0.37</td>
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<tr>
<td>II (1)</td>
<td>15.0</td>
<td>44</td>
<td>89</td>
<td>30</td>
<td>2.0</td>
<td>0 30 32 8</td>
<td>Normal</td>
<td>92</td>
<td>&lt;2.0</td>
<td>2.60</td>
<td>0.13</td>
<td></td>
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<tr>
<td>II (2)</td>
<td>12.0</td>
<td>40</td>
<td>80</td>
<td>28</td>
<td>1.8</td>
<td>0 25 33 7</td>
<td>Normal</td>
<td>95</td>
<td>&lt;2.0</td>
<td>3.95</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>II (3)</td>
<td>13.5</td>
<td>38</td>
<td>89</td>
<td>33</td>
<td>1.1</td>
<td>0 21 1 0</td>
<td>Normal</td>
<td>95</td>
<td>&lt;2.0</td>
<td>3.95</td>
<td>0.50</td>
<td></td>
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<tr>
<td>II (4)</td>
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<td>40</td>
<td>80</td>
<td>28</td>
<td>1.1</td>
<td>0 0 0 0</td>
<td>Decreased</td>
<td>145</td>
<td>2.3</td>
<td>4.00</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>II (5)</td>
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<td>40</td>
<td>88</td>
<td>29</td>
<td>2.2</td>
<td>0 50 23 7</td>
<td>Normal</td>
<td>95</td>
<td>&lt;2.0</td>
<td>3.95</td>
<td>0.50</td>
<td></td>
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<tr>
<td>II (6)</td>
<td>12.4</td>
<td>38</td>
<td>88</td>
<td>22</td>
<td>2.4</td>
<td>0 60 20 15 5</td>
<td>Decreased</td>
<td>150</td>
<td>&lt;2.0</td>
<td>4.30</td>
<td>0.35</td>
<td></td>
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<tr>
<td>II (7)</td>
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<td>39</td>
<td>89</td>
<td>29</td>
<td>2.0</td>
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<td>70</td>
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<td>II (8)</td>
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<td>38</td>
<td>85</td>
<td>28</td>
<td>1.5</td>
<td>0 45 10 5</td>
<td>Decreased</td>
<td>150</td>
<td>&lt;2.0</td>
<td>4.30</td>
<td>0.35</td>
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*Data obtained from hospital record.
In order to determine the number of elliptical cells which might be observed in a randomly selected series of blood smears the above scoring method was used to examine 50 stained smears made for routine purposes.

Hemoglobin studies

Red cell hemolysates were studied by paper, starch gel and agar electrophoresis and chromatographically on carboxymethyl cellulose, Amberlite IRC 50 and DEAE (10). Fetal hemoglobin was determined by the alkali denaturation technic. Hemoglobin A2 determinations were done by quantitative elution of the minor hemoglobin from DEAE cellulose columns.12

RESULTS

Results of the various routine hematologic investigations and hemoglobin electrophoretic studies are presented in table 1.

Eight of the 15 members studied had greater than 40 per cent elliptically shaped red cells in their peripheral smears. Of these 8, all had greater than 5 per cent rod-shaped or Type III forms. The remaining 7 family members had less than 15 per cent elliptical cells in their smears. Of this group, 2 had a few rod forms present in their peripheral smears. Evaluation of 50 randomly selected smears revealed that all contained less than 15 per cent elliptical shaped red cells with the majority containing 10 per cent or less. Most of the elliptical cells seen were of Type I and no Type III forms were seen.

On the basis of the above data we therefore selected the following criteria as evidence for the presence of hereditary elliptocytosis: (1) greater than 40 per cent of the red cells examined are elliptically shaped; (2) the presence of some Type III or rod form elliptocytes. The latter were considered particularly significant when found in numbers greater than 5 per cent and when unaccompanied by any other red cell morphologic change such as targeting, hypochromia or microcytosis.

The G-6-PD screening test was negative in all members tested.

DISCUSSION

The ethnic origin of the present family, combined with the demonstration of elevated Hb A2 levels, targeting, hypochromia, microcytosis, decreased red cell fragility and normal serum iron levels leaves little doubt that a gene for beta-thalassemia is present in certain members of the family described.

Proof of the presence of a gene for hereditary elliptocytosis is less direct since, at the present time, this disorder is based primarily on morphologic features. There is usually little difficulty in establishing the presence of this gene when a whole family is investigated. In the family under discussion, however, the problem is complicated by the presence of the beta-thalassemia gene, a disorder characterized by a slight-to-moderate degree of secondary elliptocytosis. Because of this, precise morphologic criteria for establishing the presence of the elliptocytic gene are therefore of extreme importance if one is to avoid misinterpreting the significance of elliptocytes in the peripheral blood of certain individuals.

In the present study, after examining and scoring the number and type of
elliptical cells in the family members and a group of randomly selected blood
smears, we selected the criteria described above for the diagnosis of hered-
itary elliptocytosis. These criteria agreed closely with those previously de-
scribed. Florman and Wintrobe suggested that at least 40 per cent of the
red cells should show some degree of elliptocytosis, with at least 10 per cent
showing marked degrees of elliptocytosis. Goodall et al., considered 33 per
cent as the minimum for diagnostic purposes. Bannerman took 25 per cent
eelliptocytosis as the required amount for the diagnosis of hereditaryelli-
tocytosis, and considered the presence of rod forms as particularly signifi-
cant. Most authors, as in our survey, have found that normal blood usually con-
tains less than 15 per cent elliptical forms with the majority of elliptical cells
being of Type I, i.e., roundish or oval. Of additional differential diagnostic
significance is the observation that uncomplicated cases of hereditary el-
tiptocytosis, as a general rule, show no other red cell morphologic abnor-
malities except the varying degrees of elliptical shapes. Thalassemia, on the
other hand, usually is accompanied by other red cell changes in addition to
eelliptocytosis.
Utilizing the above information in interpreting the results of the genetic
study of Family A we feel there is good evidence for concluding that the
two genes involved are neither allelic nor closely linked. These conclusions
are based on establishing that II-8 is doubly heterozygous for both genes.
Evidence for beta-thalassemia is the following: (1) hypochromia, micro-
cytosis and targetting in her peripheral blood smear; (2) decreased red cell
fragility; (3) elevated levels of Hb A2; (4) elevated levels of serum iron and
bilirubin.
Proof of the presence of the elliptocytosis gene in the cells of II-8 is based
upon the following morphologic observations: (1) the blood smear of the
husband (II-7) was morphologically normal and contained no elliptical cells
when scored by the method employed in this study. We can conclude there-
fore that he did not harbor the elliptocytosis gene. (2) The smear of the
daughter (III-7) showed marked elliptocytosis with 60 per cent of the cells
counted showing varying degrees of elliptocytosis, including 5 per cent rod
forms (fig. 2). This finding, in conjunction with the absence of any other red
cell morphologic abnormalities, and the normal hemoglobin electrophoretic
studies, seem adequate proof that the daughter has inherited the ellipto-
cytosis gene. Since there is no reason to question the paternity of III-7, we
can conclude that she inherited the elliptocytosis gene from her mother (II-8).
Additional, but less conclusive evidence in this regard, can be obtained from
the results of the examination of the blood smear of II-8. Forty per cent of
her red blood cells showed varying degrees of elliptocytosis. Although this
degree of elliptocytosis can be seen in heterozygous thalassemia alone, it is
not typical. Further, the two remaining family members thought to have
heterozygous beta-thalassemia showed only slight elliptocytosis (see II-5,
III-6, table 1, fig. 2).
Accepting that II-8 is carrying the genes for beta-thalassemia and ellli-
tocytosis, what is the evidence for nonallelism of these two genes? If the genes
Fig. 2.—Photomicrographs of peripheral blood smears of pertinent members of a family. Pedigree designations are the same as in figure 1.

are alleles than every offspring of II-8 should inherit one or the other gene. If they are not allelomorphs, then it is possible for II-8 to have an offspring who carries neither or both genes. We have established that 1 daughter of II-8 is heterozygous for elliptocytosis alone (III-7). Examination and scoring of the blood smear from the remaining daughter of II-8 (III-8) revealed no red cell abnormalities, with only 2 per cent of her cells showing type 1 elliptical forms (fig. 2). Based upon the criterion we selected for the diagnosis
of the elliptocytic gene, it seems reasonable to conclude that III-8 did not inherit the gene for elliptocytosis. Electrophoretic analysis of red cell hemolysates from III-8 demonstrated normal values for Hb A2 and alkali-resistant hemoglobin. We conclude therefore, that III-8 is normal with regard to the genes for elliptocytosis and beta-thalassemia.

Based upon the above genetic data we then have reasonably good evidence that the genes for beta-thalassemia and elliptocytosis found in the present family are not allelic. The data also permit certain conclusions regarding the genetic linkage of both factors.

If the two factors are closely linked so that cross-over phenomena do not present a serious consideration, they may be linked either in the coupling or repulsion phases. In the coupling phase, the offspring of II-8 should have both abnormalities or neither, whereas in the repulsion phase either offspring could have one or the other abnormality but not both or neither. Since neither of these situations exists in the present kinship in that one offspring (III-7) has inherited one factor alone, and the remaining offspring (III-8) has not inherited either factor, we can conclude that the two genes are not closely linked. The genetic data indicate rather that the two genes are either very distantly linked or not linked at all.

Previous reports of the association of elliptocytosis with other inherited red cell defects did not appear to show mutual enhancement of any of the involved genes. It is possible that the hemolytic anemia present in II-8 represents a summation of the clinical effects of both genes. Admittedly, the recognized variability of the thalassemic and elliptocytic states and the small sample available in the present family does not permit a definite conclusion in this regard. Although 8 members of the family have inherited the elliptocytosis gene (table 1) only II-8, the double heterozygote, has good evidence for the presence of an uncompensated hemolytic anemia. This would seem to be significant since overt hemolysis is unusual in elliptocytosis and when present usually affects several members of the same family. If one concludes that the hemolytic anemia present in II-8 was a manifestation of the beta-thalassemic gene, it is interesting that she is the only carrier of the beta-thalassemia gene in the family with a frank hemolytic anemia.

Further studies and follow up of this and similar families may clarify this question of mutual enhancement of gene expressivity.

SUMMARY

The association of the genes for beta-thalassemia and hereditary elliptocytosis are described in an Italian family. Genetic study of this family indicates that the involved genes are not allelic and are not closely linked. Clinical and laboratory data further suggest that the effects of both genes are summated when present in the same individual.

SUMMARIO IN INTERLINGUA

Es describite le association del genes pro thalassemia beta e pro elliptocytosis in un familia italian. Studios genetic de iste familia indica que le genes
BETA-THALASSEMA WITH HEREDITARY ELLIPTOCYTOSIS

in question non es allelic e non es interconnectite intimemente. Datos clinic e laboratorial suggere in plus que le effectos de ambe le mentionate genes es summate quando illos occurre in le mesme individuo.

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