Thalassemia Minor Associated with Hemoglobin-B<sub>2</sub> Heterozygosity. A Family Report

By Titus H. J. Huisman, K. Punt and J. D. G. Schaad

In a previous article<sup>1</sup> genetic and structural studies were reported concerning the minor hemoglobin component Hb-B<sub>2</sub>, which is present in about two per cent of the Negro population of the State of Georgia. This component, which is most likely the same as that reported by Kunkel, Ceppellini et al.<sup>2,3</sup> is an abnormality of the normally occurring Hb-A<sub>2</sub> fraction. Only one structural difference was found between Hb-B<sub>2</sub> and Hb-A<sub>2</sub>, namely, the introduction of an arginine residue in peptide number 12.<sup>1</sup> This difference was detected through study of a tryptic digest of the pure Hb-B<sub>2</sub> by the “fingerprinting” technic originally described by Ingram.<sup>4</sup> This fact, together with the discovery of an individual homozygous for the Hb-B<sub>2</sub> abnormality<sup>1</sup> showing complete absence of Hb-A<sub>2</sub>, proved the allelism of Hb-A<sub>2</sub> and Hb-B<sub>2</sub>, as already assumed by Ceppellini.<sup>3</sup>

In his report<sup>6</sup> Ceppellini presented a family in which the Hb-B<sub>2</sub> abnormality occurred in conjunction with thalassemia minor. The father was a heterozygous carrier of Hb-B<sub>2</sub> (Hb-A<sub>2</sub>: 1.1 per cent, Hb-B<sub>2</sub>: 0.9 per cent, normal Hb-A<sub>2</sub> value: 2.0–2.4 per cent), while the mother was suffering from thalassemia minor (Hb-A<sub>2</sub>: 5.6 per cent). Two children were found to be normal (Hb-A<sub>2</sub>: 2.1 and 2.4 per cent); three were heterozygous Hb-B<sub>2</sub> carriers (Hb-A<sub>2</sub>: 0.9–1.3 per cent, Hb-B<sub>2</sub>: 0.8–1.1 per cent), one suffered from a thalassemia trait (Hb-A<sub>2</sub>: 5.2 per cent) and two showed a double heterozygosity. These last two cases are of special interest, because it was found that, when Hb-B<sub>2</sub> is present in a thalassemia trait carrier, the values of Hb-A<sub>2</sub> (2.9 and 3.1 per cent) as well as Hb-B<sub>2</sub> (2.2 and 2.5 per cent) are doubled, the sum being the same as the high level of Hb-A<sub>2</sub> typical for most cases of thalassemia minor.

In recent years we have studied a family originating from Surinam, in which a similar abnormality, that is, the occurrence of the Hb-B<sub>2</sub> fraction together with thalassemia minor, was present. From the results of this study some conclusions will be drawn as to the genetic relationship between the thalassemia gene and the Hb-A<sub>2</sub> gene. In this paper a description of the family will be given. Preliminary results have been reported in earlier communications<sup>5–6</sup> in which the abnormal minor hemoglobin component Hb-B<sub>2</sub> was described as Hb-A<sub>2</sub><sup>1</sup>.

Materials and Methods

Family report. The family K. originates from Surinam (South America) and is of mixed Negro, European and Jewish extraction. Some members of the family live in the Nether-...
lands. The pedigree of family K. is presented in figure 1. Propositus (IV-2), a 1½ year old boy, was admitted to the Department of Pediatrics, University of Utrecht, the Netherlands (Prof. Dr. W. K. Dicke), in March 1958 because of pneumonia of the right upper lobe, that rapidly cleared up under appropriate treatment. At that time a hypochromic anemia was noted which required further study. Physical examination revealed no abnormalities, the spleen particularly was not palpable. The results of the hematologic studies will be presented below. Those relatives of this patient who could be investigated all stated that they were in good health. Only two (III-4 and III-6) had previously been treated for anemia. In one of them (III-4) the spleen was palpable just below the costal margin; in all other cases the physical examination was negative.

Hematologic methods. Routine hematologic investigations were carried out following accepted methods. Additional studies included the quantitative fecal excretion of urobilinogen, the serum iron concentration and the serum iron-binding capacity. Iron in bone marrow smears was stained by a modification of the method of Kaplan, Zuelzer and Mouriquand.

Hemoglobin studies. Hemoglobin analyses were performed by the starch electrophoretic method described by Gerald and Diamond and by starch gel electrophoresis at pH 8.0, following the description of Smithies. The possible presence of fetal hemoglobin was studied using the alkali denaturation method of Jonxis and Visser. Quantitative Hb separations were made by CM-cellulose chromatography. The abnormal minor component was isolated in pure form following the same technic as that described for Hb-B₄. In the structural studies Ingram’s fingerprinting technic was used in the same way as mentioned in the previous article describing the characteristic properties of Hb-B₄.

RESULTS

Hematologic Studies

Results of the various hematologic investigations are presented in table 1. In 14 members of 3 generations of this family (Cases IV, 1 and 2; III, 2, 4, 6, 13, 17, 18, 19, 20, 22; II, 2, 7 and 8), a more or less marked hypochromic anemia was present. This anemia was characterized by anisocytosis and poikilocytosis of the erythrocytes and by the occurrence of target cells (table 1; data on 7 cases are presented). In some cases basophilic stippling was found. Sometimes a slight reticulocytosis was noted. A typical blood smear is shown in figure 2.

![Pedigree of family K.](image)
### Table 1.—Hematologic Data of Several Members of Family K.

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex &amp; Age</th>
<th>Hb (Gm. %)</th>
<th>RBC (10⁶/mm³)</th>
<th>P.V. %</th>
<th>MCV (c/u)</th>
<th>Aniso-</th>
<th>Microcytosis</th>
<th>Target Cell</th>
<th>Basophilic Stippling</th>
<th>Reticulocytes (%)</th>
<th>Onset of Fragility 0.35% NaCl (% hemol.)</th>
<th>Feces urobilin (mg./24 hrs.)</th>
<th>Serum Iron</th>
<th>Bone Marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal values</td>
<td>M 14-17.2</td>
<td>M 4.5-5.5</td>
<td>M 42-52</td>
<td>78-98</td>
<td>9.2-2</td>
<td>91-98</td>
<td>25-180</td>
<td>0.2-2</td>
<td>91-98</td>
<td>25-180</td>
<td>0.2-2</td>
<td>91-98</td>
<td>25-180</td>
<td>0.2-2</td>
</tr>
<tr>
<td>IV-2 (Propos.)</td>
<td>M 2½</td>
<td>9.4</td>
<td>4.6</td>
<td>32.5</td>
<td>71</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IV-1</td>
<td>F 6</td>
<td>11.6</td>
<td>4.6</td>
<td>39.0</td>
<td>85</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>III-2</td>
<td>F 28</td>
<td>11.3</td>
<td>5.6</td>
<td>43.5</td>
<td>77</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>III-4</td>
<td>M 26</td>
<td>12.1</td>
<td>5.3</td>
<td>41.5</td>
<td>78</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>III-6</td>
<td>F 14</td>
<td>9.7</td>
<td>4.4</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>III-13</td>
<td>M 21</td>
<td>11.2</td>
<td>6.0</td>
<td>41.0</td>
<td>68</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>II-2</td>
<td>F 47</td>
<td>9.4</td>
<td>3.4</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>III-1</td>
<td>M 25</td>
<td>15.6</td>
<td>4.6</td>
<td>49.0</td>
<td>106</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>III-3</td>
<td>M 28</td>
<td>14.0</td>
<td>4.8</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>III-7</td>
<td>M 17</td>
<td>15.0</td>
<td>4.6</td>
<td>44.0</td>
<td>95</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>III-14</td>
<td>F 20</td>
<td>11.1</td>
<td>4.1</td>
<td>34.0</td>
<td>83</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**M.E. ratio**: 1:1

**RES iron**: 35±11

**Sideroblasts**: 2.5-5

**Iron**: 5.3

**Hemolysis**: ++

**P.C.I.**: 37.3

**P.C.I.**: 35.5

**S.C.I.**: 27.0

**S.C.I.**: 27.0
In 5 of these 7 cases the osmotic fragility of the erythrocytes were determined, and was found to be definitely decreased. The serum iron concentration and saturation index were normal or increased. The bone marrow (Cases IV, 1; III, 2; and III, 13) showed an increased rate of erythropoiesis, an increased amount of iron in the bone marrow fragments, while the number of sideroblasts was also increased. The urobilinogen content of the feces was normal in 3 cases although in one the hemolytic index was increased. The hematologic data of 12 other members of family K. (Cases III, 1, 3, 7, 14, 21, 23, 24; II, 1, 4, 6, 9 and 11; see also table 1) are within the normal limits.

In all 14 cases with hematologic abnormalities, analysis of the hemoglobin by starch gel electrophoresis revealed a slow moving minor Hb-component in addition to the normal Hb-A₂ (fig. 3). The electrophoretic mobility of this abnormal Hb-component was identical with that of Hb-B₂ as described earlier. Although the electrophoretic method yields data only roughly quantitative, it is clear from the picture that the amounts of Hb-A₂ as well as of Hb-B₂ in the blood of these heterozygous carriers are each of the same order of magnitude as the amount of Hb-A₂ found in the blood of a normal individual homozygous for Hb-A₂. The percentages of the different minor Hb-fractions were determined by CM-cellulose chromatography and the resulting figures are listed in table 2. In the 14 abnormal cases these percentages ranged from 1.7 to 2.4 per cent (Hb-A₂) and from 1.6 to 2.2 per cent (Hb-B₂). The sum of the two minor Hb-fractions adds up to values ranging from 3.5 to 4.5 per cent. The percentages of Hb-F in these 14 cases were normal (below two per cent). CMC-chromatography of the hemoglobin of the 12 other members of the fam-
Fig. 3.—Starch gel electrophoretic patterns of the hemoglobins of cases II, 8; III, 17, 18, 19, 20, 21 and 22. A discontinuous buffer system was used: the starch gel was prepared with a 0.03 M Tris-EDTA-Borate buffer pH 8.0 while the trays contained a 0.3 M boric acid—0.06 M NaOH buffer of pH 9.0 as bridge solution. The benzidine stain is presented.

ily K. revealed normal values for Hb-A$_2$ (1.7–2.5 per cent) and no abnormal component was detectable.

Studies on the Isolated Hb Fraction

In order to add more evidence for the identity of the abnormal minor Hb fraction with the Hb-B$_2$ described earlier$^1$ the isolated abnormal Hb-fraction was digested with trypsin and the resulting peptide mixture was studied by the “fingerprinting” technic.$^4$ The results obtained were compared with those found for the abnormal Hb-B$_2$.$^1$ Five different preparations, isolated from the blood of different family members were analyzed; figure 4 presents tracings of the fingerprints obtained. No difference was detectable between the fingerprints of the abnormal minor Hb and Hb-B$_2$. The fingerprints of these two components and of Hb-A$_2$ differ from those of normal Hb-A in the peptides 12 and 26 and in the presence of a new peptide appearing on the negative side of peptide number 11. However, both slow-moving abnormal minor Hb-fractions showed a similar difference from Hb-A$_2$: spot number 12 contains arginine as well as tryptophane (peptide 12 of Hb-A$_2$ is arginine negative). There is a possibility that spot number 12 found in the fingerprints of Hb-B$_2$ (and also of the minor abnormal component found in family K.) represents two different peptides with identical electrophoretic properties but with a slight difference in chromatographic behavior, one peptide being tryptophane positive and the
Table 2.—The Percentages of Hb-A₂ and Hb-B₂ Present in the Blood of Members of Family K.

<table>
<thead>
<tr>
<th>Case</th>
<th>Hb-A₂</th>
<th>Hb-B₂</th>
<th>Total</th>
<th>Case</th>
<th>Hb-A₂</th>
<th>Hb-B₂</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV-2</td>
<td>1.8</td>
<td>2.2</td>
<td>4.0</td>
<td>III-1</td>
<td>2.2</td>
<td>0</td>
<td>2.2</td>
</tr>
<tr>
<td>IV-1</td>
<td>1.8</td>
<td>2.2</td>
<td>4.0</td>
<td>III-3</td>
<td>2.2</td>
<td>0</td>
<td>2.2</td>
</tr>
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<td>2.0</td>
<td>4.3</td>
<td>III-7</td>
<td>2.0</td>
<td>0</td>
<td>2.0</td>
</tr>
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<td>III-4</td>
<td>1.9</td>
<td>1.9</td>
<td>3.8</td>
<td>III-14</td>
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<td>4.0</td>
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<td>3.8</td>
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<td>2.1</td>
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<td>4.2</td>
<td></td>
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</table>

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Fig. 4.—Tracing of fingerprints of the isolated abnormal minor Hb-fraction and of Hb-B₂⁺.

other arginine positive. Similar experiments performed with the isolated δ chains of Hb-A₂ (designated as αδ₂δ₂⁺) and of Hb-B₂ (designated as αδ₂δ₂⁺) have shown that the abnormality is located in the δ polypeptide chain. It seems, therefore, that the abnormality present in both components is limited to the replacement of one-unknown-amino acid residue of peptide 12 for arginine. Since this change involves most probably the introduction of two net positive charges per Hb-molecule, it offers also an explanation for the observed difference in electrophoretic mobility. The identical behavior in electrophoresis...
and in chromatography of the abnormal minor Hb-fraction found in the blood of members of family K. and of the Hb-B₂ together with the results of the structural studies presented above justify the statement that these two abnormal minor hemoglobins are the same. As definite proof, however, can only be obtained by complete structural analyses of the two abnormal Hb-fractions. From the differences found in the fingerprinting studies and the discovery of a homozygous Hb-B₂ carrier (with no detectable amount of Hb-A₂),¹ it may be concluded that Hb-B₂ is the product of an allele of the A₂ locus (i.e. a $\delta'$ chain abnormality).

**Discussion**

In this family 14 members had hematological findings suggestive of thalassemia minor (hypochromic anemia, target cells, decreased osmotic fragility of the red cells, normal or elevated serum iron values). At the same time an abnormal slow moving minor hemoglobin component, which is indistinguishable from the abnormal Hb-B₂, was demonstrated in the blood of all 14 patients. It seems, therefore, that all 14 are heterozygous for thalassemia as well as for the Hb-B₂, which is an abnormal Hb-A₂. In individuals heterozygous for the Hb-B₂ abnormality alone, the amount of Hb-A₂ is decreased to half its value, while Hb-B₂ is present in the same low amount.²,¹ The occurrence of the Hb-B₂ abnormality in our thalassemia heterozygous carriers results in a doubling of the values of both minor fractions which is in accordance with the findings of Ceppellini.³ From a genetic point of view, an interesting finding is the fact that in this family all individuals are either entirely normal (III, 1, 3, 7, 14, 21, 23 and 24; II, 1, 4, 6, 9 and 11) or are heterozygous for both thalassemia and Hb-B₂ (IV, 1 and 2; III, 2, 4, 6, 13, 17, 18, 19, 20 and 22; II, 2, 7 and 8). Carriers heterozygous for one of the two abnormalities have not been encountered.

The possibility that the double heterozygous state in all these 14 cases (of 26 studied) occurred only by coincidence is statistically less than one in a hundred.⁴ A linkage between the two loci, as suggested in figure 5, seems, therefore, likely. No crossing over is observed which would suggest a close linkage. An estimation of the relative distance between the loci, however, requires still more family data. This hypothesis can be applied to other kindreds. The only other known kindred in the literature is that described by Ceppellini.³ Figure 6 shows that the same explanation can be applied to this pedigree. The suggested linkage of the t-T- and the A₂-B₂-loci implies that in one pedigree (that of Ceppellini) the A₂-T-chromosome, and in the second (the present pedigree), the B₂-T-chromosome, may be present.

The reason for the percentage increase of Hb-A₂ (and eventually of Hb-A₂ and Hb-B₂) in thalassemia minor is not clear. Although this phenomenon occurs rather constantly in this anomaly, cases of apparent thalassemia minor with normal Hb-A₂ levels have been reported.¹⁸ Ingram and Stretton¹⁹ have suggested the existence of at least two types of thalassemia, one with a hidden

*P value < 0.005 as calculated by the $\chi^2$ test for small numbers, using Yates correction. (F. Croxton: Elementary Statistics, New York, Dover Publ., Inc. 1959, pp. 275–276.)
abnormality of the α-chain and the second with a hidden abnormality of the β-chain. One of the characteristics of the β-chain thalassemia is considered to be an elevated level of the Hb-A₂ component. In several members of our family the sum of the percentages of Hb-A₂ and Hb-B₂, which was found to be abnormal in the δ chain, was increased to values characteristic for carriers of the β-thalassemia. It seems, therefore, that the abnormalities present in our family have to be considered as products of an allele of the α-chain locus (β-thalassemia) and of an allele of the δ chain locus (Hb-B₂ heterozygosity), both being present on the same member of a pair of homologous chromosomes and closely linked. The same genetic abnormalities are present in members of the family.

Fig. 5.—Supposed genetic patterns of family K.

Fig. 6.—Supposed genetic patterns of family of Ceppellini (3). For legends, see figure 5.
described by Ceppellini, the two different alleles, however, each being present on an opposite member.

Ceppellini also described a family with a double heterozygosity for Hb-S and for Hb-B2. The genetic patterns in this family and in a family described earlier are suggestive of a linkage of the Hb-S (or β-chain) locus and the Hb-B2 (or δ-chain) locus. He also produced evidence for a linkage of the loci for thalassemia and Hb-S. Our data on the possible linkage of the thalassemia and the Hb-B2 abnormalities are not incompatible with this proposition.

SUMMARY AND CONCLUSIONS

1. In 14 members of 26 cases of a family originating from Surinam a double heterozygosity (for thalassemia and for the abnormal minor component Hb-B2) was demonstrated. Thalassemia heterozygous carriers or individuals heterozygous for the Hb-B2 abnormality alone were not encountered.

2. The diagnosis of thalassemia minor was based on hematologic data. The abnormal minor Hb-component present in these cases was identified using the conventional electrophoretic and chromatographic technics and the fingerprinting method as being Hb-B2 (an abnormal Hb-A2). The percentages of the two minor Hb-fractions (Hb-A2 and Hb-B2) in these thalassemia heterozygous carriers are doubled, their sum being similar to the high level of Hb-A2 typical for most cases of thalassemia trait.

3. The genetic relationship between thalassemia minor (genotype: tT) and Hb-B2 heterozygosity (genotype: A2/B2) is discussed. The data on this pedigree are suggestive of a close linkage of the t-T and A2/B2-loci.

SUMMARY IN INTERLINGUA

1. In 14 inter 26 membros de un familia originari de Surinam un duple heterozygositate pro thalassemia e pro le anormal componente minor hemoglobina B2 esseva demonstrate. Portatores heterozygotic de thalassemia o subjectos heterozygotic pro solmente le anormalitate de hemoglobina B2 non esseva incontre.

2. Le diagnose de thalassemia minor esseva basate in datos hematologic. Le anormal componente minor, i.e. le hemoglobina B2, presente in iste casos esseva identificate con le uso de technicas conventional de electrophorese e chromatographia. Le procentages del duo minor fractiones hemoglobina A2 e hemoglobina B2 (iste secunde es un forma anormal del prime) esseva duplate in iste portatores heterozygotic de thalassemia. Le summa del duo esseva simile al alte nivello de hemoglobina A2 que es typic del majoritate del casos de character de thalassemia.


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THALASSEMA MINOR WITH HEMOGLOBIN-B2 HETEROZYGOSITY

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