Coincidental Nontransfusional Iron Overload and Thalassemia Minor: Association With HLA-Linked Hemochromatosis

By Corwin Q. Edwards, Mark H. Skolnick, and James P. Kushner

A pedigree was studied in which five individuals with \( \beta \)-thalassemia minor were found to have nontransfusional hemochromatosis. Three were children under the age of 10 and two were young male adults, ages 28 and 33. A 5-yr-old child without evidence of thalassemia also had hemochromatosis. Since hemochromatosis is transmitted as an HLA-linked autosomal recessive disorder, HLA haplotypes serve as markers of hemochromatosis alleles. In this pedigree, five identifiable HLA haplotypes were associated with hemochromatosis alleles. Only individuals with two hemochromatosis alleles (homozygosity) had heavy iron loads, whether \( \beta \)-thalassemia minor was present or not.

HEAVY IRON LOADING is a rare clinical finding in patients with \( \beta \)-thalassemia minor.\(^6\) The anemia of \( \beta \)-thalassemia minor is generally modest and often mistaken for iron deficiency anemia. Medicinal iron intake has been implicated in the pathogenesis of the hemochromatosis that has occasionally been described in association with \( \beta \)-thalassemia minor.\(^7\)

Recent studies have revealed that most patients with idiopathic hemochromatosis actually have an inherited disorder.\(^8\) The hemochromatosis allele is located on chromosome six and is tightly linked to the histocompatibility locus on the same chromosome.\(^9\) Thalassemia is not linked to the HLA region.\(^10\) Pedigree analyses employing both measurements of body iron stores and histocompatibility antigen (HLA) typing have established that hemochromatosis is inherited as an autosomal recessive trait.\(^11\) It has been estimated that nearly 10% of the white population is heterozygous for the hemochromatosis allele.\(^12\)\(^13\) The disease frequency (homozygosity) is approximately 3/1000.\(^14\)\(^15\)

We recently studied a family in which \( \beta \)-thalassemia minor and hemochromatosis genes coexisted. Five individuals with \( \beta \)-thalassemia minor were found to have hemochromatosis, three of whom were young children. All five individuals were homozygous for the hemochromatosis allele. The proband, with \( \beta \)-thalassaemia minor, died of cardiac complications of hemochromatosis at the age of 28.

It is proposed that homozygosity for the hemochromatosis allele is the major pathogenic abnormality responsible for severe iron loading in occasional patients with \( \beta \)-thalassemia minor. The contribution of the \( \beta \)-thalassemia minor to the development of the hemochromatosis appears to be less important.

MATERIALS AND METHODS

\( \beta \)-Thalassemia

The following tests were performed on pedigree members to determine the presence of \( \beta \)-thalassemia minor: erythrocyte indices, reticulocyte count, examination of peripheral blood smears, and hemoglobin electrophoresis. Individuals were considered to have \( \beta \)-thalassemia minor if their erythrocytes had the following features: mean corpuscular volume (MCV) less than 75 \( \mu \)l, mean corpuscular hemoglobin (MCH) less than 22 \( \mu \)g, hemoglobin electrophoresis revealing increased amounts of hemoglobins A\(_2\) and F, and A Wright’s stained peripheral blood smear demonstrating basophilic stippling, target cells, anisocytosis, and poikilocytosis.

Assessment of Iron Stores

Serum iron, total iron-binding capacity, percent saturation of transferrin, and serum ferritin were determined\(^16\) in all pedigree members available for study. Twenty-four-hour urinary iron excretion following intramuscular injection of deferoxamine (15 mg/kg body weight) was determined in five subjects. Hepatic parenchymal cell stainable iron was graded, and hepatic iron concentration was determined\(^17\) on liver biopsy specimens from five individuals.

A major iron load was considered present if the transferrin saturation was greater than 70%, the deferoxamine-induced urinary cell stainable iron grade was 3 or 4, or hepatic iron concentration was greater than 250 \( \mu \)g/100 mg wet weight liver.\(^18\) A minor iron load was considered present when transferrin saturation was 51%–70%, hepatic iron was grade 2, or hepatic iron concentration was 40–250 \( \mu \)g/100 mg wet weight liver.

HLA Typing

Histocompatibility testing was performed by a standard method.\(^19\) HLA haplotypes were deduced in three of the deceased individuals.
Fig. 1. The pedigree studied in which \( \beta \)-thalassemia minor and hemochromatosis coexisted. Eight individuals with \( \beta \)-thalassemia minor (asterisks) were identified, five of whom had a major iron load (\( \bullet \)) associated with HLA-linked hemochromatosis. Two individuals had a minor iron load (\( \circ \)). Individuals with normal iron stores are indicated by open squares and circles. The arrow indicates the proband. Numbers above the circles and squares are pedigree numbers; numbers below are the HLA haplotypes determined (no parentheses) or deduced (haplotypes in parentheses). (\( \circ \)) Decreased individuals; (\( \bullet \)) not studied; (\( \square \)-\( \circ \)) consanguinity.

RESULTS

Subjects Studied

The pedigree studied is represented in Fig. 1. The proband, a 28-yr-old white male with \( \beta \)-thalassemia minor, who died of hemochromatotic cardiomyopathy, and 27 members of the family were evaluated for the presence of thalassemia and hemochromatosis.

The proband (III-8, Fig. 1) was hospitalized at age 28 for the management of congestive heart failure and cardiac arrhythmias. Physical examination revealed cardiomegaly and signs of congestive failure as well as gray-bronze skin, hepatomegaly, and splenomegaly. A modest anemia was noted (VPRC 36%), and a diagnosis of thalassemia minor was made on the basis of abnormal erythrocyte morphology and characteristic red cell indices (Table 1). Other laboratory abnormalities included: total bilirubin 1.7 mg/dl; serum glutamic-oxaloacetic transaminase 154 lU/liter; serum iron 159 µg/dl, and transferrin saturation 96%. Percutaneous needle biopsy of the liver revealed prominent fibrosis and grade 4 hepatic parenchymal cell stainable iron (Table 2). The patient was a teetotaler and had never received medicinal iron.

The congestive heart failure proved refractory to therapy and the proband died of a cardiac arrhythmia (ventricular fibrillation). Autopsy findings included chronic passive congestion and edema of the lungs, cardiomegaly, hepatomegaly, and splenomegaly with chronic passive congestion, and marked iron deposition in the heart and liver. The hepatic iron concentration was 861 µg/100 mg wet weight (Table 2).

Table 1. Hematologic Data in Eight Relatives With \( \beta \)-Thalassemia Minor

<table>
<thead>
<tr>
<th>Individual</th>
<th>Age (yr)</th>
<th>VPRC (%)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>HbA (%)</th>
<th>HbA2 (%)</th>
<th>HbF (%)</th>
<th>Characteristic Erythrocyte Morphology*</th>
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<tbody>
<tr>
<td>Normal adult values</td>
<td></td>
<td>39-47</td>
<td>85-100</td>
<td>27-33</td>
<td>95.5-98</td>
<td>1-4.5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>I-2</td>
<td>90</td>
<td>44</td>
<td>70</td>
<td>21</td>
<td>92.9</td>
<td>7.1</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>II-1</td>
<td>68</td>
<td>35</td>
<td>62</td>
<td>22</td>
<td>91.9</td>
<td>8.1</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>II-4</td>
<td>65</td>
<td>41</td>
<td>70</td>
<td>23</td>
<td>89.9</td>
<td>6.5</td>
<td>3.6</td>
<td>+</td>
</tr>
<tr>
<td>III-6</td>
<td>33</td>
<td>40</td>
<td>73</td>
<td>23</td>
<td>91.1</td>
<td>6.8</td>
<td>2.1</td>
<td>+</td>
</tr>
<tr>
<td>III-8</td>
<td>28</td>
<td>36</td>
<td>70</td>
<td>22</td>
<td>89.1</td>
<td>8.2</td>
<td>2.7</td>
<td>+</td>
</tr>
<tr>
<td>IV-4</td>
<td>10</td>
<td>35</td>
<td>62</td>
<td>22</td>
<td>87.6</td>
<td>7.6</td>
<td>4.8</td>
<td>+</td>
</tr>
<tr>
<td>IV-5</td>
<td>6</td>
<td>35</td>
<td>60</td>
<td>21</td>
<td>87.6</td>
<td>7.6</td>
<td>4.8</td>
<td>+</td>
</tr>
<tr>
<td>IV-7</td>
<td>2</td>
<td>31</td>
<td>63</td>
<td>20</td>
<td>78.8</td>
<td>6.2</td>
<td>15.0</td>
<td>+</td>
</tr>
</tbody>
</table>

*Microcytosis, anisocytosis, poikilocytosis, target cells, basophilic stippling.
serum ferritin concentration as the only laboratory evidence of increased body iron stores (I-2 and II-1, Table 2). Five of the eight had a transferrin saturation of 70% or greater (III-6, III-8, IV-4, IV-5, IV-7). Four of the individuals with transferrin saturations greater than 70% had liver biopsies that demonstrated marked iron loading. Two with iron-laden livers were children ages 10 and 6 (IV-4 and IV-5). Individual IV-7, a 2 yr of age, has not yet undergone liver biopsy.

Of the 21 nonthalassemic pedigree members studied, 3 had laboratory evidence of increased body iron stores. Individual II-12 died of cirrhosis and heart disease at age 45, 10 yr before this pedigree was studied. Hospital records revealed a transferrin saturation of 99% and an autopsy revealed hepatic fibrosis and heavy iron loading of the liver. Individual III-9, a 29-yr-old woman, had a transferrin saturation of 66%. Individual IV-6, a 5-yr-old girl, had a transferrin saturation of 76%.

**HLA Typing in the Pedigree**

HLA typing was done on 27 members of the pedigree (Fig. 1). Haplotypes were deduced for three deceased individuals (I-1, III-3, and III-8) after determining the HLA haplotypes in their spouses and offspring (Fig. 1). The HLA haplotypes found in individuals with an increased transferrin saturation are shown in Table 3.

**DISCUSSION**

Four children in this pedigree, under the age of 10, were found to have major iron loads. Child IV-6, a 5-yr-old girl, had no evidence of β-thalassemia minor. Her transferrin saturation was 76%. A transferrin saturation of this magnitude, in so young a child, is strong evidence for homozygosity for the hemochromatosis allele. Since the hemochromatosis gene is tightly linked to the HLA region on chromosome 6, both of this child’s HLA haplotypes (A26,B15 and A31,B15) can be considered markers for the hemochromatosis allele.

**Table 3. HLA Haplotypes Found in Pedigree Members With Increased Transferrin Saturation**

<table>
<thead>
<tr>
<th>HLA Haplotypes</th>
<th>A9,B40*</th>
<th>A31,B15</th>
<th>A2,B12(a)</th>
<th>A26,B15</th>
<th>A29,B7</th>
<th>A10,B16</th>
</tr>
</thead>
<tbody>
<tr>
<td>A9,B40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>A31,B15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2,B12(a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>A26,B15</td>
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<tr>
<td>A29,B7</td>
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<td></td>
<td></td>
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<tr>
<td>A10,B16</td>
<td></td>
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</tbody>
</table>

*A9,B40 was not linked to the hemochromatosis gene (see text).*
The three other children with major iron loads all had \( \beta \)-thalassemia minor (IV-4, IV-5, and IV-7). Children IV-4 and IV-5 had liver biopsies as part of the evaluation of iron loading. The degree of hepatic iron loading found was similar to that detected in nonthalassemic children with hemochromatosis. Thus, it seems likely that children IV-4, IV-5, and IV-7 are homozygous for the hemochromatosis allele and that HLA haplotypes A10, B16 and A2, B12(a) are also markers for hemochromatosis alleles in the pedigree. Strong evidence for this hypothesis was found in the older generations of the pedigree.

The proband (III-8) and his HLA-identical brother (III-6) both had overt hemochromatosis and \( \beta \)-thalassemia minor. Both had two HLA haplotypes linked to a hemochromatosis allele identified in generation IV [A31, B15 and A2, B12(a)]. The spouse (III-7) of III-6 had one HLA haplotype linked to the hemochromatosis allele (A10, B16). Thus, individual III-7, a 31-yr-old woman, is heterozygous for hemochromatosis. The finding of normal body iron stores in her is in keeping with the finding that over 80% of heterozygous females have no evidence of iron loading.

The spouse (III-9) of the proband, a 29-yr-old woman, had a transferrin saturation of 66% and one of the hemochromatosis-linked HLA haplotypes identified in generation IV (A26, B15). Thus, individual III-9 is at least heterozygous for hemochromatosis. Since sex and age influence the transferrin saturation in individuals homozygous for hemochromatosis, it is possible that this young woman is indeed a homozygote. Were this so, the A29, B7 haplotype would also be linked to the hemochromatosis allele, a possibility that gains support by study of generation II of the pedigree. If individual III-9 is indeed homozygous for the hemochromatosis allele, her marriage to the proband would represent the first reported homozygous–homozygous pairing.

In generation II, individual II-12 had clear evidence of hemochromatosis at autopsy. This individual and his sister (II-11) were the offspring of consanguineous parents (first cousins). It is very unlikely that II-11 and II-12 are HLA identical, since the transferrin saturation of II-11 is normal. They may have shared the A29, B7 haplotype, however, making II-11 heterozygous and supporting the possibility that III-9 is homozygous for hemochromatosis.

Individual II-10 had one marker haplotype (A26, B15), making him heterozygous for hemochromatosis. His transferrin saturation was normal, as is the case in two-thirds of heterozygous males.

Individuals II-4 and II-5 both had one marker haplotype. Individual II-5, a 62-yr-old woman, had the marker haplotype A2, B12(a) and a normal transferrin saturation, findings compatible with heterozygosity for hemochromatosis. Individual II-4, a 65-yr-old man with \( \beta \)-thalassemia minor, had the marker haplotype A31, B15. His transferrin saturation was elevated at 54%, but values for serum ferritin and deferoxamine-induced urinary iron excretion were normal (Table 2). These values are compatible with heterozygosity for the hemochromatosis gene but not homozygosity. The A9, B40 haplotype is therefore unlikely to be linked to a hemochromatosis allele.

The results of the iron studies in individual II-4, heterozygous for both \( \beta \)-thalassemia and hemochromatosis, were no different from those found in males heterozygous for hemochromatosis alone. This finding supports the conclusion that the heavily iron-laden individuals in generations III and IV were homozygous for hemochromatosis.

The serum ferritin concentration has been reported to be a reliable indicator of iron overload in individuals homozygous for the hemochromatosis gene. In previous studies we have noted occasional female homozygotes with increased transferrin saturation and normal serum ferritin concentration. In the pedigree described here, a 29-yr-old female homozygote had an increased transferrin saturation and normal serum ferritin concentration (III-9), as did 3 young, homozygous children (IV-4, IV-6, IV-7). These findings confirm our impression that increased transferrin saturation is the most reliable phenotypic marker for the hemochromatosis genotype and may be found before the serum ferritin concentration becomes elevated. The serum ferritin concentration is rarely elevated in heterozygous individuals yet the transferrin saturation is elevated in one-third of the male and one-sixth of the female homozygotes.

There are at least five marker haplotypes in this pedigree. If our assumption that II-11 and II-12 share one haplotype is correct, then there are six marker haplotypes. The incidence of heterozygosity for the hemochromatosis allele in the white population is approximately 10%, and a pedigree containing seven marker haplotypes has been reported. If a single hemochromatosis allele, interacting with \( \beta \)-thalassemia minor, were sufficient to cause massive iron overload, then 10% of individuals with \( \beta \)-thalassemia minor might have overt hemochromatosis. This clearly is not the case as heavy iron loading in individuals with \( \beta \)-thalassemia minor is observed infrequently. It seems probable that those rare individuals with \( \beta \)-thalassemia minor and heavy iron loads are in fact homozygous for hemochromatosis. The pedigree reported here is unique in that five such individuals were identified by careful study of relatives of the proband.
REFERENCES

Coincidental nontransfusional iron overload and thalassemia minor: association with HLA-linked hemochromatosis

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