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Expression of Hemochromatosis in Homozygous Subjects
Implications for Early Diagnosis and Prevention

LAWRIE W. POWELL, KIM M. SUMMERS, PHILLIP G. BOARD, ELIZABETH AXELEN, SONJA WEBB, and JUNE W. HALLIDAY
Department of Medicine, University of Queensland, Brisbane, Australia; and The John Curtin School of Medical Research, Australian National University, Canberra, Australia

This study looks at expression of genetic hemochromatosis in the homozygous and heterozygous states. Two hundred nine subjects in 40 families with confirmed hemochromatosis and clear evidence of HLA linkage in symptomatic individuals were studied prospectively for up to 24 yr. The study group consisted of 40 probands, 51 subjects sharing two HLA haplotypes with affected relatives (putative homozygotes), 98 putative heterozygotes, and 20 putative normal homozygotes. Forty-eight of 51 subjects predicted to be homozygous showed increased hepatic iron stores as assessed by liver biopsy and quantitative phlebotomy. If not evident initially, this developed in 1-8 yr. In the 3 subjects predicted by HLA typing to be homozygous but in whom there was no progressive iron accumulation, results of studies using another chromosome 6 genetic marker (Factor 13 A subunit) were consistent with chromosomal recombination, presumably separating one hemochromatosis allele from the HLA markers. No heterozygous subject developed overt hemochromatosis during the period of follow-up, although 1 showed evidence of iron overload at initial assessment. Genetic recombination is again thought to have separated the hemochromatosis allele from the HLA markers here. The present findings favor a location of the hemochromatosis locus telomeric to HLA-A. It is concluded that, in this population, hemochromatosis is apparently always HLA linked, and homozygous subjects will develop iron overload in the absence of chromosomal recombination or blood loss.

Hemochromatosis, an inherited disorder of iron metabolism characterized by progressive iron loading of parenchymal cells of the liver and other organs, is now known to be much more prevalent than was previously believed: recent estimates of the gene frequency in Caucasian populations have been as high as 1:20 (1-3). Moreover, patients diagnosed in the early precirrhotic stage of the disease and treated by phlebotomy therapy have been shown to have a normal life expectancy, in contrast to cirrhotic patients who die from complications including liver failure and primary hepatocellular carcinoma (4-6).

Reports of a tight linkage between hemochromatosis and the human leukocyte antigen (HLA)-A locus on chromosome 6 (7-9) have led to clarification of the genetic nature and mode of inheritance of the disease and also the early identification of homozygous subjects (10-13). However, it is still unclear what proportion of subjects predicted to be homozygous for the disease alleles by HLA typing develop iron overload, a point with obvious implications for family screening and management. A further unanswered question is whether all hemochromatosis other than that caused by secondary iron overload in certain anemias is HLA related.

This report describes the results of a prospective study of 40 families with hemochromatosis for up to 24 yr to determine what proportion of putative homozygous subjects will eventually develop iron overload.

Patients and Methods

Subjects

A total of 209 individuals from 40 families were studied with respect to hemochromatosis disease status and HLA type and followed up to 24 yr (median, 8 yr; range, 1-24 yr). The diagnosis of hemochromatosis in probands was made according to previously defined criteria (14). All available first- and second-degree relatives were investigated; in each instance, a clinical history was obtained and a physical examination was performed. Ethanol intake, iron
medications, menstrual loss, blood donations, and other causes of chronic blood loss were recorded. Blood was drawn between 8 AM and 9 AM for the assessment of iron status and HLA typing. Percutaneous liver biopsy was performed in 98 subjects (40 probands and 58 relatives) in whom HLA typing indicated probable homozygosity for the disease or in whom either the transferrin saturation or the serum ferritin level was abnormal on more than one occasion.

**Determination of Iron Status**

Serum iron concentration, total iron-binding capacity, and transferrin saturation were determined according to standard techniques as previously described (15). Serum ferritin concentration was measured by a solid-phase immunoradiometric assay (normal range, 20-200 μg/L for males and 10-150 μg/L for females) (16). Serum ferritin was not measured before 1974, and values at diagnosis are therefore not available for subjects seen initially before this time. Liver biopsy sections were stained with H&E, a reticulin stain, and Perls' Prussian blue stain for iron. The amount of stainable iron in hepatocytes was graded 0-4 (17), and hepatic iron concentration was measured on tissue dried to constant weight as previously described (18). The hepatic iron index was derived by dividing the hepatic iron concentration by the age in years at the time of biopsy (18). Increased iron stores were confirmed by quantitative phlebotomy in all homozygous subjects with evidence of iron overload.

**Genetic Markers on Chromosome 6**

Thirty-one HLA-A and HLA-B locus antigens were examined using the standard microlymphocytotoxicity test, and 10 HLA-DR antigens were tested in some families using the extended microlymphocytotoxicity test on purified B lymphocyte suspensions (19). In some instances, HLA-DR typing was defined by genotyping (20).

Determination of coagulation factor XIII A (F13A) phenotype was carried out by electrophoresis of plasma in agarose gels and specific staining as previously described (21).

**Assignment of Genotype in Family Members**

Results of previous studies by the authors (22) have shown that hemochromatosis is HLA-linked in symptomatic members of these families. Therefore, in each family the proband was assigned the status of homozygote, and each of the two HLA haplotypes identified in these subjects was assumed to be linked to a hemochromatosis allele. In families in which additional sibships showed evidence of disease, other HLA haplotypes associated with the disease were determined from the HLA type of affected individuals. Thus, for each family, two or more HLA haplotypes were disease linked. All family members could be classified on the basis of HLA typing as homozygous, with two disease-linked haplotypes (51 individuals), heterozygous, with one disease-linked haplotype (98 individuals), or homozygous normal, without disease-linked haplotypes (20 individuals).

HLA typing showed that homozygous-heterozygous mating had occurred at least 15 times. In each of these families, iron overload was present only in offspring carrying the same HLA haplotype from the unaffected (presumed heterozygous) parent.

**Prospective Evaluation**

All subjects were reviewed at predetermined intervals according to their hemochromatosis genotype as designated using the HLA type as described above. Thus, all presumed homozygous subjects were reviewed every 6-12 mo, heterozygous subjects were examined every 2 yr, and homozygous normal subjects were seen every 5 yr (13,15,23). This review was undertaken either by the authors or by a local physician. Liver biopsy was performed if there was a consistent increase in either the transferrin saturation or serum ferritin concentration.

**Results**

**Probands**

All 40 probands had increased iron stores as assessed by one or more of the following (in the absence of known causes of secondary iron overload): hepatic iron concentration >60 μmol/g dry weight (Figure 1); hepatic iron index >2 (18) (Figure 1); grade 3 or 4 stainable iron in the liver (17); >5 g iron removed by quantitative phlebotomy; and urinary excretion of >2.2 mg iron after administration of 0.5 g desferrioxamine (24). There were 33 male and 7 female probands, the median ages at presentation being 41 yr (range, 20-71 yr) for males and 38 yr (range, 18-74 yr) for females. This value for females is influenced by the presence of 2 young probands, aged 18 and 24 yr at presentation, with clear evidence of disease (hepatic iron concentrations of 128 and 74 μmol/g dry weight, respectively). Alcohol consumption was greater than 50 g/day in 11 (34.4%) of the males and in 1 of the females. Twenty-six of the 40 probands were recorded having symptoms relevant to hemochromatosis (hepatomegaly, diabetes, arthropathy, pigmentation, or testicular atrophy). Twelve probands had evidence of cirrhosis at biopsy, and 8 had evidence of fibrosis. As can be seen in Figure 1, all probands who had serum ferritin levels estimated had levels of greater than 200 μg/L at diagnosis but about one fifth had transferrin saturations of less than 6270, the value used by others (3) as being diagnostic of hemochromatosis. However, all probands with low transferrin saturations had high serum ferritin levels, ranging from 360 μg/L in a menstruating female to 4000 μg/L in an elderly male. All but 1 proband had grade 3 or 4 stainable iron (Table 1); the single individual with grade 2 stainable iron was a young male. The hepatic iron index was greater than 2.5 in all probands except for 1 elderly female with an index of 1.0. Body iron
stores, as estimated from the amount of blood removed before iron indices returned to normal levels, ranged from 3.5-56 g (median, 17; n = 20) in males and from 3.5-49 g (median, 14; n = 6) in females, with the lower values being found in younger probands.

**Putative Homozygous Relatives**

Twenty-eight male and 23 female relatives predicted to be homozygous because they carried two

HLA haplotypes associated with hemochromatosis in their families were followed for a median of 5 yr (range, 1-23 yr). The median age at first investigation was 27.5 yr (range, 7-61 yr) in males and 33 yr (range, 8-66 yr) in females. Iron indices at biopsy for this group are shown in Figure 1. Levels of serum ferritin, transferrin saturation, and hepatic iron concentration were widely scattered, with medians being lower than those for probands. A much broader spread was also seen in the amount of stainable iron detected (Table 1).
Table 1. Stainable Iron Graded at Liver Biopsy of Hemochromatosis Probands and Relatives

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yr)</th>
<th>Period of follow-up (yr)</th>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
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</thead>
<tbody>
<tr>
<td>Male probands</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female probands</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male hh relatives</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>11</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female hh relatives</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>9</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male Hh relatives</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female Hh relatives</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

hh Relatives, putative homozygous affected relatives; Hh relatives, putative heterozygous relatives. Includes case 6 from Table 2.

Table 2. Follow-up of Homozygous Relatives With Initial Equivocal Results

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yr)</th>
<th>Initial</th>
<th>At diagnosis</th>
<th>Hepatic iron</th>
<th>Body iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21/F</td>
<td>36/190</td>
<td>48/465</td>
<td>3/141</td>
<td>5.6/8.0</td>
</tr>
<tr>
<td>2</td>
<td>24/M</td>
<td>33/549</td>
<td>64/123</td>
<td>2/26</td>
<td>2.9/2.5</td>
</tr>
<tr>
<td>3</td>
<td>28/M</td>
<td>53/319</td>
<td>73/62</td>
<td>2/32</td>
<td>2.7/2.3</td>
</tr>
<tr>
<td>4</td>
<td>10/M</td>
<td>71/73</td>
<td>95/100</td>
<td>1/50</td>
<td>1.8/1.8</td>
</tr>
<tr>
<td>5</td>
<td>8/F</td>
<td>61/65</td>
<td>95/100</td>
<td>3/63</td>
<td>5.0/5.0</td>
</tr>
<tr>
<td>6</td>
<td>11/F</td>
<td>72/73</td>
<td>95/100</td>
<td>5/66</td>
<td>2/2</td>
</tr>
<tr>
<td>7</td>
<td>7/M</td>
<td>51/67</td>
<td>95/100</td>
<td>2/29</td>
<td>2/2</td>
</tr>
<tr>
<td>8</td>
<td>22/M</td>
<td>52/83</td>
<td>95/100</td>
<td>2/29</td>
<td>2/2</td>
</tr>
<tr>
<td>9</td>
<td>26/F</td>
<td>58/83</td>
<td>95/100</td>
<td>2/29</td>
<td>2/2</td>
</tr>
<tr>
<td>10</td>
<td>27/M</td>
<td>58/83</td>
<td>95/100</td>
<td>2/29</td>
<td>2/2</td>
</tr>
<tr>
<td>11</td>
<td>29/M</td>
<td>58/83</td>
<td>95/100</td>
<td>2/29</td>
<td>2/2</td>
</tr>
<tr>
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<td>29/M</td>
<td>12/30</td>
<td>95/100</td>
<td>2/29</td>
<td>2/2</td>
</tr>
<tr>
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<td>42/F</td>
<td>27/31</td>
<td>95/100</td>
<td>2/29</td>
<td>2/2</td>
</tr>
<tr>
<td>14</td>
<td>49/F</td>
<td>44/102</td>
<td>95/100</td>
<td>2/29</td>
<td>2/2</td>
</tr>
<tr>
<td>15</td>
<td>72/F</td>
<td>85/30</td>
<td>95/100</td>
<td>2/29</td>
<td>2/2</td>
</tr>
<tr>
<td>16</td>
<td>40/F</td>
<td>75/96</td>
<td>95/100</td>
<td>2/29</td>
<td>2/2</td>
</tr>
</tbody>
</table>

*Age at initial assessment. *From initial assessment to diagnosis of hemochromatosis or to most recent assessment if no evidence of iron overload. *Most recent value if no increase in iron stores. *Measures hepatic parenchymal cell stainable iron, graded microscopically on a scale of 0-4 (17). *Hepatic iron concentration divided by age in years (19). *Amount of iron removed by quantitative phlebotomy. *This patient was followed for 6 yr without evidence of disease before initial examination reported here. Early values not available. *Initial value unavailable; patient informed that he was normal. *Diagnosed before age 20 yr; iron stores high for age. *Values in this subject have fluctuated over the 4-yr period. At the time of liver biopsy, TS = 71% and SF = 250 µg/L. *This patient was followed for 6 yr without evidence of disease, before initial examination reported here. Early values not available. TS, transferrin saturation; SF, serum ferritin concentration; NV, not venesected; IBD, inflammatory bowel disease.

Alcohol consumption was greater than 50 g/day in 3 of 17 males (17.6%) for whom information was available and in none of the females. Only 13 of the 51 putative homozygous relatives had symptoms relevant to hemochromatosis. Cirrhosis was found at two biopsies and fibrosis at three biopsies from this group. Of those for whom hepatic iron concentration was measured, 3 had an hepatic iron index of less than 2. One of these, with an hepatic iron index of 1.7, is the sister of the female proband with an index of 1.9. The other two will be discussed. Body iron stores ranged from 2.5-25 g (median, 6; n = 9) in males and from 1.8-12 g (median, 5; n = 6) in females. Again, the lower values were found in young subjects, including subjects 5 and 6 in Table 2, who were aged 12 and 15 yr at diagnosis. Of the 51 subjects in this category, 35 showed clear evidence of increased iron stores on the first investigation, whereas 16 had equivocal results on initial investigation. The relevant details of these 16 subjects are shown in Table 2. Eleven subjects developed evidence of increased iron stores during the period of observation, whereas one (patient 16) has not yet been fully assessed.

Four putative homozygous subjects (patients 8, 13, 14, and 15 in Table 2) did not manifest an increase in body iron stores during the period of prospective evaluation (4-13 yr). Subject 13 (II-3 in reference 25) was from a family that has been reported in detail previously (12). The development of increased iron stores in this subject was delayed because she suffered from severe inflammatory bowel disease, which resulted in her death in 1985 at age 50 yr. However, during a 2-yr period of remission of the bowel disease she manifested an increase in body iron stores (25), consistent with homozygosity for hemochromatosis.

For the remaining three putative homozygous subjects who did not develop progressive iron overload (subjects 8, 14, and 15 in Table 2), one explanation would be genetic recombination resulting in separa-
tion of a hemochromatotic allele from the marker HLA haplotype. In each HLA-identical sibling pair the F13A phenotypes were not identical, indicating that chromosomal crossover had occurred between the HLA-A and F13A loci. These results are given in Table 3. In subject 15, aged 82 yr, evaluation over 16 yr (including 10 yr after normal liver biopsy results and repeated serological indices for iron storage) has indicated no increase in body iron stores during this period. Except for some angina pectoris, she remains well, with no evidence of iron overload. Genetic study of HLA and F13A markers in this family (Table 3) suggests that chromosome 6 recombination has caused the separation of one hemochromatosis allele from the HLA haplotype. This would be consistent with a location for the hemochromatosis disease locus between HLA and F13A. Subject 14 has been evaluated prospectively over a period of 13 yr, including 4 liver biopsies with estimation of hepatic iron concentration (26). The most recent (March 1989) showed a hepatic iron concentration of 50 μmol/g dry weight at age 62 yr (hepatic iron index, 0.8). She shows no evidence of iron overload or progressive increase in iron stores. Segregation of F13A alleles in this family is also consistent with chromosomal recombination between F13A and HLA-DR loci (26). In subject 14, the recombination breakpoint would have been between HLA-DR and the hemochromatosis locus. Because of homozygosity for HLA-A and HLA-B in a parent, it is not possible to determine in this family whether the hemochromatosis locus is telomeric or centromeric to HLA-A. In subject 8 (Table 2), there was also evidence of chromosomal recombination (Table 3) and no progressive increase in iron stores over the 4-yr period of follow-up, although the values for both serum ferritin and transferrin saturation have fluctuated from 135-366 μg/L and from 44%-84%, respectively. In this case, too, the hemochromatotic allele could have segregated with F13A, away from HLA-A, again localizing the hemochromatotic gene telomeric to HLA-A.

Table 3. Genetic Analysis of Predicted Homozygous Relatives Without Increased Iron Stores

<table>
<thead>
<tr>
<th>Family</th>
<th>HLA alleles</th>
<th>F13A alleles</th>
<th>ID no.</th>
<th>HLA alleles</th>
<th>F13A alleles</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>A1 D44 DR4</td>
<td>2-1</td>
<td>0</td>
<td>A1 D44 DR4</td>
<td>1-1</td>
</tr>
<tr>
<td></td>
<td>A3 B7 DR2</td>
<td></td>
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<td>A3 B7 DR2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>A2 B12 DR4</td>
<td>1-1</td>
<td>14</td>
<td>A2 B12 DR4</td>
<td>2-1</td>
</tr>
<tr>
<td></td>
<td>A11 B27 DR4</td>
<td></td>
<td></td>
<td>A11 B27 DR4</td>
<td></td>
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<td>3</td>
<td>A3 B27</td>
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<td>2-1</td>
</tr>
<tr>
<td></td>
<td>A11 B35</td>
<td></td>
<td></td>
<td>A11 B35</td>
<td></td>
</tr>
</tbody>
</table>

*As determined by F13A phenotyping (21). † As listed in Table 2. ID, identification.

Putative Heterozygous Relatives

There were 98 subjects in this group, 49 males and 49 females, whose ages at first examination ranged from 13-70 yr (median, 42 yr) and 15-69 yr (median, 46 yr), respectively. As shown in Figure 1, approximately one third (37/98) showed some phenotypic expression (elevated serum transferrin saturation and/or serum ferritin level) as has been found in previous studies (11,16,27). Twenty-five of these showed increased serum ferritin alone, 6 showed increases in both serum ferritin concentration and transferrin saturation. Alcohol consumption was greater than 50 g/day in 4 of 21 males (19%) and 1 of 25 females (4%) for whom we have this information. Thirteen putative heterozygous subjects whose serum ferritin concentration or transferrin saturation was found to be increased on more than one occasion underwent liver biopsy; the median hepatic iron concentrations that were measured were 39.5 μmol/g dry weight (range, 27-59 μmol/g) in 6 males and 48.4 μmol/g dry weight (range, 28-63 μmol/g) in 4 females and with stainable iron ranging from grades 1-3 (Table 1). Liver biopsy was not considered justified in those heterozygous subjects with normal transferrin saturation and serum ferritin levels, because previous studies in this and other centers have shown that if both these values are repeatedly normal, the probability of the subject having increased iron stores is virtually zero (13,28). Two female putative heterozygotes had iron stores calculated to be 1.5 and 1.8 g after short periods of repeated phlebotomy.

One subject in this group showed marginally increased iron stores based on the biopsy (hepatic iron concentration, 59 μmol/g dry weight; hepatic iron index, 1.1, stainable iron grade, 2), although a liver biopsy performed 10 yr earlier was normal without stainable iron. This subject is known to consume excessive alcohol, and his results are consistent with his being heterozygous for hemochromatosis with a small increase in iron stores associated with excessive alcohol consumption (29). An HLA-identical sister shows no signs of progressively increasing iron stores. A second putative heterozygote had substantial parenchymal cell iron stores (grade 3) at biopsy at age 45 yr and iron stores of 8.3 g removed by repeated phlebotomy. Thus, the latter patient had evidence of increased iron stores although sharing only one HLA haplotype with affected siblings. None of the other 96 putative heterozygous subjects showed any evidence of increasing iron stores during the period of study.

Putative Haplozygous Normal Relatives

Nine male and 10 female subjects designated normal homozygous by HLA typing showed normal transferrin saturation levels, although 4 males and 2
females showed increased levels of serum ferritin (Figure 1). None of these patients underwent liver biopsy. One male had a serum ferritin level of 540 μg/L and a transferrin saturation of 55%, and a biopsy was performed. The hepatic iron concentration was 8 μmol/g dry weight with a hepatic iron index of 0.3. At investigation, the age ranges of these subjects were 20–72 yr (male) and 17–77 yr (female). During the period of follow-up, none of these has shown evidence of increasing iron stores.

Discussion

In this study of 40 families with hemochromatosis, homozygosity for the disease as predicted by HLA typing was correlated with development of iron overload in 47/50 individuals who have been investigated more than once. Amongst these were 11 individuals who developed evidence of increased iron stores during the period of observation and 1 (subject 13 in Table 1) who developed the disease only after control of intestinal blood loss. One of the 98 individuals predicted to be heterozygous for the disease by HLA typing had clear evidence of iron overload upon first assessment. No putative heterozygous subject developed evidence of iron overload consistent with hemochromatosis during the period of follow-up.

Three individuals (2 females and 1 male) who were HLA-identical to affected siblings and therefore predicted to be homozygous for hemochromatosis showed no evidence of increased or increasing iron stores during periods of follow-up of from 4–10 yr when assessed by serial biochemical tests and multiple liver biopsies. Previous studies by the authors have indicated that homozygous subjects accumulate approximately 0.5 g storage iron per yr and that this is reflected in their serum ferritin levels which increase by an average 65 μg/L per yr in the absence of blood loss (13). In contrast, subjects with only one abnormal allele do not develop progressive iron overload even though approximately 25% of them show some phenotypic expression in the biochemical indices (11,15). The clinical picture of these 3 individuals is therefore like that of heterozygotes rather than homozygotes.

There are several possible explanations for this result. First, it is possible that these 3 subjects represent rare nonexpressing homozygotes. There is no evidence of overt or occult blood loss in the 2 females, both postmenopausal since ascertainment, or the young healthy male. In the face of distinctive and readily diagnosable disease in 47/50 putative homozygous individuals, including female siblings and subjects under 20 yr, this explanation seems unlikely. Although other investigators (11) have found that a proportion of female homozygotes do not express the expected clinical or laboratory phenotype of hemochromatosis, it is noteworthy that 43% (20/47) of the affected HLA-identical relatives are female, and that this is not significantly different from the proportion expected (50%) if males and females are equally likely to be affected (x² = 1.04; p > 0.3).

A second possibility is that these families represent a rare form of hemochromatosis that is not HLA-linked. The worldwide association of hemochromatosis with HLA-A3 in populations and with HLA haplotypes in families suggests that the same locus is involved in most or all cases. In 38 of the 40 families in this study the disease was clearly HLA-linked. In addition, data from the extended family of subject 14 (family 23 in reference 22), including a second affected generation, indicate strong linkage with HLA in this family. The families of subjects 8 and 15 are small, and further pedigree analysis is not possible. However, in both cases, the HLA-A3 allele is found to be associated with the disease. Because this allele is in strong linkage disequilibrium with hemochromatosis in all populations studied (2,30), including our own (22), it seems likely that the disease in these two families is HLA-linked. This suggests that there is a single, HLA-linked, hemochromatosis locus, although it is not clear whether there are one or several disease alleles at this locus.

A third explanation, supported by the present results, is that chromosomal recombination had occurred to separate the hemochromatosis gene and the marker HLA allele in these cases. In all 3 cases, phenotyping of F13A indicated that chromosomal recombination had occurred involving the relevant region of chromosome 6. The F13A subunit gene has recently been mapped to bands 6p24–25 (31), placing it distal to HLA-A, and the genetic distance between HLA and F13A seems to be at least 20 centimorgans (32,33), which could span a physical distance of more than 20 million bases. This means that recombination between HLA-A and F13A would be expected to be quite common, especially in the maternal chromosomes. However, if the hemochromatosis allele lies telomeric to HLA-A, a subset of such recombinants will in fact represent a crossover between the HLA markers and the hemochromatosis allele. This would explain the lack of expression of the disease in subjects 8, 14, and 15, who have HLA-identical but F13A-nonidentical affected siblings. The exact location of the hemochromatosis gene is as yet unknown, but the results in the families of subjects 8 and 15 could argue that it lies between HLA-A and F13A.

A further observation of interest in this study was that only 1 of 98 putative heterozygous subjects studied for up to 23 yr showed evidence of increased iron stores, even when there was excessive alcohol consumption. In this group of heterozygotes, there was no clinical or histological evidence of iron overload, with only 1 having marginally increased hepatic iron con-
centration. These data confirm earlier reports suggesting that hemochromatosis is not fully expressed unless two abnormal alleles are present, even with the addition of alcoholism or hemolytic anemias (26,34-36).

One individual who was predicted to be heterozygous showed clinical symptoms of hemochromatosis at initial investigation. This is unlikely to be caused by a homozygous-heterozygous mating, because if this were the case, HLA typing would predict the mother to be a homozygote, and she shows no signs of increasing iron stores or symptoms at age 84 yr (transferrin saturation, 26%; serum ferritin concentration, 105 µg/L; excretion of 0.5 mg iron in 24 h after injection of 0.5 g of desferrioxamine). Linkage of hemochromatosis to HLA haplotype is indicated by examination of other affected members of this family (family 14 in reference 22). If the hemochromatosis locus lies telomeric to HLA-A, this individual probably carries a recombinant haplotype, resulting from a cross-over between HLA-A and the hemochromatotic locus. This family was not informative for studies of F13A phenotype.

Analysis of three families with apparent recombinants (the families of subjects 8 and 15 in Table 3 and of the predicted heterozygote discussed above) shows that the hemochromatotic allele can apparently be separated from the complete HLA haplotype by a chromosomal cross-over. This is consistent with the hypothesis that the hemochromatosis locus is telomeric to HLA-A and therefore outside the HLA region. This location has been suggested by other authors (37). The result with subject 14 (Table 1) shows the hemochromatosis locus segregating away from HLA-DR, also consistent with the notion of a location close to HLA-A, although not eliminating the possibility of a location between HLA-A and HLA-B, as indicated by the results of Edwards et al (38). Resolution of these differences will come from analysis of further genetic markers in the critical region of chromosome 6 and ultimately from the molecular cloning of the gene or genes responsible for the disease.

Approximately one fourth of heterozygotes and of homozygous normal individuals had increased serum ferritin levels, with transferrin saturation less than 50%. This is consistent with the results of our population study (1), which showed that an unexplained elevation of serum ferritin concentration above 300 µg/L occurred in 172 of 704 males and 21 of 627 females. The reasons for this are unclear but may relate to diet. The result for normal homozygotes shows that serum ferritin levels can be increased in the absence of any copies of the hemochromatosis gene. In addition, about one fifth of the patients had transferrin saturation levels of less than 62% in the presence of grossly increased parenchymal iron stores, as shown by biopsy, indicating that this value, which has been used by others to discriminate homozygotes from heterozygotes (3), may be too high to detect all affected individuals. Because transferrin saturation values were not calculated after an overnight fast, it could be expected that some of the present values would be increased by dietary factors, but this does not explain the low values.

In this study, fewer family members predicted to be homozygous for the normal allele at the hemochromatotic locus were found than would be expected. This is probably the result of an ascertainment bias, because these studies have concentrated on families with more than one affected member in one or more generations, resulting in the inclusion of 15 confirmed homozygous-heterozygous matings. It should be noted that the population frequency of the hemochromatosis allele (1-3) is sufficiently high that at least 10% of known homozygotes will marry heterozygotes, with frequencies of affected and heterozygous offspring each of 50%.

This study has shown the practical clinical value of HLA typing in the management of families with hemochromatosis. HLA-identical siblings of affected individuals had lower hepatic iron concentrations and serum ferritin levels at diagnosis and were less likely to have irreversible liver damage. This illustrates the efficiency of obtaining HLA typing of siblings and initiating clinical investigation as early as possible to prevent serious complications. The distinction between homozygotes, heterozygotes, and normal relatives is not otherwise possible in the early stages of the disease because the underlying metabolic defect has not yet been identified. Almost all individuals predicted to be homozygous by HLA typing carry two hemochromatosis alleles and will develop progressive iron overload requiring phlebotomy therapy to maintain body iron stores in the normal range. Relatives who have only one HLA haplotype identical to that in the proband have a low risk of developing iron overload. However, it is important to be aware that misclassification may occasionally occur, either because of chromosomal recombination or a homozygous-heterozygous mating; hence, it is advisable to review putative heterozygotes at regular intervals of 2-3 yr. Relatives who have neither HLA haplotype identical to the proband can be reassured that the risk of developing iron overload is negligible.

References

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Address requests for reprints to: Professor L. W. Powell, Department of Medicine, University of Queensland, Clinical Sciences Building, Royal Brisbane Hospital, Brisbane, Queensland, Australia 4029.

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