

CLINICAL AND BIOCHEMICAL ABNORMALITIES IN PEOPLE HETEROZYGOUS FOR HEMOCHROMATOSIS

ZANETA J. BULAJ, M.D., LINDA M. GRIFFEN, B.A., LYNN B. JORDE, PH.D., CORWIN Q. EDWARDS, M.D.,
AND JAMES P. KUSHNER, M.D.

ABSTRACT

Background Ten percent of whites are heterozygous for the HLA-linked hemochromatosis mutation. We performed a cross-sectional analysis of 1058 genotyped heterozygotes to define the effects of age and sex on the phenotype.

Methods The heterozygous genotype was assigned to 505 male and 553 female members of 202 pedigrees, each with an HLA-typed homozygous proband. We measured serum iron, transferrin saturation, and ferritin in all heterozygotes and in 321 genetically normal subjects (unaffected family members or spouses of family members). Liver biopsies were performed in a subgroup of heterozygotes.

Results The mean serum iron concentrations and transferrin-saturation values were higher in heterozygotes than in normal subjects and did not increase with age. Initial transferrin-saturation levels exceeding the threshold associated with the homozygous genotype were found in 4 percent of male and 8 percent of female heterozygotes. The geometric mean serum ferritin concentration was higher in heterozygotes than in normal subjects and increased with age. Higher-than-normal values were found in 20 percent of male and 8 percent of female heterozygotes. The clinical and biochemical expression of hemochromatosis was more marked in heterozygotes with paternally transmitted mutations than in those with maternally transmitted mutations. Liver-biopsy abnormalities were generally associated with alcohol abuse, hepatitis, or porphyria cutanea tarda.

Conclusions The phenotype of persons heterozygous for hemochromatosis differs from that of normal subjects, but complications due to iron overload alone in these heterozygotes are extremely rare. (N Engl J Med 1996;335:1799-805.)

©1996, Massachusetts Medical Society.

HEMOCHROMATOSIS is transmitted as an autosomal recessive trait.^{1,2} The responsible gene is tightly linked to the HLA class I region on chromosome 6, and a candidate locus has recently been identified.³⁻⁵ The clinical phenotype of skin pigmentation and organ damage occurs only in homozygotes.^{6,7} Penetrance is age-related and may be incomplete, particularly in women.^{1,6,8} In whites, homozygosity occurs with a frequency of 0.005 to 0.008 and heterozygosity with a frequency of 0.100 to 0.130.^{1,3,9}

Linkage of hemochromatosis to HLA permits assignment of the heterozygous genotype to family

members whose HLA haplotypes are 50 percent homologous to that of a homozygous proband.^{10,11} Laboratory abnormalities of iron metabolism have been detected in 15 to 25 percent of heterozygotes, but the frequency of complications due to iron overload has not been defined.^{1,6,10,11} Complications have been recognized only when other disorders, such as porphyria cutanea tarda¹² and chronic anemia,¹³⁻¹⁵ are also present.

Regular measurement of iron stores in heterozygotes would require many decades. The alternative is to identify a large number of heterozygotes and study each one once. Therefore, we studied the clinical and biochemical expression of hemochromatosis in 1058 persons who were heterozygous for the disorder and analyzed the effects of age, sex, sex of the parent transmitting the affected chromosome, and specific HLA haplotypes on iron stores.

METHODS**Informed Consent**

The protocol was approved by the University of Utah Institutional Review Board, and all study subjects gave written informed consent. Subjects who agreed to undergo a liver biopsy received a careful explanation of the risks associated with this procedure and provided written informed consent.

Genotypic Assignment

We studied members of 202 pedigrees identified between 1975 and 1995. In each pedigree there was a well-characterized, HLA-typed homozygous proband. The heterozygous genotype was assigned to 958 pedigree members sharing one HLA haplotype (HLA-A and B) with a homozygous proband. One hundred pedigree members were considered obligate heterozygotes (children or parents of a homozygote) and did not undergo HLA typing.

A normal genotype was assigned to 321 subjects: 140 were related by blood to a homozygous proband but did not share a haplotype with the proband and 181 were married to a family member. The spouses were considered to have a normal genotype if they had a normal transferrin-saturation value and if marriages to genotyped pedigree members produced children with normal transferrin-saturation values.

HLA Typing

Serotyping of HLA-A and B was performed as previously described.¹⁶ The antiserum used was capable of identifying 18 HLA-A and 33 HLA-B alloantigens.

From the Departments of Internal Medicine (Z.J.B., L.M.G., C.Q.E., J.P.K.) and Human Genetics (L.B.J.), University of Utah School of Medicine, and Latter Day Saints Hospital (C.Q.E.) — both in Salt Lake City. Address reprint requests to Dr. Kushner at 4C-416, Division of Hematology-Oncology, University of Utah School of Medicine, Salt Lake City, UT 84132.

Blood Tests of Iron Stores

Serum iron and transferrin saturation were measured as previously described.¹⁷ When possible, samples were obtained while subjects were fasting. Serum ferritin was measured with radioimmunoassay kits (Ramco Laboratories, Houston).

Liver Biopsies

Hepatic iron was assessed by light microscopy (graded on a scale of 0 to 4 according to the method of Scheuer et al.¹⁸) and by atomic absorption spectrophotometry.¹⁷ The normal value for hepatocellular stainable iron is grade 0 or 1. Normal values for hepatic iron concentration are less than 25 μmol per gram of dry weight.¹⁹

Statistical Analysis

Statistical analyses used the Prophet computer software package (Bolt Beranek and Newmann, Cambridge, Mass.). Measurements of serum iron and transferrin saturation were compared between groups of subjects with Student's t-test (when normally distributed) or the Mann-Whitney U test (when not normally distributed). Serum ferritin values were analyzed after logarithmic transformation in order to achieve a normal distribution. The Shapiro-Wilk test was used to test for the normality of the distribution. Chi-square analysis was used to compare the frequencies of iron deficiency in heterozygotes and normal subjects. Haplotype frequencies in normal and affected chromosomes were compared with Fisher's exact test. All statistical tests were two-sided.

RESULTS

Phenotypic Expression of Hemochromatosis

The subjects were divided into three groups according to age: 1 to 30, 31 to 60, and 61 to 90 years. Values for serum iron, transferrin saturation, and serum ferritin are shown in Table 1. The mean serum iron concentration and mean percentage of saturation of transferrin were higher in heterozygotes than in normal subjects in all age groups and did not increase with age. The mean values for transferrin saturation in the normal subjects closely approximated those reported by McLaren et al. in a population that excluded subjects with iron deficiency or iron overload and was studied as part of the Second National Health and Nutrition Examination Survey (NHANES II).²⁰ A frequency distribution plot of transferrin-saturation values in heterozygotes and normal subjects illustrates the shift toward higher values in heterozygotes (Fig. 1).

Transferrin saturation was determined in the fasting state in approximately two thirds of subjects; when samples obtained at random times yielded values higher than 50 percent, additional samples were obtained while the subjects were fasting. When val-

TABLE 1. IRON VALUES IN 1058 SUBJECTS WHO WERE HETEROZYGOUS FOR HEMOCHROMATOSIS AND IN 321 NORMAL SUBJECTS.*

SEX AND AGE GROUP	SERUM IRON (RANGE)			TRANSFERRIN SATURATION (RANGE)			GEOMETRIC AND ARITHMETIC MEAN SERUM FERRITIN (95% CI)‡		
	HETEROZY-GOTES	NORMAL SUBJECTS	P VALUE	HETEROZY-GOTES	NORMAL SUBJECTS	P VALUE	HETEROZY-GOTES	NORMAL SUBJECTS	P VALUE
	$\mu\text{g/dl}\dagger$			%			$\mu\text{g/liter}$		
Males									
1-30 yr	132±47 (36-309)	111±46 (30-264)	0.005§	38±14 (7-82)	29±11 (10-58)	<0.001§	49/82 (43-56)	37/59 (26-50)	0.09¶
31-60 yr	124±41 (42-246)	108±35 (21-231)	0.006§	37±12 (10-85)	30±9 (4-58)	<0.001¶	131/181 (115-144)	60/83 (48-75)	<0.001¶
61-90 yr	117±38 (33-226)	99±27 (54-150)	0.009¶	38±14 (9-81)	30±8 (16-45)	<0.001¶	130/204 (103-160)	116/162 (84-157)	0.60¶
Females									
1-30 yr	118±47 (24-276)	98±37 (36-192)	0.01¶	33±13 (6-81)	26±11 (10-53)	0.003¶	25/37 (22-28)	27/46 (19-37)	0.80¶
31-60 yr	103±42 (6-240)	93±44 (15-225)	0.02§	30±13 (2-77)	25±12 (3-57)	0.002§	35/62 (30-39)	24/40 (21-26)	0.001¶
61-90 yr	103±37 (28-195)	90±31 (36-171)	0.04¶	31±13 (7-71)	25±10 (5-51)	0.008¶	79/132 (61-98)	48/73 (35-66)	0.016¶

*Plus-minus values are means \pm SD. There were 210 male heterozygotes who were 1 to 30 years of age, 209 who were 31 to 60 years of age, and 86 who were 61 to 90 years of age. There were 38 normal male subjects who were 1 to 30 years of age, 66 who were 31 to 60 years of age, and 28 who were 61 to 90 years of age. There were 201 female heterozygotes who were 1 to 30 years of age, 260 who were 31 to 60 years of age, and 92 who were 61 to 90 years of age. There were 40 normal female subjects who were 1 to 30 years of age, 105 who were 31 to 60 years of age, and 44 who were 61 to 90 years of age.

†To convert values for serum iron concentrations to micromoles per liter, multiply by 0.178.

‡The geometric mean values and 95 percent confidence intervals (CI) were obtained after logarithmic transformation.

§The P value was calculated with the Mann-Whitney U test.

¶The P value was calculated with Student's t-test.

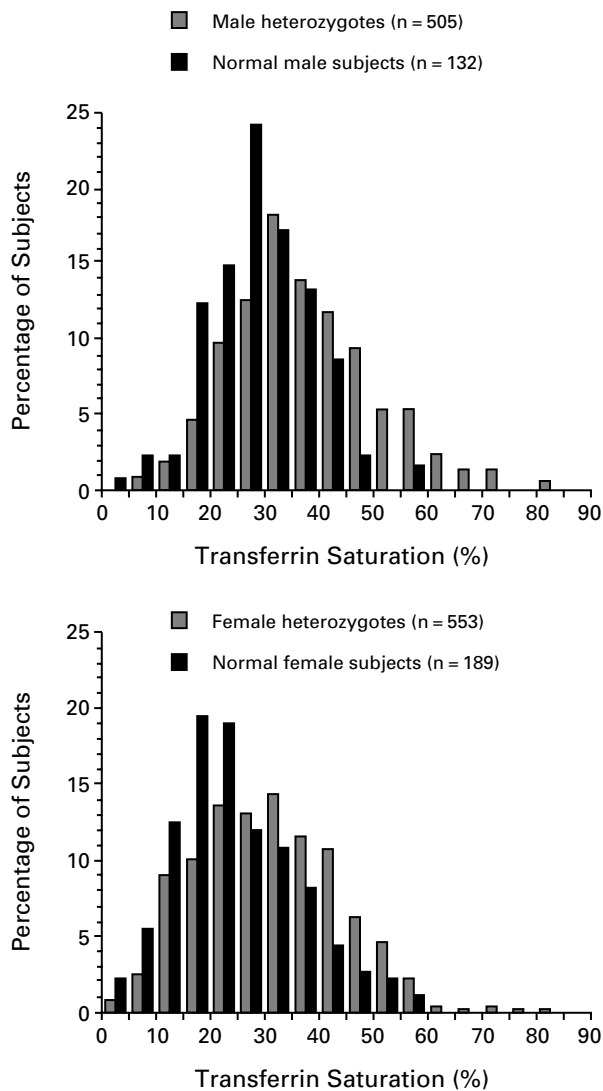


Figure 1. Distribution of Transferrin-Saturation Values in Subjects Heterozygous for Hemochromatosis and Normal Subjects, According to Sex.

ues obtained during fasting were compared between heterozygotes of either sex and normal subjects, the differences between groups remained the same (data not shown). The proportion of samples obtained during fasting was approximately the same in heterozygotes and normal subjects. We measured transferrin saturation every 2 hours for 24 hours in 19 heterozygotes and 10 normal subjects. The differences between groups remained roughly constant at all times (data not shown).

Four percent of the male heterozygotes (22 of 505) had an initial transferrin-saturation level of more than 62 percent (range, 63 to 85 percent), a threshold associated with the homozygous genotype in men.²⁰⁻²² Thirteen of these heterozygotes were

available for repeated testing in the fasting state; the values recorded during repeated testing were all 62 percent or less (range, 32 to 62 percent). Eight percent (44 of 553) of the female heterozygotes had an initial transferrin-saturation level of more than 50 percent, a threshold associated with the homozygous genotype in women.²² Thirteen of these subjects were available for repeated testing while fasting; in two the transferrin-saturation level still exceeded 50 percent (56 percent and 57 percent). Two of 132 normal men (2 percent) and 6 of 189 normal women (3 percent) had transferrin-saturation levels exceeding 50 percent.

The geometric mean serum ferritin concentration was higher in heterozygotes of both sexes than in normal subjects, with the exception of the youngest group of female subjects (Table 1). This difference was significant only among men 31 to 60 years of age and among women in the two older age groups (31 to 60 and 61 to 90 years of age). Twenty percent of male heterozygotes (101 of 505) had ferritin concentrations that exceeded the 95th percentile value for the age-matched male controls. The highest value recorded was 988 μg per liter in a 21-year-old man; his transferrin-saturation level was 58 percent. The geometric mean serum ferritin concentration in male heterozygotes rose gradually until the sixth decade and declined slightly thereafter (Fig. 2). Eight percent of female heterozygotes (46 of 553) had ferritin values that exceeded the 95th percentile value for the age-matched female controls. The highest value recorded was 737 μg per liter in a 64-year-old woman; her transferrin-saturation level was 49 percent. In heterozygous women the geometric mean serum ferritin concentration rose until the seventh decade (Fig. 2).

Iron deficiency has been defined as a serum ferritin concentration below 12 μg per liter.²³ Among the female subjects of reproductive age (12 to 50 years), ferritin concentrations below 12 μg per liter were found in 72 of 350 heterozygotes (21 percent) and in 35 of 108 normal subjects (32 percent, $P=0.02$). Among men over 18 years of age, serum ferritin concentrations below 12 μg per liter were found in 7 of 374 heterozygotes (2 percent) and 4 of 108 normal subjects (4 percent, $P=0.44$).

Liver Biopsy

When our studies began, the histology of the liver in people heterozygous for hemochromatosis had not been studied, and the relation between hepatic iron stores and serum ferritin concentrations had not been defined. We performed percutaneous liver biopsies in 39 heterozygotes. Seventeen (12 male and 5 female subjects; ages, 8 to 65 years) had normal transferrin-saturation levels and normal serum ferritin concentrations. The mean hepatic iron concentration was 23 μmol per gram of dry weight. The

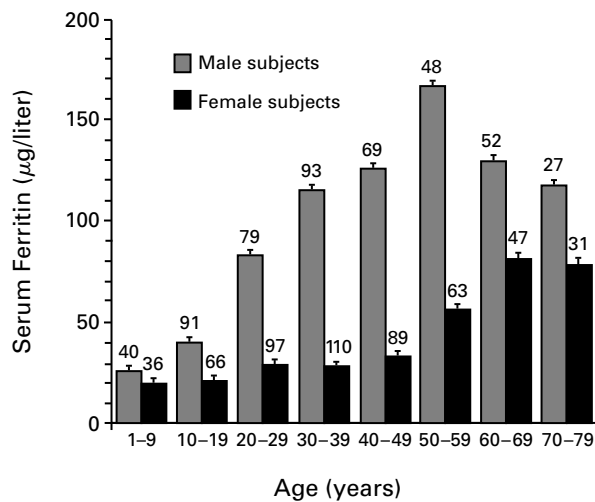


Figure 2. Effect of Age on the Serum Ferritin Concentration in Subjects Heterozygous for Hemochromatosis.

The geometric mean values (\pm SD) are shown. The numbers above the bars are the number of heterozygotes in each group. Data were not included for the four men and two women who were 80 to 90 years of age.

hepatocellular stainable-iron grade ranged from 0 to 2, with a mean of 0.82. Only two biopsy specimens were scored as grade 2. One was obtained from an 8-year-old boy with hereditary spherocytosis, the other from a healthy 65-year-old man. Two liver biopsies showed evidence of liver damage. The liver biopsy of a 36-year-old woman showed fibrosis and cirrhosis associated with steatohepatitis with a chronic inflammatory infiltrate. Serologic studies for both hepatitis B and C were negative. The stainable-iron grade was 0, and the iron concentration was only 9 μ mol per gram of dry weight. The liver biopsy of the healthy 65-year-old man with grade 2 hepatocellular iron showed mild periportal fibrosis without an inflammatory infiltrate. This man did not consume alcohol and had no laboratory evidence of hepatitis B or C.

The other 22 heterozygotes (18 male and 4 female subjects; age, 17 to 71 years) who underwent biopsy had elevated transferrin-saturation levels, increased serum ferritin concentrations, or both. The mean hepatic iron concentration was 32 μ mol per gram of dry weight. The stainable-iron grade ranged from 0 to 3, with a mean of 1.3. With two exceptions, the highest iron grades (grades 2 and 3) occurred in heterozygotes with serum ferritin concentrations of more than 300 μ g per liter. The three biopsy specimens scored as grade 3 were obtained from heterozygotes who drank more than 70 g of alcohol daily. Two of these subjects had porphyria cutanea tarda: a 32-year-old woman without laboratory evidence of previous exposure to either hepatitis B or C and a 51-year-old man with a positive test for hepatitis B

surface antibody but no laboratory evidence of exposure to hepatitis C. Six biopsy specimens — all from men — were scored as grade 2. Four of these men (age, 21 to 61 years) drank more than 70 g of alcohol daily. The mean iron concentration associated with grade 2 and 3 iron overload was 47 μ mol per gram of dry weight. Fibrosis was found in the specimens from both patients with porphyria cutanea tarda and in two of the six specimens scored as grade 2. One of these, from a 54-year-old man who drank heavily, also revealed cirrhosis. Serologic studies for hepatitis C antibody were positive. The other fibrotic specimen was from a 59-year-old man with alcoholic hepatitis and no evidence of hepatitis B or C.

HLA Haplotypes in Heterozygotes

The two haplotypes identified in the homozygous proband in each pedigree were considered to be linked to the hemochromatosis locus. The haplotype shared with the proband identified the affected chromosome in heterozygotes, and the unshared haplotype was considered to represent a normal chromosome. In each pedigree an affected or normal haplotype was counted only once. Three hundred forty-five affected chromosomes and 835 normal chromosomes were identified. Additional normal haplotypes were found in the genotypically normal spouses of family members. HLA-A3 is the most common HLA-A allele found in chromosomes bearing the hemochromatosis mutation, and HLA-A3,B7 is the most common HLA haplotype on these chromosomes. As expected, the HLA-A3 allele was overrepresented in affected chromosomes, and HLA-A3,B7 was the most common affected haplotype in the subjects we studied (Table 2).

We found no significant difference in the geometric mean serum ferritin concentrations between heterozygotes with an HLA-A3 allele and those with any other HLA-A allele (data not shown).

Effect of the Parental Genotype on the Phenotypic Expression of Hemochromatosis in Heterozygotes

HLA haplotyping was performed in the parents of 650 heterozygotes whose haplotypes were known. This permitted an analysis of the effect of the parental genotype on the phenotypic expression of hemochromatosis in heterozygotes (Table 3). The only component of the phenotype that is age-dependent is the serum ferritin concentration (Fig. 2 and Table 1). An affected paternal chromosome had a greater effect than an affected maternal chromosome on the phenotype in female heterozygotes of all ages. Female heterozygotes who inherited the affected paternal chromosome were similar in age to those who inherited the affected maternal chromosome, excluding an effect of age on the phenotype. The same general pattern was seen in heterozygous men, although the differences were not statistically significant.

DISCUSSION

The phenotype of our large group of people heterozygous for hemochromatosis clearly differed from that characteristic of normal subjects, but many of the heterozygotes were indistinguishable from normal subjects. Genotypic assignment within pedigrees was rigorous, but rare homozygotes may have been misclassified as heterozygotes if penetrance of the homozygous phenotype in the parent of a proband was incomplete. Misclassification of the offspring of probands may also have occurred if a phenotypically normal spouse was a heterozygote. Such misclassifications of homozygotes as heterozygotes would make any estimates of differences between normal subjects and heterozygotes less conservative.

The assignment of a normal genotype was also subject to a very small rate of error; some spouses of family members may have been heterozygotes misclassified as normal subjects. Misclassifying normal subjects as heterozygotes would make any estimates of differences in the phenotype between normal and heterozygous subjects more conservative.

We found that 18 percent of male heterozygotes and 11 percent of female heterozygotes had transferrin-saturation levels that were more than 2 SD above the mean values for normal subjects. Only 2 percent of the normal men and 3 percent of the normal women had transferrin-saturation levels exceeding 50 percent. We previously suggested that a reasonable threshold for the transferrin-saturation level indicating the homozygous genotype in women is 50 percent.²² Eight percent of the female heterozygotes had an initial transferrin-saturation level exceeding that threshold. Thus, we confirmed our earlier finding¹ that transferrin-saturation levels above the threshold associated with the homozygous genotype occur infrequently in heterozygotes.

The serum ferritin concentration serves as a useful index of body iron content.^{6,8,24} Twenty percent of male heterozygotes and 8 percent of female heterozygotes had serum ferritin concentrations that exceeded the 95th percentile values in our normal population. Values obtained in our normal population were similar to those reported in NHANES II.²⁵ The increased body iron burden in heterozygotes appears to have minimal consequences, but there have been very few liver biopsies in clinically normal heterozygotes. Most liver biopsies reported previously were performed in people with abnormal liver function or a history of heavy alcohol use.^{10,11} We performed biopsies in heterozygotes with abnormal phenotypes and normal phenotypes. Six of 39 biopsy specimens showed hepatic damage — fibrosis in 5 and fibrosis and cirrhosis in 1. Associated conditions (hepatitis, porphyria cutanea tarda, and alcoholism) were identified in five of the six patients, supporting the concept that heterozygosity for he-

TABLE 2. HLA HAPLOTYPES OF 345 HEMOCHROMATOSIS CHROMOSOMES AND 835 NORMAL CHROMOSOMES.

HAPLOTYPE*	HEMOCHROMATOSIS CHROMOSOMES (N=345)	NORMAL CHROMOSOMES (N=835)	P VALUE†
	no. with listed alleles (frequency of haplotype)		
A3	126 (37)	96 (11.5)	<0.001
A3, B7	82 (24)	41 (5.0)	<0.001
A3, B14	15 (4.4)	5 (0.6)	<0.001
A3, B62	7 (2.0)	3 (0.4)	0.008
A3, B44	9 (2.6)	6 (0.7)	0.01
A3, B51	5 (1.5)	2 (0.2)	0.02
A1, B8	18 (5.2)	62 (7.4)	0.20
A1, B57	8 (2.3)	5 (0.6)	0.02
A2, B7	16 (4.6)	32 (3.8)	0.51
A2, B44	15 (4.4)	43 (5.1)	0.65
A2, B51	2 (0.6)	14 (1.7)	0.17
A2, B62	11 (3.2)	21 (2.5)	0.55
A2, B60	2 (0.6)	16 (1.9)	0.11
A11, B35	2 (0.6)	18 (2.2)	0.07
A28, B44	7 (2.0)	11 (1.3)	0.43
A29, B44	7 (2.0)	15 (1.8)	0.81

*Haplotypes that were identified in less than 1 percent of normal or affected chromosomes are not shown.

†The P values were calculated with Fisher's exact test.

TABLE 3. EFFECT OF THE PARENTAL GENOTYPE ON THE PHENOTYPE OF HETEROZYGOES.*

VARIABLE	MATERNAL HEMOCHROMATOSIS CHROMOSOME	PATERNAL HEMOCHROMATOSIS CHROMOSOME	P VALUE
No. of males	132	178	
Mean age — yr	28	29	
No. of females	144	196	
Mean age — yr	30	30	
Serum iron — μg/dl‡			
Males	126±43	131±49	0.54‡
Females	103±44	114±46	0.04‡
Transferrin saturation — %			
Males	36±13	39±14	0.16§
Females	29±12	33±12	0.003§
Geometric mean serum ferritin — μg/liter (95% CI)			
Males	72 (61–86)	74 (64–86)	0.78§
Females	23 (19–27)	29 (26–34)	0.03§

*Plus-minus values are means ±SD. CI denotes confidence interval.

‡To convert values for serum iron concentration to micromoles per liter, multiply by 0.178.

‡The P value was calculated with the Mann-Whitney U test.

§The P value was calculated with Student's t-test.

mochromatosis is rarely associated with liver damage due to iron overload alone.

The HLA-A3 allele is found more often than would be expected by chance in patients with hemochromatosis, suggesting that an initial mutation causing hemochromatosis occurred on a chromosome bearing the HLA-A3 allele.⁴ This possibility has been supported by the demonstration of a preserved HLA-A3-associated haplotype and specific alleles of multiple markers distal to the HLA class I region.^{5,26,27}

The presence of hemochromatosis mutations on chromosomes not bearing the HLA-A3 allele might be explained either by the occurrence of new mutations or by genetic recombination. Crawford et al. suggested that in heterozygous women an HLA-A3-associated mutation has a greater effect on the phenotype than do mutations associated with other HLA-A alleles.²⁸ We found no difference in the phenotype of heterozygotes of either sex between those with an HLA-A3-associated mutation and those without such a mutation. The recombination hypothesis is supported by the preservation of a segment of the HLA-A3-associated haplotype on chromosomes with the hemochromatosis mutation but no HLA-A3 allele. The conserved region is telomeric to the class I region.^{5,26}

The high frequency of the hemochromatosis mutation suggests that it has been preserved because it may have once represented an advantage rather than a liability.^{4,28} The mutation may still offer a selective advantage to heterozygous women. For example, women who are less likely to have iron deficiency may have a reproductive advantage. This concept is supported by our finding that low serum ferritin concentrations were significantly less common in heterozygous women than in normal women.

Paternal hemochromatosis alleles had a greater effect on the phenotype of heterozygotes than maternal alleles, suggesting a parent-of-origin effect of this gene. Parent-of-origin effects ("imprinting") are well established for several diseases, including the Prader-Willi and Angelman's syndromes,²⁹ Wilms' tumor,³⁰ insulin-dependent diabetes,³¹ and bipolar affective disorders.³² Parent-of-origin effects are often associated with modifications of DNA, including methylation and condensation of chromatin.³³ The molecular mechanism responsible for a parent-of-origin effect in hemochromatosis can be studied once the gene has been confirmed.⁵

Screening for persons who are homozygous for hemochromatosis is cost effective.³⁴ A simple, effective therapy (repeated phlebotomies) can be used before organ damage occurs. The transferrin-saturation level has proved to be highly reliable in identifying homozygotes.^{1,8,21} Our data emphasize that very few heterozygotes will be misclassified as homozygotes when appropriate threshold values for transferrin saturation are used. When it is not possible to

determine with certainty whether a person is heterozygous or homozygous, it seems prudent to recommend therapeutic phlebotomies until iron depletion has been achieved.

Supported by grants (DK 20630 and RR 00064) from the National Institutes of Health.

We are indebted to Bernard La Salle for management of the computerized data base and to the staff of the General Clinical Research Center at the University of Utah for their invaluable assistance.

REFERENCES

1. Edwards CQ, Dadone MM, Skolnick MH, Kushner JP. Hereditary haemochromatosis. *Clin Haematol* 1982;11:411-35.
2. Simon M, Bourel M, Genetet B, Fauchet R. Idiopathic hemochromatosis: demonstration of recessive transmission and early detection by family HLA typing. *N Engl J Med* 1977;297:1017-21.
3. Edwards CQ, Skolnick MH, Kushner JP. Hereditary hemochromatosis: contributions of genetic analyses. *Prog Hematol* 1981;12:43-71.
4. Simon M, Le Mignon L, Fauchet R, et al. A study of 609 HLA haplotypes marking for the hemochromatosis gene: (1) mapping of the gene near the HLA-A locus and characters required to define a heterozygous population and (2) hypothesis concerning the underlying cause of hemochromatosis-HLA association. *Am J Hum Genet* 1987;41:89-105.
5. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 1996;13:399-408.
6. Cartwright GE, Edwards CQ, Kravitz K, et al. Hereditary hemochromatosis: phenotypic expression of the disease. *N Engl J Med* 1979;301:175-9.
7. Edwards CQ. Hemochromatosis and other iron storage disorders. In: Lee GR, Bithell TC, Foerster J, Athens JW, Lukens JN, eds. *Wintrobe's clinical hematology*. 9th ed. Vol. 1. Philadelphia: Lea & Febiger, 1993:872-84.
8. Edwards CQ, Griffen LM, Goldgar D, Drummond C, Skolnick MH, Kushner JP. Prevalence of hemochromatosis among 11,065 presumably healthy blood donors. *N Engl J Med* 1988;318:1355-62.
9. Edwards CQ, Griffen LM, Kushner JP. Southern Blood Club Symposium: an update on selected aspects of hemochromatosis. *Am J Med Sci* 1990;300:245-50.
10. Adams PC. Prevalence of abnormal iron studies in heterozygotes for hereditary hemochromatosis: an analysis of 255 heterozygotes. *Am J Hematol* 1994;45:146-9.
11. Bassett ML, Halliday JW, Powell LW. HLA typing in idiopathic hemochromatosis: distinction between homozygotes and heterozygotes with biochemical expression. *Hepatology* 1981;1:120-6.
12. Edwards CQ, Griffen LM, Goldgar DE, Skolnick MH, Kushner JP. HLA-linked hemochromatosis alleles in sporadic porphyria cutanea tarda. *Gastroenterology* 1989;97:972-81.
13. Edwards CQ, Skolnick MH, Kushner JP. Coincidental nontransfusional iron overload and thalassemia minor: association with HLA-linked hemochromatosis. *Blood* 1981;58:844-8.
14. Cartwright GE, Edwards CQ, Skolnick MH, Amos DB. Association of HLA-linked hemochromatosis with idiopathic refractory sideroblastic anemia. *J Clin Invest* 1980;65:989-92.
15. Fargion S, Cappellini MD, Piperno A, Panajotopoulos N, Ronchi G, Fiorelli G. Association of hereditary spherocytosis and idiopathic hemochromatosis: a synergistic effect in determining iron overload. *Am J Clin Pathol* 1986;86:645-9.
16. Ray JG Jr, Hare DB, Pederson PD, Mullally DI. NIH lymphocyte microcytotoxicity technique. In: *NIAID manual of tissue typing techniques*. Bethesda, Md.: National Institute of Allergy and Infectious Diseases, 1976: 22-4. (DHEW publication no. (NIH) 76-545.)
17. Edwards CQ, Carroll M, Bray P, Cartwright GE. Hereditary hemochromatosis: diagnosis in siblings and children. *N Engl J Med* 1977;297:7-13.
18. Scheuer PJ, Williams R, Muir AR. Hepatic pathology in relatives of patients with haemochromatosis. *J Pathol Bacteriol* 1962;84:53-64.
19. Witte DL, Crosby WH, Edwards CQ, Fairbanks VF, Mitros FA. Hereditary hemochromatosis. *Clin Chim Acta* 1996;245:139-200.
20. McLaren CE, Gordeuk VR, Looker AC, et al. Prevalence of heterozygotes for hemochromatosis in the white population of the United States. *Blood* 1995;86:2021-7.
21. Dadone MM, Kushner JP, Edwards CQ, Bishop DT, Skolnick MH.

Hereditary hemochromatosis: analysis of laboratory expression of the disease by genotype in 18 pedigrees. *Am J Clin Pathol* 1982;78:196-207.

22. Edwards CQ, Kushner JP. Screening for hemochromatosis. *N Engl J Med* 1993;328:1616-20.
23. Cook JD. Clinical evaluation of iron deficiency. *Semin Hematol* 1982; 19:6-18.
24. Valberg LS, Lloyd DA, Ghent CN, et al. Clinical and biochemical expression of the genetic abnormality in idiopathic hemochromatosis. *Gastroenterology* 1980;79:884-92.
25. Cook JD, Skikne BS, Lynch SR, Reusser ME. Estimates of iron sufficiency in the US population. *Blood* 1986;68:726-31.
26. Raha-Chowdhury R, Bowen DJ, Burnett AK, Worwood M. Allelic associations and homozygosity at loci from HLA-B to D6S299 in genetic haemochromatosis. *J Med Genet* 1995;32:446-52.
27. Jazwinska EC, Pyper WR, Burt MJ, et al. Haplotype analysis in Australian hemochromatosis patients: evidence for a predominant ancestral haplotype exclusively associated with hemochromatosis. *Am J Hum Genet* 1995;56:428-33.
28. Crawford DHG, Powell LW, Leggett BA, et al. Evidence that the ancestral haplotype in Australian hemochromatosis patients may be associated with a common mutation in the gene. *Am J Hum Genet* 1995;57: 362-7.
29. Buiting K, Saitoh S, Gross S, et al. Inherited microdeletions in the Angelman and Prader-Willi syndromes define an imprinting centre on human chromosome 15. *Nat Genet* 1995;9:395-400. [Erratum, *Nat Genet* 1995; 10:249.]
30. Rainier S, Johnson LA, Dobry CJ, Ping AJ, Grundy PE, Feinberg AP. Relaxation of imprinted genes in human cancer. *Nature* 1993;362:747-9.
31. Margaritte-Jeannin P, Clerget-Darpoux F, Hors J, Deschamps I. Testing parental imprinting in insulin-dependent diabetes mellitus by the marker-association-segregation- χ^2 method. *Am J Hum Genet* 1995;56: 1080-7.
32. McMahon FJ, Stine OC, Meyers DA, Simpson SG, DePaulo JR. Patterns of maternal transmission in bipolar affective disorder. *Am J Hum Genet* 1995;56:1277-86.
33. Barlow DP. Gametic imprinting in mammals. *Science* 1995;270:1610-3.
34. Balan V, Baldus W, Fairbanks V, Michels V, Burritt M, Klee G. Screening for hemochromatosis: a cost-effectiveness study based on 12,258 patients. *Gastroenterology* 1994;107:453-9.