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## *HFE* Genotype in Patients with Hemochromatosis and Other Liver Diseases

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**Background:** Hereditary hemochromatosis is a common inherited disorder of iron metabolism. The gene *HFE*, which contains two missense mutations (C282Y and H63D), was recently identified.

**Objective:** To determine how *HFE* genotyping for the C282Y and H63D mutations contributes to the diagnosis of hemochromatosis and to determine the prevalence of *HFE* mutations in a group of patients with liver disease.

**Design:** Cross-sectional study.

**Setting:** Academic medical center.

**Patients:** 66 patients with hereditary hemochromatosis and 132 referred patients with other liver diseases.

**Measurements:** At initial diagnosis, fasting transferrin saturation, ferritin level, routine chemistry panel, and complete blood count were determined. Percutaneous liver biopsy was done on all patients for histologic analysis and measurement of hepatic iron concentration and hepatic iron index. *HFE* genotyping for the C282Y and H63D mutations was done on all patients by using genomic DNA samples.

**Results:** Of the 66 patients with hemochromatosis diagnosed on the basis of serum iron studies and liver biopsy findings, 60 (91%) were C282Y homozygotes, 2 (3%) were compound heterozygotes, 1 (1.5%) was a C282Y heterozygote, 2 (3%) were H63D heterozygotes, and 1 (1.5%) was negative for both mutations. Of the 132 patients with liver disease, 6 (5%) were C282Y homozygotes, 8 (6%) were compound heterozygotes, 6 (5%) were C282Y heterozygotes, 5 (4%) were H63D homozygotes, 20 (15%) were H63D heterozygotes, and 87 (66%) were negative for both mutations. All 66 C282Y homozygotes had an elevated hepatic iron concentration, and 65 of the 66 patients (98%) had a transferrin saturation of at least 45%. Ten of the 66 patients (15% [95% CI, 7.5% to 26%]) had a hepatic iron index less than 1.9 mmol/kg per year; hemochromatosis was not suspected in 6 of the 10 patients before genotyping. Cirrhosis or substantial hepatic fibrosis was not seen in any (0% [CI, 0% to 18%]) of the 19 patients younger than 40 years of age who were homozygous for the C282Y mutation.

**Conclusions:** All 66 patients homozygous for the C282Y mutation of *HFE* had an elevated hepatic iron concentra-

tion, but approximately 15% of these patients did not meet a previous diagnostic criterion for hemochromatosis (hepatic iron index > 1.9 mmol/kg per year). Determination of *HFE* genotype is clinically useful in patients with liver disease and suspected iron overload and may lead to identification of otherwise unsuspected C282Y homozygotes.

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Hereditary hemochromatosis is a common inherited disorder of iron metabolism that affects between 1 in 200 and 1 in 400 persons of northern European descent (1). With early diagnosis and appropriate treatment, survival of patients is normal (1, 2). Recently, researchers identified a novel MHC class 1-like gene, *HFE*, which contains two missense mutations (3). Eighty-three percent of 178 typical patients with hemochromatosis were homozygous for one of these mutations (Cys282→Tyr [C282Y]) (3). Subsequent studies from the United States, Australia, France, and Italy showed homozygosity for the C282Y mutation in 64% to 100% of patients with hemochromatosis (4–7). Heterozygosity for the second mutation (His63→Asp [H63D]) is seen in 15% to 20% of the general population; this mutation is not believed to cause the same extent of progressive iron loading (3–7). The estimated allelic frequency of these mutations in white populations is 0.04 for the C282Y mutation and 0.14 for the H63D mutation (1). The presence of C282Y homozygosity and direct (elevated hepatic iron concentration) or indirect (elevated transferrin saturation or ferritin

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level) evidence of increased iron stores constitute the current gold standard for a definitive diagnosis of hemochromatosis (1, 8).

Approximately 40% to 50% of patients with alcoholic liver disease (9), chronic viral hepatitis (10), and nonalcoholic steatohepatitis (11) have abnormal results on blood iron studies. About 5% to 10% of these patients have a modestly increased hepatic iron concentration, but not to the degree seen in typical patients with hemochromatosis. Clinicians have suspected that some of these patients are heterozygous for hemochromatosis; however, without pedigree studies (using HLA haplotyping) of a family with hemochromatosis, this interpretation has been only speculative (10, 11). A high prevalence of C282Y heterozygosity was found in patients with nonalcoholic steatohepatitis (12, 13). In patients with hepatitis C (14–16) and patients with alcoholic liver disease (17), researchers have found an incidence of C282Y heterozygosity equivalent to that in control populations.

Recently, a genetic test for hemochromatosis that analyzes the C282Y and H63D mutations has become available. This test allows genotyping of patients who have typical hemochromatosis and those who have liver disease with or without abnormal results on iron studies. We evaluated the contribution of *HFE* genotyping to the diagnosis of hemochromatosis and determined the prevalence of *HFE* mutations in a group of patients with liver disease.

## Methods

### Patients with Hemochromatosis

Between September 1990 and September 1997, clinical hemochromatosis was newly diagnosed in 66 patients by using one of two criteria: 1) a compatible result on liver biopsy (iron deposits of 2+, 3+, or 4+, predominantly in hepatocytes) and a hepatic iron index greater than 1.9 mmol/kg per year (18–22) or 2) HLA identity to a proband. A subset of these 66 patients ( $n = 44$ ) was included in a previous study (3) that identified the *HFE* gene.

### Patients with Liver Disease

Between January 1996 and September 1997, we performed *HFE* genotyping on 132 patients with various liver diseases for whom we had also obtained a hepatic iron concentration. Nineteen of these patients were referred for suspected hemochromatosis on the basis of abnormal results on iron studies. The cause of liver disease was thoroughly evaluated in these 132 patients by examination of history of alcohol consumption, viral serologic studies for hepatitis B and C, autoimmune

markers (antinuclear antibody, anti-smooth-muscle antibody, and antimitochondrial antibody), and markers of inherited metabolic diseases (transferrin saturation, ferritin level, ceruloplasmin level, and  $\alpha_1$ -antitrypsin level and protein typing).

### Laboratory Studies

To aid in initial diagnosis, fasting transferrin saturation (reference range, 0.16 to 0.5), ferritin level (reference range, 15 to 200  $\mu\text{g/L}$  in women and 30 to 300  $\mu\text{g/L}$  in men), routine chemistry panel, and complete blood count were obtained for all patients. Specific serologic studies were also done, as appropriate. All studies were performed at routine clinical laboratories.

After obtaining informed consent from all patients, we performed standard percutaneous liver biopsy. Sections of liver tissue were prepared in a routine manner. Staining was done with hematoxylin and eosin, the periodic acid–Schiff test after diastase digestion, Masson trichrome stain, Sweet reticulin stain, and Perls Prussian blue stain. Iron deposits in hepatocytes were graded from 0 to 4+ (23). Hepatic iron concentration was measured on a portion of the liver biopsy sample, as described by Torrance and Bothwell (24). The upper limit of normal used in our laboratory is 26.8 mmol/kg dry weight (1500  $\mu\text{g/g}$  dry weight). Hepatic iron index was calculated as hepatic iron concentration (mmol/kg) divided by age (years) (18).

*HFE* genotyping for the C282Y and H63D mutations was performed by oligonucleotide ligation assays on polymerase chain reaction–amplified genomic samples of DNA taken from each patient (3). After obtaining informed consent, we drew blood for *HFE* genotyping from all patients with liver disease and from all patients with hemochromatosis (before the test became commercially available) by using protocols approved by the institutional review board of Saint Louis University.

### Statistical Analysis

Data are presented as the median and range. Statistical comparisons among the cumulative distributions of the groups were performed by using the exact Komolgorov–Smirnov two-sample test. A *P* value less than 0.01 was considered statistically significant.

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**Table 1. HFE Genotype, Age, Serum Iron Studies, and Hepatic Iron Studies in 198 Patients with Hemochromatosis and Other Liver Diseases**

Genotype	Interpretation	Patients	Median Age (Range)	Serum Iron Studies		Hepatic Iron Studies	
				Median Transferrin Saturation* (Range)	Median Ferritin Concentration† (Range)	Median Hepatic Iron Concentration‡ (Range)	Median Hepatic Iron Index§ (Range)
				<i>n</i>	<i>y</i>	$\mu\text{g/L}$	$\mu\text{g/g dry weight}$
C282Y/C282Y	C282Y homozygote	66	45 (23–74)	0.87 (0.35–1.05)	742 (183–5090)	8910 (2355–27 130)	3.25 (0.80–13.0)
C282Y/wild-type	C282Y heterozygote	7	54 (37–61)	0.41 (0.33–0.56)	685 (45–1496)	1103 (235–22 570)	0.30 (0.10–7.30)
C282Y/H63D	Compound heterozygote	10	46 (20–59)	0.58 (0.16–1.00)	495 (103–1380)	2021 (624–8158)	0.80 (0.50–2.50)
H63D/H63D	H63D homozygote	5	43 (35–54)	0.50 (0.28–0.64)	404 (30–729)	800 (38–2169)	0.40 (0.02–0.70)
H63D/wild-type	H63D heterozygote	22	48 (31–66)	0.58 (0.24–1.00)	475 (34–1907)	976 (62–10 874)	0.50 (0.03–4.04)
Wild-type/wild-type	Neither mutation	88	45 (27–84)	0.44 (0.14–1.00)	310 (33–7520)	696 (122–41 040)	0.27 (0.04–22.6)

\* Reference range, 0.16–0.50.

† Reference range, 15–200  $\mu\text{g/L}$  (women) and 30–300  $\mu\text{g/L}$  (men).‡ Reference range, 0–1500  $\mu\text{g/g dry weight}$ ; to convert  $\mu\text{g/g dry weight}$  to  $\text{mmol/kg dry weight}$ , multiply by 0.0179.§ Reference range, <1.9  $\text{mmol/kg per year}$ .

## Results

### Genotype by Patient Group

Of the 66 patients who had clinically diagnosed hemochromatosis, 60 (91%) were C282Y homozygotes, 2 (3%) were compound heterozygotes, 1 (1.5%) was a C282Y heterozygote, 2 (3%) were H63D heterozygotes, and 1 (1.5%) was negative for both mutations. Of the 132 patients with liver disease, 80 had chronic hepatitis C, 19 had abnormal results on serum iron studies and had been referred for evaluation of iron overload or suspected hemochromatosis, 17 had nonalcoholic steatohepatitis, 4 had primary biliary cirrhosis or primary sclerosing cholangitis, and 12 had other liver disorders. Of these 132 patients, 6 (5%) were C282Y homozygotes, 8 (6%) were compound heterozygotes, 6 (5%) were C282Y heterozygotes, 5 (4%) were H63D homozygotes, 20 (15%) were H63D heterozygotes, and 87 (66%) were negative for both mutations. In the group of 19 patients with abnormal results on iron studies who were referred for evaluation of iron overload, 5 (26.5%) were C282Y homozygotes, 1 (5%) was a C282Y heterozygote, 1 (5%) was an H63D homozygote, 5 (26.5%) were H63D heterozygotes, and 7 (37%) were negative for both mutations.

### Iron Status by HFE Genotype

To define and analyze the iron status of all patients for whom genotyping had been performed, we grouped patients with hemochromatosis and patients with liver disease together and divided them according to genotype (Table 1). Additional information about patients with certain genotypes is presented in Tables 2 through 4.

### C282Y Homozygotes

Of the 66 patients who were homozygous for the C282Y mutation (C282Y/C282Y), 40 were men and

26 were women. Median age at diagnosis was 45 years and 49 years, respectively. At diagnosis, men and women had similar transferrin saturations ( $P > 0.2$ ) and ferritin levels ( $P = 0.07$ ). The hepatic iron concentration (median, 159.1  $\text{mmol/kg dry weight}$  [8910  $\mu\text{g/g dry weight}$ ];  $P < 0.01$ ) and the hepatic iron index (median, 3.25  $\text{mmol/kg per year}$ ;  $P < 0.01$ ) were higher in C282Y homozygotes than in the groups of patients with other genotypes (Figure 1). The hepatic iron concentration and the hepatic iron index were similar in male and female C282Y homozygotes ( $P > 0.2$  [data not shown]). On biopsy, all patients had hepatic iron deposition of grade 2+ to 4+. Three women and 9 men had substantial fibrosis or cirrhosis on liver biopsy; 6 of these patients had alanine aminotransferase levels or aspartate aminotransferase levels above the upper limit of normal. The median age of patients with fibrosis was 54 years (range, 40 to 72 years). The youngest patient with cirrhosis was a 40-year-old man with concomitant chronic hepatitis C. The youngest patients with cirrhosis or substantial fibrosis who were homozygous for the C282Y mutation and did not have contributing factors were a 47-year-old man and a 47-year-old woman.

Ten of 66 patients (15% [CI, 7.5% to 26%]) who were C282Y homozygotes had a hepatic iron index less than 1.9  $\text{mmol/kg per year}$ . Specific findings of these patients are detailed in Table 2. Six of these 10 patients were men; 1 was a blood donor. Four of these patients (patients 1, 2, 5, and 8) were identified by HLA haplotyping in family studies; the other 6 were identified from the group of patients with liver disease who were not suspected of having hemochromatosis at the time of initial evaluation. These patients were thought to be heterozygotes or to have concomitant nonalcoholic steatohepatitis. Nine of the 10 patients had a transferrin saturation of at least 45%, and all 10 patients had a hepatic iron concentration above the upper limit of normal.

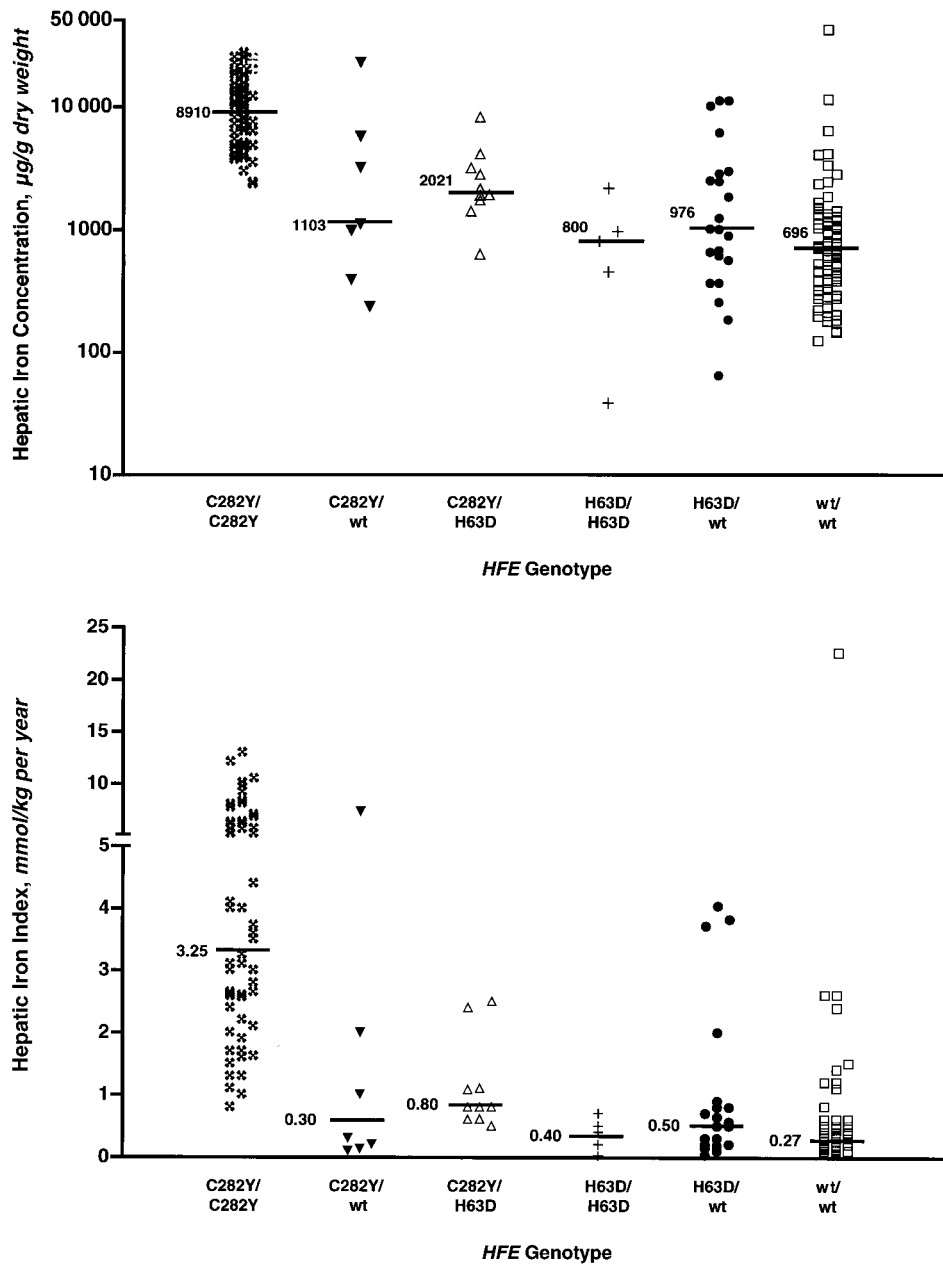
All patients had hepatic iron deposition of grade 2+ to 4+, and only 1 had minimal fibrosis on biopsy.

When C282Y homozygosity is used as the gold standard for the diagnosis of hemochromatosis, the sensitivity and specificity for an elevated hepatic iron concentration are 100% (CI, 93% to 100%) and 74% (CI, 60% to 85%), respectively; the sensitivity and specificity for a hepatic iron index greater than 1.9 mmol/kg per year are 85% (CI, 73% to 93%) and 90% (CI, 81% to 96%), respectively.

### C282Y Heterozygotes

Clinical and laboratory characteristics of the C282Y heterozygotes (C282Y/wild-type) are pre-

sented in **Table 3**. For these patients, hepatic iron concentration was similar to that in patients who did not have either mutation (median, 19.7 mmol/kg [1103  $\mu\text{g/g}$ ] compared with 12.4 mmol/kg [696  $\mu\text{g/g}$ ];  $P > 0.2$ ). The hepatic iron index in both groups of patients was also similar (median, 0.30 mmol/kg per year compared with 0.27 mmol/kg per year;  $P > 0.2$ ) (**Figure 1**). Two of the 7 patients who were heterozygous for the C282Y mutation (patients 1 and 3) had an elevated hepatic iron concentration greater than 26.8 mmol/kg [1500  $\mu\text{g/g}$ ] and a hepatic iron index greater than 1.9 mmol/kg per year; these findings were probably due to secondary iron overload associated with hemolytic anemia and myelo-



**Figure 1.** Hepatic iron concentration (top) and hepatic iron index (bottom) according to HFE genotype. Iron concentrations are expressed on a logarithmic scale; the median value for each group is shown. Hepatic iron index values greater than 5 are shown using a compressed scale. To convert  $\mu\text{g/g}$  to mmol/kg, multiply by 0.0179. wt = wild-type.

**Table 2. Characteristics of C282Y Homozygotes with a Hepatic Iron Index Less Than 1.9 mmol/kg per Year\***

Patient†	Age	Sex	Clinical Diagnosis	ALT Level‡	AST Level§	Transferrin Saturation	Ferritin Level¶	Hepatic Iron Concentration**	Hepatic Iron Index††	Grade of Hepatic Iron Deposition Found on Biopsy‡‡	Fibrosis Found on Biopsy‡‡
<i>y</i>				<i>nkat/L (IU/L)</i>	<i>μkat/L (IU/L)</i>	<i>μg/L</i>	<i>μg/L</i>	<i>mmol/kg dry weight (μg/g dry weight)</i>	<i>mmol/kg per year</i>		
1	68	Female	HH	567 (34)	0.60 (36)	0.38	332	74.8 (4190)	1.1	3–4	1
2	68	Female	HH	433 (26)	0.38 (23)	0.63	405	86.2 (4826)	1.3	3	1
3	55	Female	NASH/presumed heterozygote	500 (30)	0.37 (22)	0.87	310	43.5 (2438)	0.8	2	2
4	40	Female	NASH/presumed heterozygote	1600 (96)	1.07 (64)	0.57	673	53.3 (2987)	1.3	3	1
5	71	Male	HH	467 (28)	0.43 (26)	1.00	860	66.8 (3742)	1.0	3–4	1
6	51	Male	Presumed heterozygote	1317 (79)	0.95 (57)	0.61	335	72.7 (4073)	1.6	2	1
7	43	Male	Presumed heterozygote	500 (30)	0.50 (30)	0.82	414	69.7 (3901)	1.6	2–3	1
8	48	Male	HH	1117 (67)	0.60 (36)	0.53	790	82.9 (4644)	1.7	3–4	1
9	28	Male	NASH/presumed heterozygote	1633 (98)	0.72 (43)	1.00	461	42.1 (2355)	1.5	2	1
10	45	Male	NASH/presumed heterozygote	867 (52)	0.42 (25)	0.60	492	68.2 (3821)	1.7	2–3	1

\* ALT = alanine aminotransferase; AST = aspartate aminotransferase; HH = hereditary hemochromatosis; NASH = nonalcoholic steatohepatitis.

† Patients 1, 2, 5, and 8 were considered to have hereditary hemochromatosis before genotyping on the basis of HLA haplotyping and family study. Patient 9 was a blood donor.

‡ Reference range, 83–917 nkat/L (3–55 IU/L).

§ Reference range, 0.20–0.50 μkat/L (12–50 IU/L).

|| Reference range, 0.16–0.50.

¶ Reference range, 15–200 μg/L (women) and 30–300 μg/L (men).

\*\* Reference range, 0–26.8 mmol/kg dry weight (0–1500 μg/g dry weight).

†† Reference range, <1.9 mmol/kg per year.

‡‡ 1 = no fibrosis; 2 = periportal fibrosis.

dysplastic syndrome. Another patient (patient 7) had an elevated hepatic iron concentration and cirrhosis associated with excessive alcohol consumption.

### Compound Heterozygotes

Clinical and laboratory characteristics of the compound heterozygotes (C282Y/H63D) are presented in **Table 3**. The hepatic iron concentration was lower in this group than in patients who were homozygous for the C282Y mutation (median, 36.1 mmol/kg [2021 μg/g] compared with 159.1 mmol/kg [8910 μg/g];  $P < 0.001$ ). The hepatic iron index was also lower in compound heterozygotes than in patients who were homozygous for the C282Y mutation (median, 0.80 mmol/kg per year compared with 3.25 mmol/kg per year;  $P < 0.001$ ) (**Figure 1**). However, these values for hepatic iron concentration ( $P < 0.001$ ) and the hepatic iron index ( $P < 0.001$ ) were higher in compound heterozygotes than in the patients without either mutation (median, 12.4 mmol/kg [696 μg/g] and 0.27 mmol/kg per year, respectively).

Nine of the 10 patients were men, and 8 patients had an elevated hepatic iron concentration greater than 26.8 mmol/kg (1500 μg/g). Two patients (patients 8 and 9) also had a hepatic iron index greater than 1.9 mmol/kg per year and were initially thought to have typical hemochromatosis. In two patients (patients 6 and 10), presumed heterozygosity for the C282Y mutation (C282Y/wild-type) had been clinically diagnosed on the basis of an elevated hepatic iron concentration and a normal hepatic iron index. Two additional patients (patients 1 and 3) had normal results on hepatic iron studies but substantial fibrosis or cirrhosis caused by autoim-

mune hepatitis (patient 1) or chronic hepatitis C (patient 3).

### H63D Homozygotes

Clinical and laboratory characteristics of patients who were homozygous for the H63D mutation (H63D/H63D) are shown in **Table 3**. The hepatic iron concentration in this group (median, 14.3 mmol/kg [800 μg/g]) was similar to that in patients with neither mutation (median, 12.4 mmol/kg [696 μg/g];  $P > 0.2$ ). The hepatic iron index in these two groups was also similar (median, 0.40 mmol/kg per year compared with 0.27 mmol/kg per year;  $P > 0.2$ ) (**Figure 1**). All H63D homozygotes received a clinical diagnosis of conditions other than hemochromatosis. One patient with nonalcoholic steatohepatitis (patient 3) had an elevated hepatic iron concentration and a normal hepatic iron index associated with grade 2 hepatic iron deposition and substantial fibrosis.

### H63D Heterozygotes

Clinical and laboratory characteristics of patients who were heterozygous for the H63D mutation (H63D/wild-type) are shown in **Table 3**. The hepatic iron concentration in this group (median, 17.4 mmol/kg [976 μg/g]) was similar to that in patients without either mutation (median, 12.4 mmol/kg [696 μg/g]) ( $P = 0.11$ ). The hepatic iron index in these two groups was also similar (median, 0.50 mmol/kg per year compared with 0.27 mmol/kg per year;  $P = 0.10$ ) (**Figure 1**). Nine of the 22 patients had an elevated hepatic iron concentration, and 4 patients had a hepatic iron index greater than 1.9 mmol/kg

**Table 3. Characteristics of Patients with C282Y/Wild-Type, C282Y/H63D, H63D/H63D, and H63D/Wild-Type Genotypes\***

Variable	Age	Sex	Clinical Diagnosis	ALT Level†	AST Level‡	Transferrin Saturation§	Ferritin Level
	y			nkat/L (IU/L)	μkat/L (IU/L)		μg/L
<b>C282Y/wild-type</b>							
Patient 1	51	Female	Hemolytic anemia	583 (35)	0.98 (59)	0.52	1496
Patient 2	61	Female	Porphyria cutanea tarda and autoimmune hepatitis	1283 (77)	0.83 (50)	0.40	685
Patient 3	55	Female	Myelodysplasia	550 (33)	0.55 (33)	0.56	1150
Patient 4	37	Female	HCV infection	1783 (107)	1.93 (116)	0.33	45
Patient 5	52	Male	HCV infection	1400 (84)	0.83 (50)	0.41	68
Patient 6	57	Male	NASH	1467 (88)	0.78 (47)	0.36	369
Patient 7	54	Male	Alcoholic liver disease	750 (45)	0.52 (31)	0.41	1485
<b>C282Y/H63D</b>							
Patient 1	20	Female	Autoimmune hepatitis	1817 (109)	1.07 (64)	0.16	125
Patient 2	47	Male	NASH	1067 (64)	0.65 (39)	0.54	541
Patient 3	46	Male	HCV infection	2233 (134)	1.42 (85)	0.74	375
Patient 4	46	Male	NASH	1067 (64)	0.68 (41)	0.48	451
Patient 5	56	Male	NASH	1267 (76)	0.77 (46)	0.84	495
Patient 6	29	Male	Presumed heterozygote	533 (32)	0.55 (33)	0.61	103
Patient 7	51	Male	Biliary cirrhosis	833 (50)	1.40 (84)	1.00	1059
Patient 8	31	Male	Presumed homozygote	650 (39)	0.50 (30)	0.48	517
Patient 9	59	Male	Presumed homozygote	467 (28)	0.33 (20)	0.63	1380
Patient 10	40	Male	Presumed heterozygote	800 (48)	0.52 (31)	0.54	479
<b>H63D/H63D</b>							
Patient 1	49	Female	HCV infection	7267 (436)	3.65 (219)	0.28	568
Patient 2	43	Female	HCV infection	767 (46)	0.68 (41)	0.50	30
Patient 3	54	Male	NASH	1900 (114)	0.88 (53)	0.56	729
Patient 4	38	Male	Autoimmune hepatitis	2983 (179)	1.35 (81)	0.64	241
Patient 5	35	Male	HCV infection	1750 (105)	1.05 (63)	0.39	200
<b>H63D/wild-type</b>							
Patient 1	54	Female	HCV infection	1533 (92)	0.97 (58)	0.24	86
Patient 2	47	Male	HBV infection	1050 (63)	0.73 (44)	0.24	165
Patient 3	61	Male	Presumed heterozygote	233 (14)	0.28 (17)	0.31	355
Patient 4	36	Female	HCV infection	1383 (83)	1.22 (73)	0.33	136
Patient 5	38	Female	HCV infection	1567 (94)	1.48 (89)	0.33	34
Patient 6	62	Male	Presumed heterozygote	450 (27)	0.38 (23)	0.41	808
Patient 7	40	Male	HCV infection	6833 (410)	2.80 (168)	0.43	421
Patient 8	46	Male	HCV infection	5800 (348)	3.63 (218)	0.45	138
Patient 9	50	Male	NASH	1400 (84)	0.80 (48)	0.46	1652
Patient 10	31	Male	Drug toxicity	517 (31)	0.72 (43)	0.55	229
Patient 11	40	Male	HCV infection	7000 (420)	5.57 (334)	0.60	895
Patient 12	47	Female	Thalassemia minor	800 (48)	1.12 (67)	0.63	475
Patient 13	38	Male	HCV infection	10 334 (620)	9.17 (550)	0.64	953
Patient 14	47	Male	NASH	850 (51)	0.63 (38)	0.65	784
Patient 15	64	Male	Alcoholic liver disease	1500 (90)	1.47 (88)	0.65	1605
Patient 16	49	Male	HCV infection	1000 (60)	1.23 (74)	0.80	673
Patient 17	66	Male	HBV infection	3500 (210)	3.26 (196)	0.83	898
Patient 18	52	Male	Hereditary hemochromatosis and alcoholic liver disease	717 (43)	1.60 (96)	0.87	1907
Patient 19	53	Female	Parenteral iron overload	967 (58)	0.60 (36)	0.89	792
Patient 20	49	Male	Hereditary hemochromatosis and alcoholic liver disease	417 (25)	1.03 (62)	1.00	436
Patient 21	44	Female	NASH	1783 (107)	1.55 (93)	na	99
Patient 22	36	Male	HCV infection	3000 (180)	2.97 (178)	na	na

\* ALT = alanine aminotransferase; AST = aspartate aminotransferase; HBV = hepatitis B virus; HCV = hepatitis C virus; NASH = nonalcoholic steatohepatitis.

† Reference range, 83–917 nkat/L (3–55 IU/L).

‡ Reference range, 0.20–0.50 μkat/L (12–50 IU/L).

§ Reference range, 0.16–0.50.

|| Reference range, 15–200 μg/L (women) and 30–300 μg/L (men).

¶ Reference range, 0–26.8 mmol/kg dry weight (0–1500 μg/g dry weight).

\*\* Reference range, <1.9 mmol/kg per year.

†† 1 = no fibrosis; 2 = periportal fibrosis; 3 = bridging fibrosis; 4 = cirrhosis.

per year. Of the 4 patients with an elevated hepatic iron index, 2 men were cirrhotic and had received a clinical diagnosis of hemochromatosis and alcoholic liver disease (patients 18 and 20). Two women with elevated hepatic iron indexes had no substantial fibrosis and developed secondary iron overload because of thalassemia minor (patient 12) or repeated blood transfusions after chemotherapy for cancer (patient 19).

### Wild-Type Patients

Of the wild-type patients (wild-type/wild-type), 74 had a normal hepatic iron concentration and 14 had an elevated hepatic iron concentration greater than 26.8 mmol/kg (1500 μg/g). The clinical characteristics and iron status of these 14 patients are shown in **Table 4**. Five of these 14 patients had a hepatic iron index greater than 1.9 mmol/kg per year. On

**Table 3—Continued**

Hepatic Iron Concentration†	Hepatic Iron Index**	Grade of Hepatic Iron Deposition Found on Biopsy	Fibrosis Found on Biopsy††
mmol/kg dry weight (μg/g dry weight)	mmol/kg per year		
101.9 (5704)	2.0	4	1
19.7 (1103)	0.3	1	2
403.0 (22 570)	7.3	4	1
4.2 (235)	0.1	0	1
6.9 (388)	0.1	0	1
17.4 (976)	0.2	2	1
57.1 (3195)	1.0	2	4
11.1 (624)	0.6	0	3
38.1 (2132)	0.8	2	1
25.1 (1403)	0.5	3	4
49.7 (2784)	1.1	2	1
34.1 (1910)	0.6	2	1
30.8 (1725)	0.8	2	1
56.6 (3168)	1.1	3	4
73.1 (4091)	2.4	3	1
145.7 (8158)	2.5	4	1
33.7 (1888)	0.8	3	1
8.7 (452)	0.2	0	4
0.7 (38)	0.02	0	2
38.7 (2169)	0.7	2	3
14.3 (800)	0.4	1	3
17.2 (965)	0.5	1	2
9.7 (546)	0.2	0	2
11.3 (635)	0.2	0	4
49.5 (2774)	0.8	3	1
3.2 (178)	0.1	0	2
6.3 (355)	0.2	0	1
42.8 (2397)	0.7	2	2
21.5 (1202)	0.5	1	1
6.4 (356)	0.1	0	1
32.0 (1793)	0.6	2	2
17.5 (982)	0.6	2	1
4.4 (247)	0.1	0	4
175.7 (9841)	3.8	4	1
17.4 (976)	0.5	1	2
43.6 (2439)	0.9	2	3
51.7 (2897)	0.8	3	4
15.4 (865)	0.3	1	4
11.7 (653)	0.2	1	4
193.6 (10 841)	3.7	4	4
106.2 (5947)	2.0	3-4	1
194.2 (10 874)	4.0	4	4
1.1 (62)	0.03	0	1
10.6 (594)	0.3	0	1

both clinical and histologic examination, 2 of these 5 patients had secondary iron overload, 1 had autoimmune hepatitis, 1 had iron overload consistent with hemochromatosis, and 1 had chronic hepatitis C. Family studies could not be performed for the 1 patient who had severe iron loading (patient 5) because he was adopted. Of the remaining 9 patients, 4 had chronic hepatitis C and 5 had other liver diseases. Four of the 14 patients with an ele-

vated hepatic iron concentration had cirrhosis: 1 from autoimmune hepatitis, 1 from alcoholic liver disease, 1 from iron overload alone, and 1 from chronic hepatitis C. Of the 74 patients with a hepatic iron concentration in the normal range, 32 (43%) had substantial fibrosis or cirrhosis related to their underlying chronic liver disease.

## Discussion

The purpose of our study was to determine the contribution of *HFE* genotyping to the diagnosis of hereditary hemochromatosis and to determine the prevalence of *HFE* mutations in a group of patients with various types of liver disease. In agreement with other reports (1, 8), we found that 91% of typical patients with hemochromatosis were homozygous for the C282Y mutation, whereas most patients with liver disease (87 of 132 [66%]) had neither mutation. However, 6 of the 132 patients with liver disease (5%) were homozygous for the C282Y mutation. We combined these groups of patients and subdivided them according to *HFE* genotype for further analysis. All 66 patients who were homozygous for the C282Y mutation had an elevated hepatic iron concentration, and 65 of 66 (98%) had a transferrin saturation of at least 45%. However, 10 of the 66 patients (15%) who were homozygous for the C282Y mutation had a hepatic iron index less than 1.9 mmol/kg per year. Patients with liver disease who had the *HFE* genotypes C282Y/wild-type, H63D/wild-type, and H63D/H63D had hepatic iron concentrations similar to those in patients with the wild-type/wild-type genotype. In patients who have liver disease complicated by iron overload, determination of *HFE* genotype is useful in recognizing otherwise unidentified C282Y homozygotes.

Our study provides detailed serum and hepatic iron values from a large group of patients with hemochromatosis and liver disease who had genotyping for the two mutations in the *HFE* gene. We recognize that our patients with liver disease are probably subject to a selection bias because of a referral bias caused by our interest in disorders of hepatic iron metabolism and because of the suspected effect of liver disease on hepatic iron deposition and secondary iron overload (25). Therefore, our findings should not be considered representative of the prevalence of the various *HFE* genotypes or of iron overload in the general population. However, our results can be considered representative for patients who have hemochromatosis or various other liver diseases. Of interest, 6 of the 132 patients with liver disease (5%) were C282Y heterozygotes and 20 (15%) were H63D heterozygotes; these frequencies are similar to those found for

**Table 4. Characteristics of Patients with Neither Mutation (Wild-Type/Wild-Type) Who Had Hepatic Iron Concentration Greater Than 1500  $\mu\text{g/g}$  Dry Weight**

Patient	Age	Sex	Clinical Diagnosis	ALT Level†	AST Level‡	Transferrin Saturation§	Ferritin Level	Hepatic Iron Concentration¶	Hepatic Iron Index**	Grade of Hepatic Iron Deposition Found on Biopsy	Fibrosis Found on Biopsy††
	<i>y</i>			<i>nkat/L (IU/L)</i>	$\mu\text{kat/L (IU/L)}$		$\mu\text{g/L}$	<i>mmol/kg dry weight (<math>\mu\text{g/g dry weight})</math></i>	<i>mmol/kg per year</i>		
1	73	Female	Drug toxicity	3183 (191)	6.05 (363)	0.94	1126	27.1 (1520)	0.4	2	2
2	59	Female	Sibling of patient with confirmed HH	317 (19)	0.42 (25)	0.89	248	29.4 (1644)	0.5	2	1
3	46	Male	HCV infection	2367 (142)	2.35 (141)	0.64	1088	124.4 (6966)	2.8	2	2
4	49	Male	Alcoholic liver disease, HCV infection, $\alpha_1$ -antitrypsin deficiency	417 (25)	0.75 (45)	0.99	539	27.1 (1520)	0.6	2	4
5	33	Male	HH	2367 (142)	2.53 (152)	0.80	7520	732.9 (41 040)	22.0	4	4
6	84	Female	Anemia, oral iron ingestion	383 (23)	0.6 (36)	0.28	858	200.9 (11 252)	2.4	4	1
7	43	Male	Glu-6-P dehydrogenase deficiency	583 (35)	0.67 (40)	0.83	1164	112.4 (6295)	2.6	4	2
8	63	Male	Alcoholic liver disease	617 (37)	0.85 (51)	1.00	1511	73.3 (4104)	1.2	3	4
9	29	Male	Autoimmune hepatitis	1017 (61)	1.67 (100)	1.00	1786	72.0 (4032)	2.6	4	4
10	53	Male	HCV infection	817 (49)	0.67 (40)	0.78	1166	58.9 (3301)	1.1	2	1
11	36	Male	Sibling of patient with confirmed HH	583 (35)	0.47 (28)	0.23	602	49.7 (2782)	1.4	1	1
12	29	Male	NASH	950 (57)	0.42 (25)	0.27	560	43.4 (2432)	1.5	2	1
13	36	Male	Sibling of patient with confirmed HH	600 (36)	0.43 (26)	0.76	409	41.9 (2346)	1.2	2	1
14	40	Male	HCV infection	2967 (178)	1.12 (67)	0.55	440	32.6 (1824)	0.8	2	2

\* ALT = alanine aminotransferase; AST = aspartate aminotransferase; glu-6-P = glucose-6-phosphate; HCV = hepatitis C virus; HH = hereditary hemochromatosis; NASH = nonalcoholic steatohepatitis.

† Reference range, 83–917  $n\text{kat/L}$  (3–55 IU/L).

‡ Reference range, 0.20–0.50  $\mu\text{kat/L}$  (12–50 IU/L).

§ Reference range, 0.16–0.50.

|| Reference range, 15–200  $\mu\text{g/L}$  (women) and 30–300  $\mu\text{g/L}$  (men).

¶ Reference range, 0–26.8  $\text{mmol/kg dry weight}$  (0–1500  $\mu\text{g/g dry weight}$ ).

\*\* Reference range, <1.9  $\text{mmol/kg per year}$ .

†† 1 = no fibrosis; 2 = periportal fibrosis; 3 = bridging fibrosis; 4 = cirrhosis.

these genotypes (3% and 17%, respectively) in a control population described by Feder and coworkers (3). Prospective evaluation of *HFE* genotyping in large general population studies is in progress; however, these studies will not provide the detailed biochemical and histologic data on hepatic iron that we have described.

The hepatic iron index, introduced in 1986 (18), was developed to differentiate homozygotes with hemochromatosis from heterozygotes and from patients with alcoholic liver disease complicated by secondary iron overload. However, a hepatic iron index greater than 1.9  $\text{mmol/kg per year}$  became a widely used surrogate indicator of homozygous hemochromatosis in the absence of a genetic test (19–22, 26). Our findings suggest that a hepatic iron index greater than 1.9  $\text{mmol/kg per year}$  should no longer be considered necessary for the diagnosis of hemochromatosis and that the hepatic iron index may be clinically relevant only when elevated. Furthermore, a hepatic iron index less than 1.9  $\text{mmol/kg per year}$  does not exclude the presence of the C282Y homozygous state. It may be more appropriate to confirm phenotypic expression of iron overload in patients suspected of having hemochromatosis by demonstrating a hepatic iron concentra-

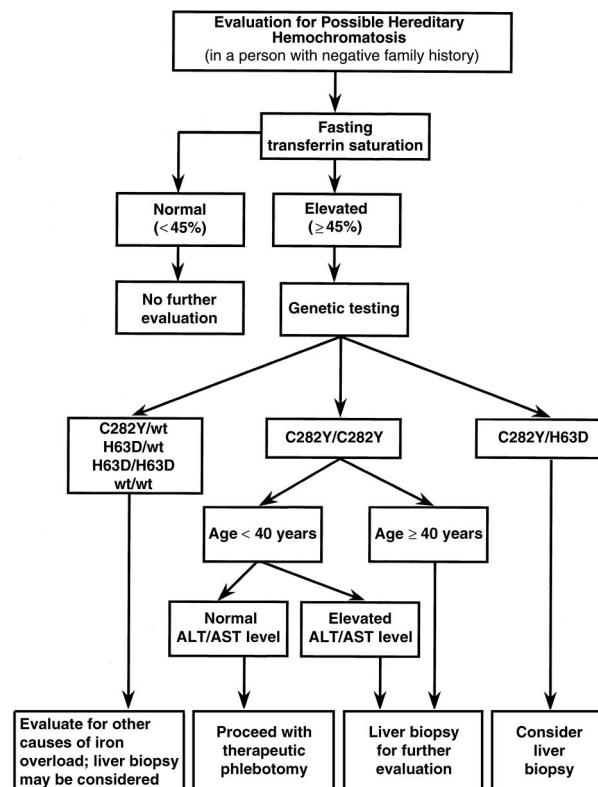
tion above the upper limit of normal and relying less on the hepatic iron index, especially because genetic testing is now available.

In our series, cirrhosis was observed only in patients with hemochromatosis who were at least 40 years of age; this finding is similar to that in a French study of 197 patients with hemochromatosis (27) in which severe fibrosis was seen only in patients older than 44 years of age. Fifty percent of patients with cirrhosis who were homozygous for the C282Y mutation had normal alanine aminotransferase or aspartate aminotransferase levels at the time of diagnosis; this finding confirms that normal aminotransferase levels cannot be used to exclude the presence of cirrhosis. On the basis of these observations, we propose an algorithm with which to evaluate patients for possible hereditary hemochromatosis (Figure 2). Fasting transferrin saturation is known to be a sensitive screening test for hemochromatosis (28, 29). Therefore, we recommend that *HFE* genotyping be performed in patients found to have an elevated fasting transferrin saturation ( $\geq 45\%$ ). In C282Y homozygous patients who have an elevated fasting transferrin saturation and are younger than 40 years of age, liver biopsy is probably not necessary because substantial fibrosis

and cirrhosis are rare. Liver biopsy is indicated in C282Y homozygotes when they are at least 40 years of age or when serum aminotransferase levels are elevated (Figure 2). When we retrospectively applied this algorithm to our patients, 19 of 66 C282Y homozygotes (29% [CI, 19% to 43%]) would not have had liver biopsy, and a diagnosis of substantial fibrosis or cirrhosis would not have been missed in any patient younger than 40 years of age who had normal liver enzyme levels and a fasting transferrin saturation of at least 45%. Identification of increased fibrosis and cirrhosis is considered the most important reason (apart from confirmation of diagnosis) for performing liver biopsy in patients with suspected hemochromatosis. The presence of cirrhosis has been associated with decreased survival in patients with hemochromatosis (30, 31), and some investigators have suggested that it is important to establish the presence of cirrhosis for prognosis and to initiate screening for hepatocellular cancer (32). Liver biopsy may also be considered for compound heterozygotes to determine hepatic iron concentration and evaluate histologic characteristics. Before general clinical application, our algorithm should be validated in a larger series of patients and particular attention should be paid to the cutoff age for liver biopsy.

In previously reported series of typical patients with hemochromatosis (1, 3–8), compound heterozygotes constituted approximately 3% to 5% of patients. This was true for our typical patients with hemochromatosis: Two of 66 (3%) had a genotype of C282Y/H63D, and 8 other compound heterozygotes were identified in the group of patients with liver disease. Of these 10 patients, 2 had an elevated hepatic iron concentration and a hepatic iron index greater than 1.9 mmol/kg per year, 2 had a normal hepatic iron concentration and a normal hepatic iron index, and 6 had an elevated hepatic iron concentration and a hepatic iron index less than 1.9 mmol/kg per year; these 6 patients were classified as having an intermediate degree of iron loading. Fasting transferrin saturation or serum ferritin levels were elevated in all except 1 of these patients, a 20-year-old woman with autoimmune hepatitis. Because of selection bias, our patients may not be representative of all patients with a compound heterozygote genotype; however, if results of serum iron studies are abnormal in a patient with liver disease who has the C282Y/H63D genotype, liver biopsy should be considered to determine the degree of iron loading and whether fibrosis is present.

We have shown that determination of *HFE* genotype is clinically useful in patients who have liver disease complicated by iron overload and may lead to the recognition of otherwise unsuspected C282Y homozygous patients. In our referral population, all



**Figure 2.** Proposed algorithm for the evaluation of possible hereditary hemochromatosis in a person with a negative family history. ALT = alanine aminotransferase; AST = aspartate aminotransferase; wt = wild-type.

66 patients who were homozygous for the C282Y mutation had an elevated hepatic iron concentration; however, approximately 15% of these patients did not meet an earlier diagnostic criterion (hepatic iron index > 1.9 mmol/kg per year). A hepatic iron concentration above the upper limit of normal may be a more reliable phenotypic indicator of hemochromatosis; however, many persons who are not homozygous for the C282Y mutation have an elevated hepatic iron concentration. On a population basis, it remains to be determined how many C282Y homozygotes will never develop clinically significant iron loading. The compound heterozygote genotype (C282Y/H63D) seems to predispose persons to elevation of hepatic iron concentration but usually not to the degree seen in C282Y homozygotes. We have proposed an algorithm for the diagnosis and management of young patients (<40 years of age) with suspected hemochromatosis that avoids the necessity of liver biopsy. This algorithm and the contribution of *HFE* genotyping to the diagnosis and management of iron overload should be confirmed by further clinical and genetic studies.

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