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A POPULATION-BASED STUDY OF THE CLINICAL EXPRESSION OF THE HEMOCHROMATOSIS GENE

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ABSTRACT

Background and Methods Hereditary hemochromatosis is associated with homozygosity for the C282Y mutation in the hemochromatosis (*HFE*) gene on chromosome 6, elevated serum transferrin saturation, and excess iron deposits throughout the body. To assess the prevalence and clinical expression of the *HFE* gene, we conducted a population-based study in Busselton, Australia. In 1994, we obtained blood samples for the determination of serum transferrin saturation and ferritin levels and the presence or absence of the C282Y mutation and the H63D mutation (which may contribute to increased hepatic iron levels) in 3011 unrelated white adults. We evaluated all subjects who had persistently elevated transferrin-saturation values (45 percent or higher) or were homozygous for the C282Y mutation. We recommended liver biopsy for subjects with serum ferritin levels of 300 ng per milliliter or higher. The subjects were followed for up to four years.

Results Sixteen of the subjects (0.5 percent) were homozygous for the C282Y mutation, and 424 (14.1 percent) were heterozygous. The serum transferrin saturation was 45 percent or higher in 15 of the 16 who were homozygous; in 1 subject it was 43 percent. Four of the homozygous subjects had previously been given a diagnosis of hemochromatosis, and 12 had not. Seven of these 12 patients had elevated serum ferritin levels in 1994; 6 of the 7 had further increases in 1998, and 1 had a decrease, although the value remained elevated. The serum ferritin levels in the four other homozygous patients remained in the normal range. Eleven of the 16 homozygous subjects underwent liver biopsy; 3 had hepatic fibrosis, and 1, who had a history of excessive alcohol consumption, had cirrhosis and mild microvesicular steatosis. Eight of the 16 homozygous subjects had clinical findings that were consistent with the presence of hereditary hemochromatosis, such as hepatomegaly, skin pigmentation, and arthritis.

Conclusions In a population of white adults of northern European ancestry, 0.5 percent were homozygous for the C282Y mutation in the *HFE* gene. However, only half of those who were homozygous had clinical features of hemochromatosis, and one quarter had serum ferritin levels that remained normal over a four-year period. (N Engl J Med 1999;341: 718-24.)

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HEREDITARY hemochromatosis is a common inherited disorder of iron metabolism.¹⁻⁴ Recently, a new major-histocompatibility-complex class I–like candidate gene (*HFE*) for hereditary hemochromatosis containing two missense mutations was identified on chromosome 6.⁵ A single mutation (G to A at nucleotide 845) in the *HFE* gene results in the substitution of tyrosine for cysteine at amino acid 282 and is termed the C282Y mutation. A second mutation (C to G at nucleotide 187) in the *HFE* gene results in the substitution of aspartate for histidine at amino acid 63 and is termed the H63D mutation. These mutations are usually detected by restriction-enzyme digestion after amplification of DNA with the polymerase chain reaction (PCR). The C282Y mutation results in the formation of a unique *Sna*BI restriction site, whereas the H63D mutation results in the loss of a *Dpn*II site.

Homozygosity for the C282Y mutation is found in 85 to 90 percent of patients of northern European origin who have typical hereditary hemochromatosis.^{3,5-14} Fifteen to 20 percent of the patient population is heterozygous for the H63D mutation. This mutation may contribute to increased hepatic iron levels but does not result in iron overload in the absence of the C282Y mutation.^{3,15,16} The diagnosis of overt hereditary hemochromatosis is usually based on clinical features, elevated serum transferrin saturation, elevated ferritin levels, characteristic findings on liver biopsy, and elevated hepatic iron levels.¹⁷ Measurement of transferrin saturation is the single best screening test.^{2,18-20}

The degree to which the hemochromatosis mutation affects the development of iron overload and clinical disease is unknown.²¹ Recent family studies of subjects of known genotype with hereditary hemochromatosis have shown that in up to 26 percent of subjects who are homozygous for the C282Y mutation, iron overload may not develop.^{22,23} These studies suggest that rates of expression of the disease may

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be variable and lower than previously believed. We conducted a population-based study to determine the prevalence of the C282Y mutation, the frequency of the clinical expression of iron overload, and genotype-phenotype correlations over a four-year period.

METHODS

Subjects

Busselton is a town in the southwestern region of Western Australia, with a population of 10,888 in 1994. The residents are predominantly of Anglo-Celtic descent. They have been prospectively studied since 1966 and, in many respects, are similar to the population of Framingham, Massachusetts.^{24,25} The most recent follow-up study of this population was performed in 1994. At this evaluation of approximately 5000 white subjects, clinical assessment was performed and whole-blood and serum samples were obtained while the subjects were fasting. From this group, we randomly selected a sample of 3011 unrelated subjects, 20 to 79 years old. The predicted rates of prevalence of heterozygosity and homozygosity for the C282Y mutation in this sample were 8 percent (95 percent confidence interval, 7 to 9 percent) and 0.3 percent (95 percent confidence interval, 0.1 to 0.6 percent), respectively.¹

Clinical, biochemical, and genotypic information was collected for all the subjects. Only in cases in which the serum transferrin saturation was elevated (≥ 45 percent) were the data on the subjects reviewed by one of the investigators and the serum iron studies repeated while the subjects were fasting. All subjects with persistently elevated transferrin-saturation values and those who were homozygous for the C282Y mutation underwent clinical evaluation. Liver biopsy was recommended if the serum ferritin level was 300 ng per milliliter or higher.

Informed consent was obtained before the biopsies were performed. The Busselton Population Medical Research Foundation and the Committee for Human Rights at the University of Western Australia granted us permission to conduct the study.

Measurement of Serum Iron, Transferrin, and Ferritin

Serum iron levels were measured by a standard colorimetric method, and serum transferrin levels were determined by rate immunoturbidimetry on an automated analyzer (model 917, Hitachi, Tokyo, Japan). Serum transferrin-saturation values were calculated as follows: $([\text{serum iron} \div 2] \times \text{serum transferrin}) \times 100$. Serum ferritin levels were measured by chemiluminescence immunoassay (ACS-180, Chiron Diagnostics, Norwood, Mass.). Hepatic iron levels were measured as previously described.²⁶

Identification of the C282Y and H63D Mutations in the *HFE* Gene

DNA was extracted from spots of whole blood collected on neonatal-type screening cards, as described by Singer-Sam and Tanguay.²⁷ PCR amplification of the regions containing the missense mutations was performed with the primer sequences of Feder et al.⁵ (GenBank accession number U60319) and the cycling conditions described by Cullen et al.²⁸ The C282Y and H63D mutations were identified with use of restriction-enzyme digestion, followed by analysis on a 3 percent agarose gel. The status of all subjects homozygous for the C282Y mutation was confirmed with the use of the primer sequence of Jeffrey et al.²⁹ Only subjects with a single C282Y mutation or persistently elevated serum iron levels underwent genotyping for the H63D mutation.

Statistical Analysis

All values are presented as means \pm SD unless otherwise specified. Comparisons between groups were made with the Mann-Whitney U test.³⁰ All P values are two-tailed.

TABLE 1. AGE AND SEX OF THE 3011 STUDY SUBJECTS.

AGE	MEN (N = 1520)	WOMEN (N = 1491)
Median (yr)	52	53
Age group (no.)		
20–29 yr	132	120
30–39 yr	275	220
40–49 yr	313	284
50–59 yr	286	294
60–69 yr	294	319
70–79 yr	220	254

RESULTS

Characteristics of the Subjects

The age and sex of the subjects are shown in Table 1. There were 1491 women and 1520 men, with an age range of 20 to 79 years. Four subjects (two men and two women) were being treated for hemochromatosis at the time of the study.

Serum Transferrin Saturation and Ferritin Levels

The frequency distributions of the serum transferrin-saturation values and serum ferritin levels are shown in Figure 1. The serum transferrin saturation had a normal distribution in both men and women, whereas the serum ferritin levels had a skewed distribution. One hundred thirty-five men and 57 women had transferrin-saturation values of 45 percent or higher; 343 men and 62 women had ferritin levels of 300 ng per milliliter or higher. Forty men and six women had both serum transferrin-saturation values of 45 percent or higher and serum ferritin levels of 300 ng per milliliter or higher.

Hemochromatosis Genotypes

The prevalence rates for the C282Y and H63D mutations are shown in Tables 2 and 3. Subjects were grouped according to whether or not they had serum transferrin-saturation values of 45 percent or higher (Table 2) and whether or not they had serum ferritin levels of 300 ng per milliliter or higher (Table 3). There were 16 subjects with the C282Y/C282Y genotype (0.5 percent), 359 with the C282Y/wild-type genotype (11.9 percent), and 65 with the C282Y/H63D genotype (2.2 percent). The remaining 2571 subjects did not have the C282Y mutation (those with the wild-type/wild-type genotype).

Of the 192 subjects with transferrin-saturation values of 45 percent or higher in 1994, 15 had the C282Y/C282Y genotype, 38 had the C282Y/wild-type genotype, 14 had the C282Y/H63D genotype, and 125 had the wild-type/wild-type genotype (Table 2). In 1994, one 53-year-old homozygous man had a serum transferrin-saturation value of 43 percent and a serum ferritin level of 647 ng per milliliter; in

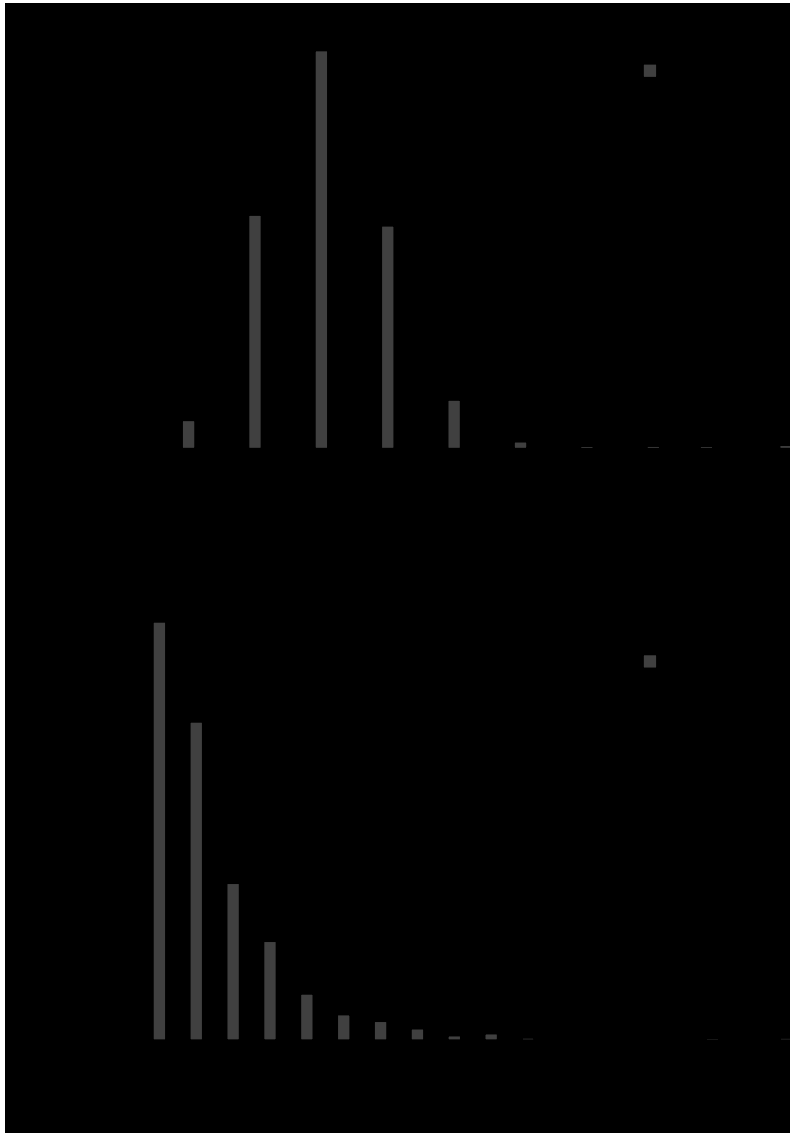


Figure 1. Frequency Distributions of Transferrin-Saturation (Panel A) and Ferritin Levels (Panel B) in Blood Samples Obtained from the Busselton, Australia, Population in 1994. Each bar represents cumulative data for the preceding 10-percent range for transferrin saturation or the preceding 50-ng-per-milliliter range for ferritin.

1998, he had a serum transferrin-saturation value of 102 percent and a serum ferritin level of 731 ng per milliliter (Subject 1, Table 4). He had no history of blood donation or blood loss at the time of the initial testing.

Only 35 of the 192 subjects with elevated transferrin-saturation values in 1994 had persistently elevated transferrin-saturation values. Of these 35, 15 had the C282Y/C282Y genotype (Subjects 2 through 16 in Table 4), 5 had the C282Y/wild-type genotype, 1 had the C282Y/H63D genotype, and 14 had the wild-type/wild-type genotype. Thus, the sensitivity, specificity, and positive predictive value of a

single serum transferrin-saturation value of 45 percent or higher (measured while the subject was fasting) for the detection of C282Y homozygosity were 94 percent, 94 percent, and 6 percent, respectively.

Of the 405 subjects who had serum ferritin levels of 300 ng per milliliter or higher in 1994, 8 had the C282Y/C282Y genotype, 47 had the C282Y/wild-type genotype, 22 had the C282Y/H63D genotype, and 328 did not have the C282Y mutation (Table 3). Thus, the sensitivity, specificity, and positive predictive value of a serum ferritin level of 300 ng per milliliter or higher for the detection of C282Y homozygosity were 50 percent, 87 percent, and 2 percent,

TABLE 2. PREVALENCE OF ELEVATED TRANSFERRIN SATURATION (≥ 45 PERCENT) IN 1994 AND 1998, ACCORDING TO THE HEMOCHROMATOSIS GENOTYPE.*

GENOTYPE	NO. OF SUBJECTS	ELEVATED TRANSFERRIN SATURATION	
		1994	1998
		no. (95% CI)†	
Wild type/wild type	2571	125 (105–149)	14 (8–23)
C282Y/wild type	359	38 (27–51)	5 (2–11)
C282Y/H63D	65	14 (8–22)	1 (0–5)
C282Y/C282Y	16	15 (11–16)‡	16 (13–16)
Total	3011	192	36

*Only subjects with elevated transferrin-saturation values in 1994 underwent another test four years later.

†CI denotes confidence interval.

‡In addition, a 53-year-old man with newly diagnosed hemochromatosis had a serum transferrin saturation of 43 percent and a serum ferritin level of 647 ng per milliliter in 1994. Before liver biopsy in 1998, his serum transferrin saturation was 102 percent, and his serum ferritin level was 731 ng per milliliter. Four of the subjects who were homozygous for the C282Y mutation had received a diagnosis of hereditary hemochromatosis before the study and were undergoing treatment.

TABLE 3. PREVALENCE OF ELEVATED SERUM FERRITIN LEVEL (≥ 300 ng PER MILLILITER) IN 1994, ACCORDING TO THE HEMOCHROMATOSIS GENOTYPE.

GENOTYPE	NO. OF SUBJECTS	ELEVATED SERUM FERRITIN
		no. (95% CI)*
Wild type/wild type	2571	328 (282–359)
C282Y/wild type	359	47 (36–61)
C282Y/H63D	65	22 (15–30)
C282Y/C282Y†	16	8 (4–12)
Total	3011	405

*CI denotes confidence interval.

†Twelve of 16 subjects who were homozygous for the C282Y mutation received a diagnosis of hemochromatosis in 1994; 7 of the 12 had ferritin levels of 300 ng per milliliter or higher.

respectively. When the four subjects who were undergoing treatment for hemochromatosis at the time of the study were excluded from the analysis of serum ferritin levels, the sensitivity, specificity, and positive predictive value were 75 percent, 87 percent, and 2 percent, respectively. Nine subjects had a persistently elevated serum ferritin level (300 ng per milliliter or higher) and a serum transferrin-saturation value of 45 percent or less.

Serum Iron Levels, Sex, and Genotype

Men and women with the C282Y/H63D genotype had significantly higher serum transferrin-saturation values (41 ± 15 percent and 36 ± 11 percent, respectively) than men and women with either the C282Y/wild-type genotype (32 ± 11 percent and 28 ± 12 percent, respectively) or the wild-type/wild-type genotype (30 ± 11 percent and 26 ± 14 percent, respectively; $P < 0.001$). Men with the C282Y/H63D genotype had significantly higher serum ferritin levels (351 ± 191 ng per milliliter) than men with the C282Y/wild-type genotype (217 ± 173 ng per milliliter) or the wild-type/wild-type genotype (221 ± 263 ng per milliliter, $P < 0.001$). There was a trend toward higher serum ferritin levels in women with the C282Y/H63D genotype than in those with the C282Y/wild-type or wild-type/wild-type genotype ($P = 0.07$). Men and women who were homozygous for the C282Y mutation had similar serum transferrin-saturation values (80 ± 24 percent and 70 ± 16 percent, respectively); the serum ferritin levels in the men were higher than those in the women but were not significantly different (609 ± 55 ng per milliliter and 334 ± 387 ng per milliliter, respectively).

Clinical Characteristics of the Subjects Who Were Homozygous for the C282Y Mutation

Of the 16 subjects with the C282Y/C282Y genotype, 12 had not previously received a diagnosis of hemochromatosis (Table 4). In 1994, 7 of these 12 subjects had elevated serum ferritin levels (≥ 300 ng per milliliter or higher); 6 of these 7 (Subjects 1, 2, 7, 8, 9, and 10) had further increases during the four-year follow-up period, and 1 (Subject 4) had a decrease although the value remained elevated. Four subjects (Subjects 3, 5, 11, and 12) had normal serum ferritin levels that did not increase substantially during the four-year follow-up period; none had a history of blood donation, gastrointestinal bleeding, or malabsorption of food. Two subjects without an increase in serum ferritin levels over the four-year period (Subjects 4 and 5) had hepatic iron levels above the normal range (reference range in our laboratory, ≤ 20 μmol per gram of liver, dry weight).

Clinical features that are consistent with the diagnosis of hereditary hemochromatosis were present at the time of diagnosis in 8 of the 16 subjects. Eleven of the 16 underwent liver biopsy. Three of the five who did not (Subjects 3, 11, and 12) did not meet the criteria for liver biopsy and had serum ferritin levels of 100 ng per milliliter or less. Two subjects (Subjects 6 and 9) met the criteria for liver biopsy but declined to undergo the procedure. All subjects who underwent liver biopsy had a hepatic iron level above the upper limit of the normal range. Three subjects had a hepatic iron index of 1.9 or less (Subjects 1, 4, and 5). Three subjects had hepatic fibrosis, and one had cirrhosis; the patient with cirrhosis also had

TABLE 4. CLINICAL CHARACTERISTICS OF THE SUBJECTS WHO WERE HOMOZYGOUS FOR THE C282Y MUTATION.

SUBJECT NO.	NEW OR PREVIOUS DIAGNOSIS*	AGE IN 1998 (YR)	SEX	TRANSFERRIN SATURATION		SERUM FERRITIN		HEPATIC IRON LEVEL	HEPATIC IRON INDEX†	FIBROSIS OR CIRRHOSIS	IRON GRADE‡	CLINICAL SIGNS
				1994	1998	1994	1998	μmol/g				
				%		ng/ml						
1	New	57	M	43	102	647	731	100	1.7	No	3	Pigmentation, arthritis, hepatomegaly
2	New	57	F	57	75	510	660	166	2.7	No	3	Arthritis
3	New	41	F	63	62	44	60	—	—	—	—	None
4	New	74	M	68	55	564	526	95	1.2	Fibrosis	3	Arthritis
5	New	45	F	70	63	189	187	69	1.4	No	1	None
6§	New	35	F	83	95	145	805	—	—	—	—	None
7	New	50	M	100	100	1677	2290	381	7.2	Cirrhosis	4	Pigmentation, hepatomegaly
8	New	42	M	113	97	606	790	796	17.3	Fibrosis	4	Pigmentation, arthritis
9§	New	73	F	105	77	870	1200	—	—	—	—	None
10	New	42	M	90	46	694	868	311	7.4	No	3	None
11	New	30	F	56	75	14	32	—	—	—	—	None
12	New	43	F	62	76	25	28	—	—	—	—	None
13	Previous	58	F	71		1047		295	4.8	No	4	Pigmentation, arthritis
14	Previous	35	M	82		31		289	7.8	No	3	Pigmentation, arthritis, hepatomegaly
15	Previous	55	M	62		44		280	6.0	Fibrosis	4	None
16	Previous	40	F	63		159		80	2.0	No	2	Pigmentation

*Subjects with previous diagnoses did not have their iron levels tested again in 1998 as part of this study.

†The hepatic iron index is the hepatic iron level divided by the age in years.

‡Iron grade denotes the degree of iron deposition, as classified by Searle et al.³¹

§Subjects 6 and 9 declined liver biopsy. Subject 9 had lymphoma and had recently been treated with chemotherapy.

mild microvascular steatosis and a history of excessive alcohol consumption (>60 g per day). None of the other homozygous subjects had a history of chronic viral hepatitis or excessive alcohol consumption.

Clinical Characteristics of the Subjects with Elevated Iron Levels Who Were Not Homozygous for the C282Y Mutation

Eleven subjects who were not homozygous for the C282Y mutation had serum transferrin-saturation values of 45 percent or higher and serum ferritin levels of 300 ng per milliliter or higher. Two of these subjects had recognizable clinical conditions resulting in elevated iron levels: one subject, who was 69 years old and had the C282Y/wild-type genotype, had myelofibrosis, and one, who was 66 years old and had the wild-type/wild-type genotype, had chronic lymphocytic leukemia. Of the other nine subjects, three between the ages of 38 and 54 years (one with the C282Y/H63D genotype, one with the C282Y/wild-type genotype, and one with the wild-type/wild-type genotype) had grade 3 hepatic iron deposition (defined as 75 to 100 percent of hepatocytes with stainable iron deposits³¹) without fibrosis and are currently undergoing phlebotomy therapy. Two subjects who were 66 and 71 years of age (one with the C282Y/

wild-type genotype and one with the wild-type/wild-type genotype) had no stainable iron or evidence of liver injury. One 77-year-old subject with the wild-type/wild-type genotype declined to undergo liver biopsy and is undergoing phlebotomy therapy. Six of the nine subjects with elevated iron levels had the wild-type/wild-type genotype, and all had histories of excessive alcohol consumption. Thus, the overall prevalence of clinically significant iron overload among the subjects who were not homozygous for the C282Y mutation and who did not have other conditions causing iron overload was 4 in 2995 (0.13 percent). In nine subjects (seven with the wild-type/wild-type genotype and two with the C282Y/wild-type genotype) the serum transferrin-saturation values were 45 percent or higher and the serum ferritin levels were normal. There was no identifiable cause of the overload in eight of these subjects; one had chronic lymphocytic leukemia and consumed an excessive amount of alcohol. In nine subjects (five with the wild-type/wild-type genotype and four with the C282Y/wild-type genotype) the serum ferritin levels were 300 ng per milliliter or higher and the serum transferrin-saturation values were 45 percent or less. Three had histories of excessive alcohol consumption, and one had chronic lymphocytic leukemia.

DISCUSSION

We evaluated the penetrance of the C282Y mutation in the *HFE* gene in a population study that was not based on blood-bank data. In our sample of 3011 white Australians, 0.5 percent of the subjects were homozygous for the mutation. Twelve percent had the C282Y/wild-type genotype, and 2 percent had the C282Y/H63D genotype. The frequency of heterozygosity for the C282Y mutation in our sample (0.141) was consistent with the frequency that would be predicted with the Hardy–Weinberg equation (0.135), on the basis of an allelic frequency of 0.076 for the C282Y mutation. These prevalence rates for the C282Y mutation are among the highest that have been reported.^{2,28,32} There is no evidence of consanguinity in this population, and it has been stable, with minimal migration in or out of the region over a period of 30 years.

All 16 subjects with the C282Y/C282Y genotype had elevated serum transferrin-saturation values or serum ferritin levels, and all the subjects with this mutation who underwent liver biopsy had hepatic iron levels above the upper limit of the normal range. However, only 8 of the 16 subjects had clinical features of hemochromatosis, and 4 had hepatic fibrosis or cirrhosis. Four subjects had no clinical symptoms or signs of disease, and their serum ferritin levels were normal over the four-year follow-up period.

Sixty percent of the subjects with newly diagnosed hemochromatosis had an increase in iron stores over the four-year follow-up period, as demonstrated by the changes in their serum ferritin levels. Four women (between the ages of 30 and 45 years) did not have elevated ferritin levels. These data are consistent with the findings reported by Crawford et al.²²; up to 30 percent of the women in their study who were homozygous for the C282Y mutation did not have iron overload.

Eleven subjects in our study had elevated serum transferrin-saturation and ferritin levels but were not homozygous for the C282Y mutation; 4 of the 11 had evidence of hepatic iron overload and are undergoing phlebotomy treatment. An additional nine subjects had elevated ferritin levels but normal serum transferrin-saturation values. These subjects may have disordered iron metabolism, which has been termed the “dysmetabolic iron overload syndrome,”³³ or their high ferritin levels may be due to an acute-phase reactant.

The C282Y/H63D genotype (compound heterozygosity) was associated with higher serum iron levels than the C282Y/wild-type or the wild-type/wild-type genotype. These data provide further support for a synergistic interaction between the two mutations.^{3,15,16}

Our data confirm and extend the recent reports of McLaren et al.²⁰ and Burt et al.² and indicate that serum transferrin saturation is a more sensitive bio-

chemical marker than the serum ferritin level for the detection of the C282Y mutation. The sensitivity, specificity, and positive predictive value of serum transferrin saturation for the detection of the homozygous C282Y mutation were 94 percent, 94 percent, and 6 percent, respectively. If the threshold level for serum transferrin saturation had been set at 50 percent, the sensitivity, specificity, and positive predictive value would have been 94 percent, 96 percent, and 16 percent, respectively. Since the serum ferritin level closely reflects total-body iron stores, however, and since one subject who was homozygous for the C282Y mutation would not have been identified if we had relied only on serum transferrin saturation as a screening test, we recommend that the initial screening also include measurement of the serum ferritin level.³⁴

McLaren et al.²⁰ predicted that a serum transferrin-saturation value of 45 percent or higher, obtained while the subject was fasting, would identify 98 percent of subjects with homozygous hemochromatosis but no normal subjects. In their control population, none of the subjects had a serum transferrin-saturation value of 45 percent or higher. In contrast, we found that 0.5 percent of subjects with the wild-type/wild-type genotype had serum transferrin-saturation values above 45 percent. The cause of this discrepancy is not clear but could be related to differences in the populations studied. The population studied by McLaren et al. was composed of employees of banks and insurance companies, whereas our sample was drawn from the general population and had a wider age range.

Five of the 12 subjects with newly diagnosed homozygous hemochromatosis had no significant changes in serum transferrin-saturation values or serum ferritin levels over the four-year study period (Subjects 3, 4, 5, 11, and 12). Two of these subjects (a woman who was 45 years old and a man who was 74) underwent liver biopsy, and both were found to have a hepatic iron index below the threshold of 1.9. The man had hepatic fibrosis. The other three subjects had no symptoms or signs of disease and had serum ferritin levels of 100 ng per milliliter or lower; none of them underwent liver biopsy. Although all the women in this group had normal menstrual cycles, two had excessive postpartum bleeding. Iron overload develops at a slower rate in women with hereditary hemochromatosis than in men with the disorder, because in women iron stores are reduced during menstruation and pregnancy.³⁴

A cross-sectional study is limited to the evaluation of data at specific times. Since hemochromatosis is a disease that changes phenotypically with time, we used initial screening information from 1994; the subjects with abnormal test results were evaluated four years later. This study design eliminated ethical issues that would have arisen if this had been a pro-

spective study. Although a four-year follow-up period is likely to be adequate for the assessment of a progressive increase in iron stores, as determined by measurements of serum ferritin levels,³³ it is possible that in some subjects iron overload progressed at a rate that was too slow to be detected after four years because of physiologic or pathologic blood loss or other factors.

Our findings have implications for population screening for hereditary hemochromatosis and for identifying the most appropriate initial test. In our view, the high prevalence of the disorder and the opportunity to detect early phenotypic expression and to intervene and prevent subsequent disease justify routine screening of asymptomatic white people of northern European ancestry. The findings of our study demonstrate that biochemical screening for hereditary hemochromatosis identifies virtually all adults who are homozygous for the C282Y mutation and who have iron overload. Alternatively, genetic screening could be performed at birth, with biochemical follow-up of persons with the homozygous mutation and those with the compound heterozygous mutation, in order to identify those who would require treatment. If hemochromatosis is detected before the age of 40 years and if the serum ferritin level is less than 1000 ng per milliliter, then hepatic fibrosis is very likely to be absent and treatment can be initiated without the need for liver biopsy.^{3,15,35,36}

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