Article

The Effect of *HFE* Genotypes on Measurements of Iron Overload in Patients Attending a Health Appraisal Clinic

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Background: **The gene that causes most cases of hereditary hemochromatosis is designated *HFE*. Three mutations exist at this locus at a relatively high gene frequency.**

Objective: **To determine the gene frequency of the three *HFE* mutations and to relate genotypes to various clinical and laboratory variables.**

Design: **Observational study.**

Setting: **Health appraisal clinic.**

Patients: **10 198 adults who registered for health appraisal and consented to DNA examination for hemochromatosis. Consenting patients were slightly older and had attained a slightly higher educational level than nonconsenting patients.**

Measurements: **Extensive medical history and laboratory tests, including complete blood count, transferrin saturation, and other chemistries; serum ferritin levels; and *HFE* genotype.**

Results: **In white participants, the gene frequencies were 0.063 for the C282Y mutation, 0.152 for the H63D mutation, and 0.016 for the S65C mutation. Gene frequencies were lower in other ethnic groups. In participants with *HFE* mutations, the average serum transferrin saturation and ferritin levels were slightly increased, as were mean hemoglobin levels and mean corpuscular volume. A transferrin saturation of 50% had a sensitivity of only 0.52 (95% CI, 0.345 to 0.686) and a specificity of 0.908 (CI, 0.902 to 0.914) for detection of homozygosity. A ferritin level of 200 μg/L in women and 250 μg/L in men had a sensitivity of 0.70 (CI, 0.540 to 0.854) and a specificity of 0.803 (CI, 0.796 to 0.811). The prevalence of iron deficiency anemia was lower in women who carried *HFE* mutations.**

Conclusions: **Screening for transferrin saturation and ferritin levels does not detect all homozygotes for the major hemochromatosis mutation. Heterozygotes for *HFE* mutations had a lower prevalence of iron deficiency anemia.**

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Once considered dismissably rare, hereditary hemochromatosis has come to be regarded as the most common genetic disease among persons of European ancestry (1). The importance of hereditary hemochromatosis is based on its prevalence, its remarkably diverse clinical spectrum, and the fact that early treatment is extremely effective in preventing and to some extent reversing its clinical manifestations. Consequently, interest has developed in detection of the presymptomatic state, in the most efficient way of accomplishing such detection, and in the clinical significance of various mutations of the *HFE* gene.

The most common mutations that cause hereditary hemochromatosis in persons of European ancestry are those of the *HFE* gene. The major mutation, c.845G→A (C282Y;845A), has achieved gene frequencies so high that almost 18% of persons in some populations are heterozygous (2, 3). The clinically milder mutation c.187C→G (H63D;187G) is even more common; in some populations, more than 25% of persons are carriers (2–4). A somewhat less prevalent mutation, c.193A→T (S65C; 193T), has an estimated heterozygote frequency of 4% and may be involved in iron storage disease (5).

Most European patients with hereditary hemochromatosis are homozygous for the C282Y mutation (6). It is less clear whether most patients who inherit the homozygous C282Y/C282Y genotype develop the clinical disorder. Studies of family members of patients with HLA-linked hemochromatosis have suggested that the penetrance of the homozygous defect is very high (7–9). A recent population study from Australia indicated that most patients who are homozygous for the C282Y mutation had elevated transferrin saturation and serum ferritin levels. It was less clear to what extent these biochemical changes were reflected in clinical disease, although cirrhosis was found on liver biopsy in some of these patients (10). We sought to determine the gene frequency of the three *HFE* mutations and to relate genotypes to various clinical and laboratory variables.

Methods

Patients

The Kaiser Permanente San Diego Health Appraisal Clinic is available to Kaiser Health Plan members who wish to have a general health assessment. Members choose to be examined in this clinic for various reasons: Some are

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Table 1. **Genotypes of the *HFE* Mutations in 9650 Study Participants Who Indicated a Single Ethnic Background**

Ethnicity	*wt/wt*	*wt*/H63D	H63D/H63D	*wt*/C282Y	C282Y/H63D	C282Y/C282Y	*wt*/S65C	H63D/S65C	C282Y/S65C	S65C/S65C	Total
	←					*n (%)*					→
White	4679 (59.51)	1809 (23.01)	194 (2.47)	750 (9.54)	150 (1.90)	38 (0.51)	184 (2.34)	42 (0.53)	16 (0.20)	2 (0.03)	7864
Hispanic	690 (71.13)	209 (21.55)	11 (1.13)	36 (3.71)	9 (0.93)	4 (0.41)	10 (1.03)	1 (0.10)	0	0	970
Asian	414 (93.02)	29 (6.53)	0	2 (0.45)	0	0	0	0	0	0	445
Black	324 (87.33)	33 (8.89)	2 (0.54)	6 (1.62)	1 (0.27)	0	4 (1.08)	0	1 (0.27)	0	371

aware of existing health problems, whereas others wish to avoid the development of preventable disease. The average age of members attending the Health Appraisal Clinic is 57 years. In any given 4-year period, 81% of adult members older than 25 years of age will have attended the clinic at least once. All patients undergo a standard set of examinations, including a complete blood count and measurement of a random serum iron and iron-binding capacity, blood glucose level, and creatinine concentration. When the initial transferrin saturation is found to be in excess of 50%, the test is repeated while the patient is in the fasting state. Patients complete a questionnaire that includes information about their past and current health and about their ethnic origin, including specific regions of Europe or Asia.

All patients older than 25 years of age who were registered with the clinic were apprised of a research project in which analysis of DNA for mutations of *HFE* and measurement of serum ferritin would be added to the tests that are usually performed. Our study was approved by the institutional review boards of Kaiser Permanente and the Scripps Clinic. All patients who consented to participate in the study and on whom *HFE* genotype data and health and demographic data had been obtained are included in our report. No patients fulfilling these criteria were excluded from the study, including patients for whom other laboratory data were incomplete and those in whom hemochromatosis was previously diagnosed.

Approximately 39% of clinic patients agreed to participate in the project. The rate of consent to participate was highest among white patients (47.4%).

In some cases, the tests ordered were not performed or the data were not available. Ferritin levels were missing for 979 participants (9.6%), transferrin saturations were missing for 551 participants (5.4%), and data on both were missing for 237 participants (2.3%). Hemoglobin concentrations were not available for 276 participants (2.7%). Missing data were related to logistic failures and were unrelated to participants' medical status.

Laboratory Analyses

Analysis of samples for the C282Y, H63D, and S65C mutations were performed by allele-specific oligonucleotide hybridization using peripheral-blood DNA amplified by polymerase chain reaction assay in a 96-well format (11). The downstream primer was designed to amplify samples with the intron 4 mutation that has been shown to be a potential cause of mistyping of samples (12). In addition to this previously known polymorphism, we have discovered a relatively common polymorphism (g.5198C→G) in intron 3 in the binding site of the upstream primer recommended by Feder and colleagues (6). One patient who was originally thought to be homozygous for C282Y was subsequently shown to be a heterozygote who carried this intron 3 mutation cis to the C282Y mutation, totally preventing amplification of the *wt* (wild-type) allele. Details of this new polymorphism have been published elsewhere (13).

With possible very rare exceptions (14), the three *HFE* mutations are mutually exclusive; any chromosome will contain only one of the three mutations. This makes possible a simplified nomenclature encompassing all four haplotypes. The most common haplotype, 187C, 193A, 845G (H63H, S65S, C282C), is designated *wt*. Each of the other three haplotypes is designated by the altered amino acid: H63D, S65C, and C282Y.

In the present analysis, the term *white* is used to designate participants who responded to the questionnaire by indicating this as their sole ancestry. Those who indicated Hispanic origin were not included in subset analysis of white participants.

Hematologic measurements were made by using a Coulter T540 counter (Beckman-Coulter, Inc., Miami,

Florida). Serum iron levels were measured by the ferrozine method using a Hitachi 717 analyzer; the total iron-binding capacity was measured by using the same instrument after alumna adsorption (Hitachi Instruments, Inc., San Jose, California). Ferritin levels were measured by using the Centaur immunoassay system (Bayer Diagnostics, Tarrytown, New York).

Statistical Analysis

All of the data were entered into an Access (Microsoft Corp., Redmond, Washington) database. Standard errors and confidence intervals were estimated by using Access or Excel software (Microsoft Corp.). Because the distribution of ferritin concentrations approximates a log-normal distribution, means and CIs of ferritin values were calculated from the logarithm of the ferritin concentration. The 95% CIs represent the mean ± 1.96 SEs. Chi-square values were calculated by using the Statmate program (GraphPad Software, San Diego, California).

Results

Patients

The **Appendix Table** shows the characteristics of patients who consented to undergo DNA analysis and those who did not. In 13 patients, hemochromatosis had been previously diagnosed; of these patients, 10 (7 men and 3 women) had undergone phlebotomy. None of the patients with a previous diagnosis had severe clinical manifestations of hemochromatosis, such as cirrhosis. Sixty-nine patients indicated that they had a family history of hemochromatosis; of these patients, 23 had the *wt/wt* genotype, 18 had the C282Y/*wt* genotype, 15 had the H63D/*wt* genotype, 5 had the C282Y/C282Y genotype, 3 had the C282Y/H63D genotype, and 5 had other genotypes.

Laboratory Analyses

Genotypes of participants who indicated that they had a single ethnic origin are shown in **Table 1**. The corresponding calculated gene frequencies for the *HFE* mutations are shown in **Table 2**.

Ferritin levels, transferrin saturation, hemoglobin levels, and mean corpuscular volume in white participants with various genotypes are shown in **Table 3**. In each case, the first value obtained was used in these analyses. This analysis was limited to white participants because of the well-known differences in hemoglobin concentration and mean corpuscular volume according to ethnicity (15); these differences are also apparent in the data shown in the **Appendix Table.**

Table 2. **HFE Allele Frequencies in 9650 Study Participants Who Indicated a Single Ethnic Background**

Ethnicity	*wt*	C282Y	H63D	S65C
White	0.770	0.063	0.152	0.016
Hispanic	0.843	0.027	0.124	0.006
Asian	0.965	0.002	0.033	0
Black	0.931	0.011	0.051	0.007

Heterozygosity for *HFE* Mutations

Simple heterozygosity for *HFE* mutations had only a minor effect on transferrin saturation and ferritin levels. However, compound heterozygotes for the C282Y and H63D mutations had considerably increased transferrin saturation and serum ferritin level. As shown in **Table 3**, heterozygotes and compound heterozygotes for each of the *HFE* mutations had an increase in the average hemoglobin concentration and mean corpuscular volume. The average mean corpuscular hemoglobin concentration was also slightly higher in white patients with *HFE* mutations by about 0.7 g/L (for example, 344.16 ± 0.10 g/L in the *wt/wt* group vs. 344.93 g/L in the C282Y/*wt* group). This seemingly negligible difference was statistically significant ($P < 0.001$) when the *wt/wt* group was compared with groups having the C282Y or the H63D mutation.

The effect of genotype on the prevalence of iron deficiency anemia is shown in **Table 4**.

Effects of Homozygosity for the C282Y Mutation

Forty-three of the 10 198 participants were homozygous for the C282Y mutation (**Table 2**), the genotype most frequently associated with hereditary hemochromatosis. Twenty-two participants were men and 21 were women; the average age was 56 years (range, 27 to 78 years). Thirteen participants had previously received a diagnosis of hemochromatosis, and 10 were already receiving treatment when they were first seen for our study.

The distributions of transferrin saturation and ferritin levels in the 33 participants with the C282Y/C282Y genotype who had not been treated by phlebotomy are shown in the **Figure**. Five of these participants were frequent blood donors. Three had estimated lifetime donations of 20 to 30 units of blood, 1 donated 31 to 100 units, and 1 donated more than 100 units. The sensitivity and specific-

Table 3. **Mean Laboratory Values in White Patients according to *HFE* Genotype***

Value	*wt/wt*	*wt*/H63D	H63D/H63D	C282Y/*wt*	C282Y/H63D
Ferritin level, μg/L					
Women	54.87 (53.0–56.8)	57.83 (54.6–61.3)	70.55 (58.9–84.5)	62.48 (57.3–68.2)	73.76 (56.5–96.3)
Men	106.79 (103.3–110.4)	118.37 (112.2–124.9)	132.97 (111.3–158.9)	117.31 (106.7–129.0)	187.25 (158.4–221.3)
Transferrin saturation					
Women	0.2233 (0.220–0.227)	0.2465 (0.240–0.253)	0.2809 (0.261–0.301)	0.2654 (0.256–0.275)	0.3190 (0.288–0.350)
Men	0.2625 (0.259–0.267)	0.2852 (0.279–0.291)	0.3341 (0.312–0.356)	0.3036 (0.293–0.314)	0.4005 (0.369–0.432)
Hemoglobin level, g/L					
Women	134.5 (134–135)	135.7 (135–136)	136.3 (134–138)	135.8 (135–137)	138 (136–140)
Men	147.8 (147–148)	149.2 (149–150)	150.5 (148–153)	150.3 (149–151)	151.3 (149–153)
Mean corpuscular volume, fL					
Women	89.91 (89.7–90.1)	90.81 (90.5–91.1)	92.01 (91.1–93.0)	91.04 (90.6–91.5)	91.87 (90.8–93.0)
Men	90.35 (90.2–90.5)	90.89 (90.6–91.2)	92.19 (91.3–93.1)	91.04 (90.6–91.5)	93.50 (92.5–94.5)

* Values in parentheses are 95% CIs.
† The CI could not be computed because there was only one patient in this category.

ity of transferrin saturations and serum ferritin levels in detecting these persons are shown in **Table 5**.

Symptoms reported by untreated participants in response to the questionnaire included arthralgias (8 of 33 participants), arrhythmias (5 of 33 participants), abdominal pains (2 of 33 participants), hearing loss (8 of 33 participants), impotence in males (2 of 15 participants), diarrhea (1 of 33 participants), and darkening of the skin (1 of 33 participants). The relation of these symptoms to hemochromatosis is uncertain; their frequency did not statistically significantly differ from that in the rest of the population, even after stratification by age or by serum ferritin levels. However, when a physician obtained a history from patients with these symptoms, the incidence of focal arthralgias, abdominal pain, and diarrhea was much higher than that obtained from the questionnaire completed by the patients themselves.

There was no evidence that participants who were homozygous for the C282Y mutation had an increased incidence of diabetes. Blood glucose levels measured more than 2 hours postprandially were available for 30 of the untreated homozygotes. Their average blood glucose level was 5.05 ± 0.12 mmol/L (91.0 ± 2.15 mg/dL); this value did not significantly differ from that found in 6064 similarly obtained blood glucose levels in patients with the *wt/wt* genotype (5.22 ± 0.02 mmol/L [94.1 ± 0.35 mg/dL]). No blood glucose levels in the 30 homozygotes for the C282Y mutation were higher than 6.67 mmol/L (120 mg/dL); of the controls, 334 (5.5%) had levels higher than 6.67 mmol/L. Similarly, the average level of aspartate aminotransferase in the 21 untreated homozygotes in whom this measurement was available was 0.52 ± 0.07 μkat/L compared with 0.53 ± 0.02 μkat/L among 397 wild-type controls in whom this measurement was obtained as part of the screening procedure.

Increased Transferrin Saturation Levels in Participants without *HFE* Mutations

If clinic patients were found to have transferrin saturations of 50% or higher, they were asked to return for a repeated test done under fasting conditions. One hundred forty-four participants who were not C282Y/C282Y homozygotes had transferrin saturations of 50% or more. The transferrin saturation was repeated in 98 of these participants, and the second value was still greater than 50% in 25 (25.5%) patients. We were unable to obtain a repeated transferrin saturation in the remaining 46 participants. Of the 25 participants with persistently elevated transferrin saturation values, 9 had the *wt/wt* genotype, 5 had the *wt*/H63D genotype, 1 had the H63D/H63D genotype, 9 had the C282Y/H63D genotype, and 1 had the H63D/S65C genotype.

Discussion

We determined the *HFE* genotype of 10 198 persons attending a health assessment clinic. This gave us a different perspective on the clinical and laboratory effects of *HFE* mutations than that obtained by studying populations of patients with hemochromatosis or their relatives. We detected the homozygous hemochromatosis genotype C282Y/C282Y in 43 persons. Transferrin saturation and serum ferritin levels of homozygotes are presented in the **Figure**. Compound heterozygotes with the C282Y/H63D genotype were found to have increased mean transferrin saturation (by 13.8% in men and 9.57% in women). A

Table 3—**Continued**

C282Y/C282Y	C282Y/S65C	S65C/S65C	H63D/S65C	*wt*/S65C
146.39 (90.4–237.2)	59.64 (25.4–140.2)	144.00†	58.36 (41.4–82.3)	57.72 (48.6–68.6)
313.97 (164.8–598.0)	185.89 (133.5–258.7)	212.00†	120.27 (85.4–169.4)	104.98 (89.2–123.5)
0.4913 (0.400–0.582)	0.360 (0.303–0.417)	0.210†	0.2517 (0.209–0.294)	0.2502 (0.230–0.271)
0.5443 (0.442–0.646)	0.3367 (0.267–0.407)	0.310†	0.3436 (0.300–0.387)	0.2767 (0.257–0.296)
138.6 (135–142)	143.1 (136–150)	126.0†	138.2 (135–141)	135.1 (133–137)
153.1 (149–158)	151.2 (145–157)	141.0†	148.7 (145–153)	147.6 (145–150)
94.02 (92.2–95.8)	93.41 (91.0–95.9)	88.6†	91.12 (89.1–93.2)	90.92 (89.9–92.0)
95.13 (92.5–97.8)	92.50 (90.6–94.4)	92.4†	94.49 (92.1–96.9)	90.39 (89.5–91.3)

small but statistically significant increase in transferrin saturation was observed even in simple heterozygotes with the C282Y/*wt* genotype, but it amounted to a mean change of only 4.11% of the transferrin saturation in men and 4.21% in women. Similarly, substantial increases in ferritin levels were documented in compound heterozygotes, but hardly any increase was noted among the simple heterozygotes.

The role of the H63D mutation in producing hemochromatosis has been questioned (16, 17), but biochemical (18, 19) and epidemiologic (20, 21) data show clearly that the H63D mutation affects iron metabolism. Data on the role of the S65C mutation, which is much less common, have been controversial: Mura and colleagues (5) found an increased prevalence of this mutation in patients with hemochromatosis, but Arya and colleagues (22) did not find an increased prevalence of the mutation in blood donors with high transferrin saturations. Our data on this mutation, which have been analyzed in greater detail elsewhere (23), clearly show that the S65C mutation affects the serum transferrin saturation, although to a lesser extent than the C282Y or the H63D mutations.

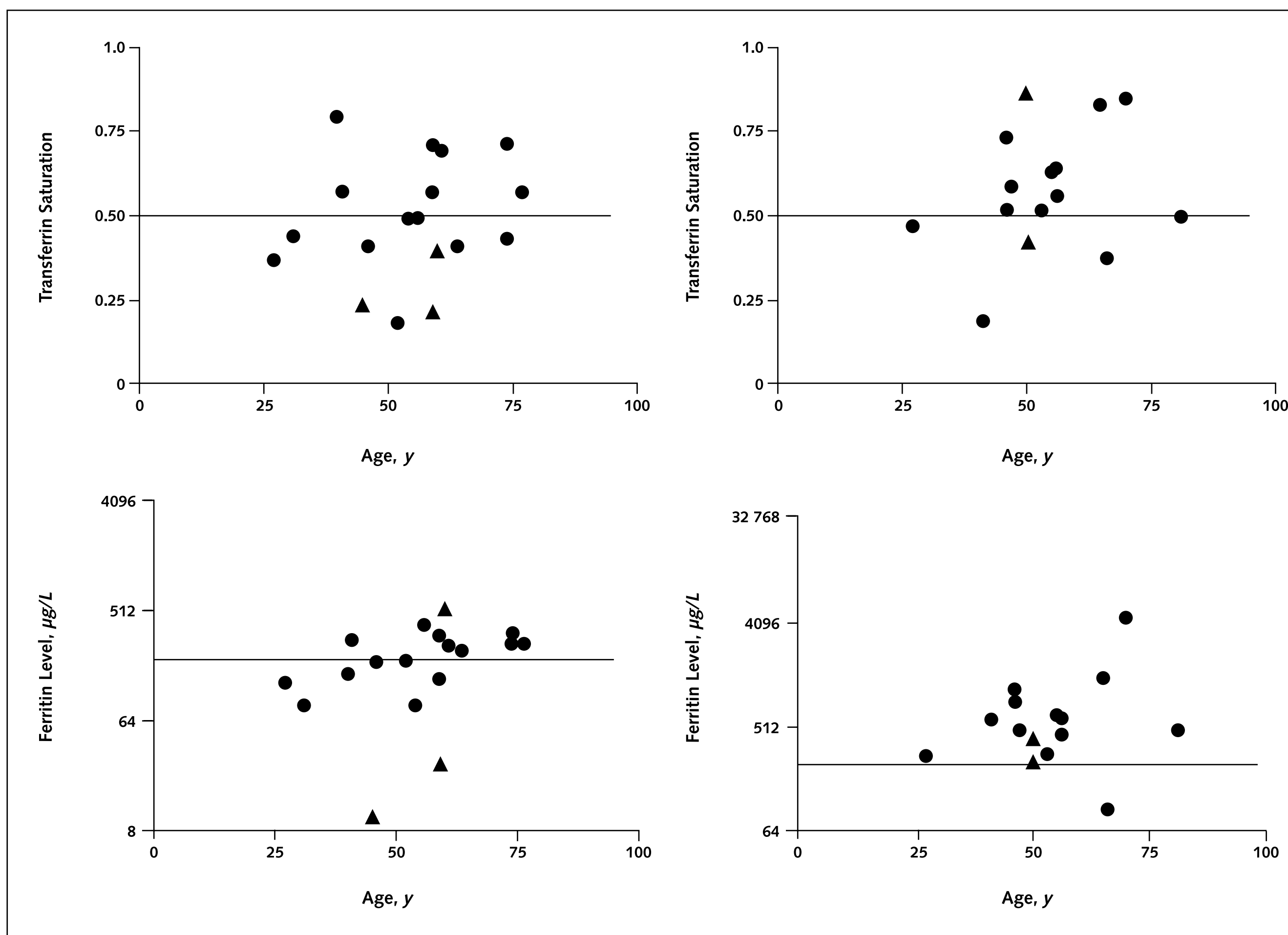
Surprisingly, the mean hemoglobin levels of persons with different genotypes differed significantly. Both men and women carrying any *HFE* mutation had slightly higher hemoglobin levels than did persons of the same sex with the normal (*wt/wt*) genotype. Although anemia was somewhat more common in women without *HFE* mutations than in those with them (**Table 4**), anemia was not the cause of the difference in hemoglobin levels among persons with different genotypes; the entire distribution of hemoglobin values was shifted, and the difference persisted even if the relatively small number of anemic persons was not considered (data not shown). In addition, the effect of *HFE* mutations on the mean corpuscular volume was significant, even in heterozygotes.

The sensitivity of transferrin saturation (**Table 5**) differs from that reported by McLaren and associates (24), who suggested that a cutoff value of 45% would identify 98% of the homozygotes for hemochromatosis. Our findings about transferrin saturation and ferritin levels also differ from those reported by Olynyk and coworkers (10), who found that although 25% of homozygotes detected by genetic analysis had normal ferritin levels, 15 of 16 (94%) homozygous patients had transferrin saturations higher than 45%. The differences between our results and those of other studies might reflect unknown genetic factors,

Table 4. ***HFE*** **Mutation and Prevalence of Anemia among White Women according to Various Definitions of Iron Deficiency**

Criterion	*wt/wt*	*wt*/H63D	H63D/H63D	C282Y/*wt*	C282Y/H63D	C282Y/C282Y
	←———			*n/n (%)*		———→
Hemoglobin level < 115 g/L	53/2092 (2.53)	12/808 (1.49)	0/84 (0)	5/367 (1.36)	1/62 (1.61)	0/20 (0)
Hemoglobin level < 115 g/L and transferrin saturation < 0.16 μg/L	26/1942 (1.34)	7/750 (0.93)	0/77 (0)	3/336 (0.89)	1/58 (1.72)	0/20 (0)
Hemoglobin level < 115 g/L and ferritin level < 20 μg/L	17/1929 (0.88)	5/741 (0.67)	0/79 (0)	2/342 (0.58)	1/58 (1.72)	0/20 (0)

Figure. **Transferrin saturation and serum ferritin levels of female (*left*) and male (*right*) C282Y/C282Y homozygotes.**



Triangles represent persons who donated more than 20 units of blood during their lifetime; circles represent those who donated 20 or fewer units of blood. The horizontal lines demarcate 50% transferrin saturation and ferritin concentrations of 200 μg/L in women and 250 μg/L in men.

dietary intake, or other environmental factors. They are not a function of the age of the population, since our patients were older than the ones studied by Olynyk and coworkers. Our results and those of other studies clearly show that neither the transferrin saturation nor the serum ferritin level can reliably identify all homozygotes for the C282Y *HFE* mutation.

The fact that patients with hemochromatosis have macrocytosis has been noted previously (25). However, we did not expect to find that the volume of erythrocytes is affected in heterozygotes for *HFE* mutations. We have demonstrated the presence of HFE messenger RNA in erythrocyte precursors (Lee P. Unpublished data), and it is possible that the mutant protein somehow affects the development program of the erythrocyte precursor. However, the relation between heterozygous *HFE* mutations and macrocytosis, although statistically significant, may not represent a cause-and-effect relationship; rather, it may reflect the fact that population clines of hemoglobin and mean corpuscular volume values and population clines of *HFE* mutations from northern to southern Europe coincide (data not shown).

In contrast to our study, in which the homozygotes detected were clinically well, studies of family members of patients with a clinical diagnosis of HLA-linked hemochromatosis have suggested that the penetrance of the homozygous defect is very high (7–9). On the other hand, in agreement with the apparently low penetrance that we observed, asymptomatic patients with the homozygous C282Y genotype were common in an elderly population

(26), and a study of patients with cirrhosis suggested that only about 2.5% of patients with the homozygous genotype develop cirrhosis (27). Relatives of clinically affected patients may be more likely than persons in the general population to manifest morbidity. Because the patients in our study were reporting for health screening rather than treatment of specific symptoms and because patients who had already received a diagnosis of hemochromatosis were not considered in evaluation of symptoms, most of the homozygotes detected were asymptomatic or presymptomatic. Questioning by a physician elicited more symptoms than did self-report on a patient questionnaire. A carefully conducted, blinded case–control study of a larger sample will be required to determine to what extent the homozygotes detected have clinical symptoms that can be attributed to hemochromatosis.

Iron deficiency anemia is one of the most prevalent diseases, particularly in women and children. Because iron deficiency leads to decreased fitness, mutations that protect against it would be subject to positive selection. A recent survey of 499 women for only the C282Y mutation showed that heterozygotes had slightly higher hemoglobin levels than did controls (28), but another study of similar size found no protection against iron deficiency (29). Moreover, Rochette and colleagues (30) have maintained that if *HFE* mutations protected against iron deficiency, one might expect that one or more of the *HFE* mutations would have reached fixation in areas in which iron deficiency was common. They suggest that *HFE* serves as a receptor for an infectious agent. In our series, the incidence of anemia was highest among patients who carried no *HFE* mutations. Because serum iron levels are not uniformly decreased in women with mild iron deficiency (31), analyses were based on criteria that included or did not include biochemical iron measurements (**Table 4**). Chi-square analysis comparing patients who did not have *HFE* mutations with those who did showed that the difference in the incidence of anemia was significant ($P = 0.01$).

A major practical implication of our study concerns the strategy that is most appropriate for screening of populations for hemochromatosis. Regardless of the cutoff value selected for transferrin saturation and regardless of whether ferritin measurement is added, an unexpectedly large number of homozygotes for the C282Y mutation will be missed. However, we do not know whether these homozygotes are at risk for clinical disease. Does their lack of elevated transferrin saturation in middle age imply that the effect of the mutation on iron saturation is so slight that these patients are unlikely to manifest phenotypic expression over the remaining decades of their lives? Furthermore, does the probability of ultimate phenotypic expression of homozygosity for the C282Y mutation differ in cases detected by using a clinically diagnosed case as the index case for screening compared with cases in which genotype was detected through mass population screening? If so, should family members of clinically diagnosed cases be screened by using genetic analysis rather than transferrin saturation or ferritin levels? Our study and others raise the question of whether the best predictor of phenotypic expression is the genotype or the pathophysiologic measure implicit in transferrin saturation. Many other investigators have discussed this problem, but no obvious solution has been found (32, 33).

Table 5. **Sensitivity and Specificity of Transferrin Saturation and Serum Ferritin Level for Detection of C282Y/C282Y Homozygosity***

Substance	Sensitivity			Specificity		
	Men	Women	Both	Men	Women	Both
Transferrin saturation						
0.50	0.67	0.389	0.52 (0.345–0.686)	0.904	0.912	0.908 (0.902–0.914)
0.45	0.80	0.5	0.64	0.88	0.901	0.891 (0.885–0.897)
0.45	0.85†	0.6†	0.70 (0.560–0.854)	0.88‡	0.90‡	0.891 (0.885–0.811)
Ferritin level						
200 μg/L		0.5			0.856	
			0.70 (0.54–0.854)			0.803 (0.796–0.811)
250 μg/L	0.93			0.749		

* Values in parentheses are 95% CIs.
† Excluding 5 participants who donated ≥20 units of blood over their lifetime.
‡ Excluding 914 participants who donated ≥20 units of blood over their lifetime.

Appendix Table. **Demographic and Clinical Characteristics of Participants and Nonparticipants***

Characteristic	Men	Women	Healthy†	Higher Education‡	Age	Hemoglobin Level	Mean Corpuscular Volume	Transferrin Saturation
	←	%		→	*y*	*g/L*	*fL*	
Men								
White								
Participant (*n* = 3911)	49.7		56.3	50.9	59.8 ± 13.61	148 ± 10.6	90.7 ± 4.54	0.28 ± 0.10
Nonparticipant (*n* = 4177)	48.7		59.2	47.1	56.0 ± 15.66	148 ± 11	90.4 ± 4.6	0.28 ± 0.10
Hispanic								
Participant (*n* = 427)	43.8		36	31	50.2 ± 14.37	150 ± 9.8	89.6 ± 4.3	0.28 ± 0.10
Nonparticipant (*n* = 1292)	43.7		67.1	21.4	44.7 ± 16.58	149 ± 9.9	88.9 ± 4.1	0.26 ± 0.10
Black								
Participant (*n* = 186)	49.7		53.9	41.1	53.4 ± 12.9	144 ± 12.3	88.0 ± 5.64	0.25 ± 0.08
Nonparticipant (*n* = 485)	43.6		66.8	32.7	48.5 ± 14.4	142 ± 10.8	86.7 ± 5.88	0.23 ± 0.09
Asian								
Participant (*n* = 199)	44.5		61.6	55.4	52.9 ± 12.65	151 ± 10.8	88.0 ± 7.01	0.29 ± 0.10
Nonparticipant (*n* = 616)	38.9		67	48.7	48.2 ± 14.8	150 ± 10.2	88.0 ± 6.58	0.28 ± 0.10
Other§								
Participant (*n* = 257)	46.9		53.8	38.9	54.5 ± 14.33	149 ± 11.3	89.9 ± 5.04	0.28 ± 0.10
Nonparticipant (*n* = 649)	43.5		60.7	31.1	46.6 ± 16.86	150 ± 9.7	88.9 ± 4.74	0.26 ± 0.09
Women								
White								
Participant (*n* = 3953)		50.3	49.2	37.4	58.9 ± 13.9	135 ± 9.5	90.4 ± 4.54	0.24 ± 0.10
Nonparticipant (*n* = 4558)		51.3	50.1	36.4	54.9 ± 16.44	134 ± 9.6	90.0 ± 4.66	0.24 ± 0.10
Hispanic								
Participant (*n* = 543)		56.2	45	22.2	48.6 ± 14.33	133 ± 9.5	88.9 ± 4.85	0.23 ± 0.10
Nonparticipant (*n* = 1741)		56.3	60.6	20.8	42.4 ± 15.88	132 ± 9.8	88.3 ± 5.22	0.21 ± 0.11
Black								
Participant (*n* = 185)		50.3	53.8	35.8	51.3 ± 12.78	127 ± 11.5	87.3 ± 6.73	0.21 ± 0.09
Nonparticipant (*n* = 629)		56.4	58.5	30.6	45.8 ± 15.48	126 ± 11.4	85.6 ± 6.5	0.20 ± 0.09
Asian								
Participant (*n* = 246)		55.5	60.3	50	50.2 ± 13.02	134 ± 9.9	88.8 ± 6.36	0.25 ± 0.10
Nonparticipant (*n* = 968)		61.1	65.6	52.4	46.3 ± 14.21	132 ± 10.2	87.1 ± 7.58	0.23 ± 0.11
Other§								
Participant (*n* = 291)		53.1	49.4	32.3	52.3 ± 15.68	134 ± 9.7	89.4 ± 5.41	0.23 ± 0.10
Nonparticipant (*n* = 860)		56.5	53.8	28.1	45.3 ± 16.64	132 ± 10.1	87.7 ± 5.78	0.22 ± 0.11

* Values with the plus/minus symbol are the mean ± SD.
† Persons who chose the response "My health allows full activity" on a questionnaire.
‡ Degree from a 4-year college or higher.
§ American Indian, Pacific Islander, mixed race, or unknown.

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