ORIGINAL ARTICLE

Hemochromatosis and Iron-Overload Screening in a Racially Diverse Population

Paul C. Adams, M.D., David M. Reboussin, Ph.D., James C. Barton, M.D.,
Christine E. McLaren, Ph.D., John H. Eckfeldt, M.D., Ph.D.,
Gordon D. McLaren, M.D., Fitzroy W. Dawkins, M.D., Ronald T. Acton, Ph.D.,
Emily L. Harris, Ph.D., M.P.H., Victor R. Gordeuk, M.D.,
Catherine Leiendecker-Foster, M.S., Mark Speechley, Ph.D.,
Beverly M. Snively, Ph.D., Joan L. Holup, M.A., Elizabeth Thomson, M.S., R.N.,
and Phyliss Sholinsky, M.S.P.H., for the Hemochromatosis and Iron Overload
Screening (HEIRS) Study Research Investigators*

ABSTRACT

BACKGROUND

Iron overload and hemochromatosis are common, treatable conditions. *HFE* genotypes, levels of serum ferritin, transferrin saturation values, and self-reported medical history were studied in a multiethnic primary care population.

METHODS

Participants were recruited from primary care practices and blood-drawing laboratories. Blood samples were tested for transferrin saturation, serum ferritin, and C282Y and H63D mutations of the *HFE* gene. Before genetic screening, participants were asked whether they had a history of medical conditions related to iron overload.

RESULTS

Of the 99,711 participants, 299 were homozygous for the C282Y mutation. The estimated prevalence of C282Y homozygotes was higher in non-Hispanic whites (0.44 percent) than in Native Americans (0.11 percent), Hispanics (0.027 percent), blacks (0.014 percent), Pacific Islanders (0.012 percent), or Asians (0.000039 percent). Among participants who were homozygous for the C282Y mutation but in whom iron overload had not been diagnosed (227 participants), serum ferritin levels were greater than 300 μg per liter in 78 of 89 men (88 percent) and greater than 200 μg per liter in 79 of 138 women (57 percent). Pacific Islanders and Asians had the highest geometric mean levels of serum ferritin and mean transferrin saturation despite having the lowest prevalence of C282Y homozygotes. There were 364 participants in whom iron overload had not been diagnosed (29 C282Y homozygotes) who had a serum ferritin level greater than 1000 μg per liter. Among men, C282Y homozygotes and compound heterozygotes were more likely to report a history of liver disease than were participants without *HFE* mutations.

CONCLUSIONS

The C282Y mutation is most common in whites, and most C282Y homozygotes have elevations in serum ferritin levels and transferrin saturation. The C282Y mutation does not account for high mean serum ferritin levels and transferrin saturation values in nonwhites.

From the Department of Medicine, London Health Sciences Centre, London, Ont., Canada (P.C.A.); the Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, N.C. (D.M.R., B.M.S.); Southern Iron Disorders Center, Birmingham, Ala. (J.C.B.); Epidemiology Division (C.E.M.) and Division of Hematology and Oncology (G.D.M.), Department of Medicine, University of California, Irvine; Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis (J.H.E., C.L.-F.); Veterans Affairs Long Beach Healthcare System, Long Beach, Calif. (G.D.M.); Department of Medicine, Howard University, Washington, D.C. (F.W.D., V.R.G.); Departments of Microbiology, Medicine, and Epidemiology and International Health, University of Alabama at Birmingham, Birmingham (R.T.A.); Kaiser Permanente Center for Health Research, Portland, Oreg. (E.L.H.), Department of Epidemiology and Biostatistics, University of Western Ontario, London, Canada (M.S.); Kaiser Permanente Center for Health Research, Honolulu (J.L.H.); National Human Genome Research Institute, Bethesda, Md. (E.T.); and the Epidemiology and Biometry Program, National Heart, Lung, and Blood Institute, Bethesda, Md. (P.S.). Address reprint requests to Dr. Adams at the Department of Medicine, London Health Sciences Centre, 339 Windermere Rd., London, ON N6A 5A5, Canada, or at padams@uwo.ca.

*Members of the HEIRS study are listed in the Appendix.

IRON OVERLOAD CAN BE ASSOCIATED WITH a wide range of genetic and environmental factors and can lead to parenchymal damage of organs. Homozygosity for the C282Y mutation of the *HFE* gene is associated with susceptibility to iron overload and is a common genetic mutation, occurring in 0.3 to 0.5 percent of white persons of northern European descent.[1,2] Phlebotomy treatment can prevent some of the major complications of iron overload, and patients have normal life expectancy if they are treated before organ damage occurs.[3] Iron overload can occur in nonwhites and may be related to as-yet-undiscovered genetic mutations, environmental factors, or both.[4-9] The Hemochromatosis and Iron Overload Screening (HEIRS) study was designed to evaluate the prevalence, genetic and environmental determinants, and potential clinical, personal, and societal effects of iron overload and hemochromatosis in a multicenter, multiethnic sample of 101,168 primary care adults 25 years of age or older. This article reports findings from the first, or screening, stage of the study.

METHODS

STUDY PARTICIPANTS

A detailed description of the HEIRS study has previously been reported.[10] Participants were recruited over a two-year period at five field centers (Washington, D.C.; Birmingham, Alabama; Irvine, California; Portland, Oregon, and Honolulu; and London, Ontario, Canada). Our target population was primary care patients, identified through primary care clinics and medical blood-drawing laboratories. Both patients and other persons accompanying the patient were potential participants. We did no advertising to recruit participants. Eligibility criteria included an age of 25 years or older and an ability to understand the informed-consent document. Participants were asked how they heard about the study and whether they had previously been given a diagnosis of iron overload or hemochromatosis.

Measurements included spectrophotometric determination of serum iron and unsaturated iron-binding capacity, turbidimetric immunoassay of serum ferritin (Hitachi 911, Roche), and calculated transferrin saturation on nonfasting blood samples. *HFE* C282Y and H63D alleles were determined from spots of whole blood with the use of a modification of the Invader assay (Third Wave Technologies) that increases the allele-specific fluorescent signal by including 12 cycles of locus-specific polymerase chain reaction before the cleavase reaction. Absence of a detectable C282Y or H63D mutation was designated as wild type.

Race or ethnic group was determined by self-reported answers to two questions, one regarding Hispanic background and one offering a nonexclusive choice of five racial groups: white, black, Asian, Pacific Islander, and American Indian. Participants affirming Hispanic background were classified as Hispanic; otherwise, they were classified according to their response to the question about race. Before receiving the results of any genetic test, all participants were asked whether they had a history of liver disease, diabetes, arthritis, congestive heart failure, impotence, or infertility. Participants with elevations in transferrin saturation and ferritin

Table 1. Prevalence of *HFE* C282Y and H63D Genotypes According to Race or Ethnic Group.*

Race or Ethnic Group	Total No. of Participants	C282Y/C282Y		C282Y/H63D		H63D/H63D	
		No.	Prevalence (95% CI) %	No.	Prevalence (95% CI) %	No.	Prevalence (95% CI) %
White	44,082	281	0.44 (0.42–0.47)	908	2.0 (2.0–2.1)	1029	2.4 (2.3–2.4)
Native American	648	1	0.11 (0.061–0.20)	7	0.77 (0.56–1.1)	7	1.3 (0.98–1.8)
Hispanic	12,459	7	0.027 (0.022–0.032)	48	0.33 (0.30–0.37)	154	1.1 (0.98–1.1)
Black	27,124	4	0.014 (0.012–0.017)	35	0.071 (0.065–0.078)	30	0.089 (0.081–0.097)
Pacific Islander	698	0	0.012 (0.0043–0.032)	0	0.096 (0.055–0.17)	0	0.20 (0.12–0.32)
Asian	12,772	0	0.000039 (0.000015–0.00010)	0	0.0055 (0.0029–0.0093)	29	0.20 (0.17–0.22)
Multiple/unknown	1928	6	—	19	—	21	—
All	99,711	299	—	1017	—	1270	—

levels and all C282Y homozygotes were invited to a clinical examination at a later stage of the study.

A total of 101,168 participants had complete data on serum ferritin levels, transferrin saturation, and *HFE* C282Y and H63D alleles, and 333 participants with complete data were homozygous for the C282Y mutation. After 1457 participants (including 34 C282Y homozygotes) who reported hearing about the study exclusively from a participating family member were excluded from all analyses, 99,711 participants remained. For the analyses involving serum ferritin levels and transferrin saturation, an additional 1182 participants who reported a previous diagnosis of hemochromatosis or iron overload were also excluded to remove possible effects of earlier phlebotomy therapy on iron tests and ascertainment bias, leaving 98,529 participants for these analyses. For the analyses presented, we assumed that all persons were unrelated.

STATISTICAL ANALYSIS

Deviations of observed frequencies of *HFE* genotypes from Hardy–Weinberg proportions were assessed by exact test[11] in the total sample and stratified according to race or ethnic group and field center. The frequencies of *HFE* genotypes were calculated separately in each stratum of race or ethnic group. To account for higher participation rates among C282Y homozygotes, maximum likelihood estimates of the frequencies of *HFE* genotypes were calculated under the assumption of Hardy–Weinberg proportions in the participants within each stratum of race or ethnic group who were not homozygous for the C282Y mutation, regardless of the number of observed C282Y homozygotes. Confidence intervals for frequencies of *HFE* genotypes were calculated on the basis of inverting the score test for a multinomial proportion.[12] Odds ratios for the effect of *HFE* genotype on self-reported medical history were estimated with the use of logistic regression. Estimates were adjusted for age, field center, and race or ethnic group. Age was modeled as a set of indicator variables for each year of age, except that all ages above 80 years were grouped. Comparisons of pairs of means among the 12 sex and genotype combinations were performed with the use of two-way analysis of variance with Scheffé's method for multiple comparisons. For serum ferritin levels, analysis was done after log transformation, and the geometric mean and antilog of the first and third quartiles are reported. For transferrin saturation, mean and standard deviation are reported. Participants with complete data on transferrin saturation, serum ferritin levels, and *HFE* genotype were included in this analysis. Serum ferritin levels below the detectable limit of 15 μg per liter were imputed as 7.5 μg per liter. Transferrin saturation values reported as less than 3 percent were imputed as 1.5 percent.

Race or Ethnic Group	Total No. of Participants	C282Y/+		H63D/+		+/+	
		No.	Prevalence (95% CI) %	No.	Prevalence (95% CI) %	No.	Prevalence (95% CI) %
White	44,082	4548	10 (10–11)	10,537	24 (24–24)	26,779	61 (60–61)
Native American	648	35	5.7 (4.2–7.7)	128	20 (17–22)	470	72 (69–76)
Hispanic	12,459	351	2.9 (2.6–3.2)	2199	18 (18–19)	9700	78 (77–78)
Black	27,124	605	2.3 (2.1–2.5)	1520	5.7 (5.4–6.0)	24,930	92 (92–92)
Pacific Islander	698	15	2.0 (1.2–3.4)	62	8.4 (6.6–11)	621	89 (87–91)
Asian	12,772	16	0.12 (0.074–0.19)	1070	8.4 (8.0–8.9)	11,657	91 (91–92)
Multiple/Unknown	1928	111	—	313	—	1458	—
All	99,711	5681	—	15,829	—	75,615	—

Table 1. (Continued.)

* All participants with complete data on *HFE* C282Y and H63D mutations, transferrin saturation, and serum ferritin levels are included, with the exception of 1457 participants who reported hearing about the study exclusively from a participating family member. Rates of prevalence were derived with Hardy–Weinberg proportions in the five groups of participants not homozygous for the C282Y/C282Y mutation within each racial or ethnic group. CI denotes confidence interval. Race or ethnic group was self-reported.

RESULTS

STUDY POPULATION

The participants included 62,749 women and 36,962 men. The predominance of female participants is representative of primary care populations. Women also have a higher participation rate than men in medical research studies. The median age was 50 years (range, 25 to 100). Among men, 23 percent were 25 to 39 years of age, 23 percent were 40 to 49, 23 percent were 50 to 59, 18 percent were 60 to 69, and 13 percent were older than 69. Among women, 27 percent were 25 to 39 years of age, 24 percent were 40 to 49, 24 percent were 50 to 59, 15 percent were 60 to 69, and 10 percent were older than 69. According to self-identified race or ethnic group, the sample included 44 percent white participants, 27 percent black, 13 percent Asian, 13 per-cent Hispanic, 0.7 percent Pacific Islander, 0.7 percent Native American, and 2 percent of mixed or unknown race.

GENOTYPIC VARIATIONS

Of the 99,711 participants, 299 were homozygous for the C282Y mutation (Table 1). The estimated prevalence of C282Y homozygotes was highest in whites (0.44 percent; 95 percent confidence interval, 0.42 to 0.47) and lowest in Asians (0.000039 percent; 95 percent confidence interval, 0.000015 to 0.00010), Pacific Islanders (0.012 percent; 95 percent confidence interval, 0.0043 to 0.032), and blacks (0.014 percent; 95 percent confidence interval, 0.012 to 0.017); among the latter three groups, the vast majority of participants (>88 percent) had the wild-type genotype. The H63D/+ genotype was common in whites (24 percent), Native Americans (20 percent), and Hispanics (18 percent). In whites, the C282Y/+ genotype was also quite common (10 percent).

These estimates of genotype frequency assume Hardy–Weinberg proportions in the groups of participants who were not homozygous for the C282Y mutation. The estimation procedure accounts for higher participation rates in C282Y homozygotes, who were observed more frequently than expected ($P<0.05$ at four of five field centers among whites).

In the unrestricted sample of 101,168 participants (which included 1457 people who heard about the study only from a participating family member), 333 were C282Y homozygotes, which exceeds the expected number of 233. In the 364 participants without a diagnosis who had a serum ferritin level above 1000 µg per liter, there were 29 C282Y homozygotes, 3 compound heterozygotes, 4 H63D homozygotes, 14 C282Y heterozygotes, 40 H63D heterozygotes, and 274 without *HFE* mutations.

PHENOTYPIC VARIATIONS

Of 1182 participants who reported having previously been told by a doctor that they had increased iron in the blood, 72 were homozygous for the C282Y mutation. These participants in whom conditions related to iron overload had been previously diagnosed were excluded to assess the distributions of transferrin saturation and serum ferritin levels.

The relationships between transferrin saturation and serum ferritin levels among *HFE* genotypes are shown in Figures 1 and 2. Both transferrin saturation and ferritin levels were higher in men than in women. In the initial screening stage of the study,
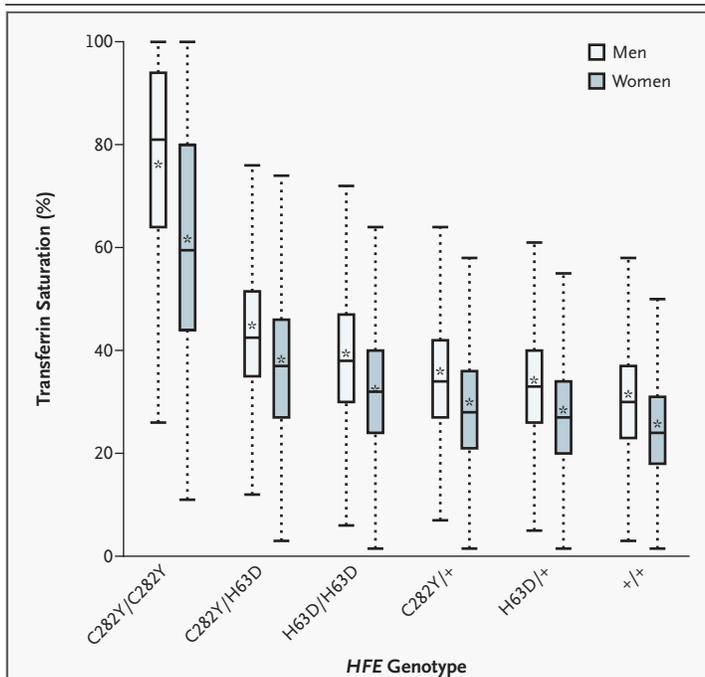

Figure 1. Nonfasting Serum Transferrin Saturation in Men and Women According to Genotype.

An elevated transferrin saturation was defined as higher than 45 percent in women and higher than 50 percent in men. Data are presented as box plots. The box stretches from the 25th to the 75th percentile. The median is shown with a line across the box, and the mean with an asterisk. The whiskers indicate 1.5 times the interquartile range above the third and below the first quartiles, or the upper or lower extreme values, whichever is closer. Participants who joined the study only because they heard about it from a participating family member or who reported a previous diagnosis of hemochromatosis or iron overload were excluded.

we defined the following phenotypic characteristics as elevated: transferrin saturation higher than 50 percent for men and higher than 45 percent for women, and serum ferritin levels greater than 300 μg per liter for men and greater than 200 μg per liter for women. In C282Y homozygotes (89 men and 138 women), transferrin saturation was higher than 50 percent in 84 percent of men and higher than 45 percent in 73 percent of women. The mean (±SD) transferrin saturation in these previously unidentified C282Y homozygotes was 76±22 percent in men and 61±24 percent in women. The geometric mean level of serum ferritin was 698 μg per liter (interquartile range, 511 to 1190) in men and 212 μg per liter (interquartile range, 111 to 529) in women. A serum ferritin level greater than 300 μg per liter was found in 88 percent of untreated male C282Y homozygotes, and a level greater than 200 μg per liter was found in 57 percent of female untreated C282Y homozygotes. A serum ferritin level of more than 1000 μg per liter, which has previously been reported to be associated with liver disease in C282Y homozygotes,[13,14] was seen in 13 percent of C282Y homozygotes. Elevations in serum ferritin levels were common in all other genotypes.

Among male participants, a ferritin level greater than 300 μg per liter was observed in 37 percent of those with the C282Y/H63D genotype, 32 percent of those with the H63D/H63D genotype, 23 percent of those with the C282Y/+ genotype, 24 percent of those with the H63D/+ genotype, and 26 percent of those with the +/+ (wild-type) genotype. Among female participants, a serum ferritin level greater than 200 μg per liter was found in 20 percent of those with the C282Y/H63D genotype, 15 percent of those with the H63D/H63D genotype, 10 percent of those with the C282Y/+ genotype, 11 percent of those with the H63D/+ genotype, and 13 percent of those with the +/+ (wild-type) *HFE* genotype.

Among male C282Y homozygotes, the geometric mean serum ferritin level (698 μg per liter) was significantly higher than the mean serum ferritin levels among males with C282Y/H63D, H63D/H63D, C282Y/+, H63D/+, and +/+ (wild-type) genotypes (range, 163 to 208 μg per liter; P<0.001 for all comparisons with homozygotes). Thus, male C282Y homozygotes had geometric mean serum ferritin levels above 300 μg per liter, whereas geometric means for all other genotype groups were below this value. Geometric mean serum ferritin levels among male participants with the C282Y/H63D, H63D/H63D, C282Y/+, and H63D/+ genotypes were

not significantly different from the levels among male participants with the wild-type genotype (P> 0.12 for all).

Similarly, among female C282Y homozygotes, the geometric mean serum ferritin level (212 μg per liter) was above the screening criteria of 200 μg per liter and significantly greater than the levels among female participants in all other genotype groups (range, 64 to 85 μg per liter; P<0.001 for all comparisons). Female participants with the C282Y/ H63D genotype had significantly greater geometric mean serum ferritin levels than did female participants with the wild-type genotype (P<0.001); however, their geometric mean was much closer to the geometric mean of the wild-type group than to the geometric mean of the C282Y homozygotes (Fig. 2). Geometric mean levels among female participants with the H63D/H63D, C282Y/+, and H63D/+ genotypes were not significantly different from those
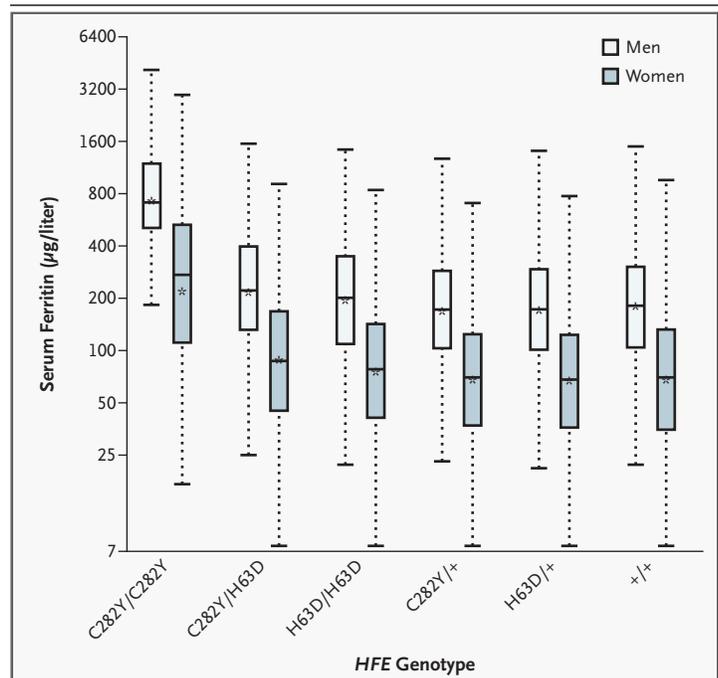

Figure 2. Serum Ferritin Levels in Men and Women According to Genotype.
An elevated serum ferritin level was defined as a level greater than 200 μg per liter in women and greater than 300 μg per liter in men. Data are presented as box plots. The box stretches from the 25th to 75th percentile. The median is shown with a line across the box, and the mean, which is a geometric mean, with an asterisk. The whiskers indicate 1.5 times the interquartile range above the third and below the first quartiles, or the upper or lower extreme values, whichever is closer. Participants who joined the study only because they heard about it from a participating family member or who reported a previous diagnosis of hemochromatosis or iron overload were excluded.

among female participants with the wild-type genotype (P>0.80 for all).

C282Y homozygotes had markedly higher transferrin saturation and serum ferritin levels, as expected. The transferrin saturation values according to sex gradually increased, from *HFE* wild type, to H63D/+, to C282Y/+, to H63D homozygote, and to compound heterozygote. Pairwise comparisons of the genotype means according to sex (Fig. 1) all showed a significant difference (P<0.01).

Descriptive statistics of transferrin saturation and ferritin levels according to race or ethnic group and sex are shown in Table 2. The threshold values used in this table correspond to those used by Ioannou et al.[15] (although we have added ferritin levels greater than 1000 µg per liter) and those used in the HEIRS study to define elevated status.

Among men, a history of liver disease was reported more often in C282Y homozygotes (odds ratio, 3.3; 95 percent confidence interval, 1.5 to 7.2; P=0.003) and compound heterozygotes (odds ratio, 1.7; 95 percent confidence interval, 1.0 to 2.7; P=0.05) than in participants without *HFE* mutations (Table 3). There were no significant differences in the incidence of self-reported diabetes between C282Y homozygotes and participants without *HFE* mutations. Among men, H63D homozygotes were more likely to report a history of arthritis (odds ratio, 1.3; 95 percent confidence interval, 1.0 to 1.6) than were participants without *HFE* mutations. Among women, H63D homozygotes were more likely to report a history of heart disease (odds ratio, 1.5; 95 percent confidence interval, 1.0 to 2.1) than were participants without *HFE* mutations.

DISCUSSION

The HEIRS study included a large group of ethnically and geographically diverse participants. Asberg et al. studied 65,238 Norwegians in a white population,[16] and Beutler et al. studied 41,038 participants from Kaiser San Diego, including 4 percent blacks, 4 percent Asians, and 10 percent Hispanics.[17] Steinberg et al. studied *HFE* mutations in 5171 anonymous samples from the National Health and Nutrition Examination Survey, but no iron tests were reported for correlations between genotype and phenotype.[18]

HFE mutations are most common in white populations, a finding consistent with the theory that hemochromatosis originated in northern Europe.[18-20] The frequency of C282Y genotypes is much lower in nonwhite populations. Despite our efforts to minimize the influence of family members of homozygotes, it is possible that they are still overrepresented in our analysis. In the HEIRS study, the prevalence of C282Y genotypes among Hispanics was higher than among Asians or blacks but lower than among whites. Previous studies have suggested a higher prevalence of C282Y mutations in Hispanic populations than in nonwhite populations.[17] Although H63D genotypes are more common than C282Y genotypes in all ethnic or racial groups and H63D has been considered to be an older mutation that possibly originated in Asia,[21] H63D mutations in our study were less common among Asians than among most other racial or ethnic groups. The prevalence of H63D genotypes was highest among whites. The prevalence among Hispanics was lower than among whites but considerably higher than among blacks and Asians. Among Asians, the prevalence of H63D genotypes was higher than among blacks but the prevalence of C282Y genotypes was lower than among blacks.

Serum ferritin levels and transferrin saturation were significantly higher in C282Y homozygotes than in those with other genotypes. In participants who were homozygous for the C282Y mutation but in whom iron overload had not previously been diagnosed, an elevated ferritin level was found in 88 percent of men and 57 percent of women, suggesting that many adults who are homozygous for the C282Y mutation may have iron overload.

The prevalence of self-reported liver disease was significantly greater in male C282Y homozygotes and compound heterozygotes than in participants without *HFE* mutations. Liver disease is one of the most well-established of the complications of iron overload, and iron-depletion therapy by means of phlebotomy has been shown to stabilize liver disease and prevent the progression to cirrhosis, which adversely affects long-term survival.[3] The self-reported history of liver disease is nonspecific and could encompass a broad range of liver diseases, including alcoholic liver disease, chronic viral hepatitis, and fatty liver, but we do not believe that these would be overrepresented in the C282Y homozygotes.[22,23] We believe that the increased self-reporting of liver disease in C282Y homozygotes points to a potential opportunity to treat and prevent the progression of liver disease.

Of 98,529 participants who had not previously been diagnosed with hemochromatosis or iron overload, 364 (0.4 percent) had a serum ferritin lev-

Table 2. Prevalence of Elevated Iron Levels According to Race or Ethnic Group.*

Iron Test	No. of Participants (N=98,529)	Native American (N=645)	Asian (N=12,672)	Black (N=26,847)	Hispanic (N=12,337)	Multiple (N=1444)	Pacific Islander (N=689)	Unknown (N=442)	White (N=43,453)
					percent ±SE				
Ferritin levels >400 μg/liter (men) or >300 μg/liter (women)	8652	6.36±0.96	19.00±0.35	9.67±0.18	5.26±0.20	12.53±0.87	25.40±1.66	7.69±1.27	5.91±0.11
Ferritin levels >500 μg/liter (men) or >400 μg/liter (women)	4816	3.26±0.70	11.26±0.28	5.37±0.14	2.69±0.15	7.76±0.70	16.69±1.42	4.52±0.99	3.10±0.08
Ferritin levels >600 μg/liter (men) or >500 μg/liter (women)	2777	1.71±0.51	6.54±0.22	3.27±0.11	1.46±0.11	4.78±0.56	11.76±1.23	2.71±0.77	1.65±0.06
Ferritin levels >1000 μg/liter	364	0.62±0.31	0.58±0.07	0.53±0.04	0.28±0.05	0.69±0.22	1.31±0.43	0.45±0.32	0.20±0.02
Transferrin saturation >45%	7763	6.67±0.98	13.97±0.31	4.52±0.13	5.93±0.21	6.72±0.66	6.68±0.95	7.24±1.23	8.81±0.14
Transferrin saturation >50%	4322	3.72±0.75	7.68±0.24	2.55±0.10	3.37±0.16	3.60±0.49	3.05±0.65	4.30±0.96	4.91±0.10
Transferrin saturation >60%	1497	1.71±0.51	2.33±0.13	1.06±0.06	0.92±0.09	1.52±0.32	1.31±0.43	1.13±0.50	1.74±0.06
Ferritin levels >400 μg/liter (men) or >300 μg/liter (women), and transferrin saturation >45%	1732	0.93±0.38	4.43±0.18	1.34±0.07	1.08±0.09	2.08±0.38	2.90±0.64	1.13±0.50	1.42±0.06
Ferritin levels >500 μg/liter (men) or >400 μg/liter (women), and transferrin saturation >45%	812	0.47±0.27	1.87±0.12	0.75±0.05	0.47±0.06	0.90±0.25	1.02±0.38	0.23±0.23	0.67±0.04
Ferritin levels >400 μg/liter (men) or >300 μg/liter (women), and transferrin saturation >50%	356	0.31±0.22	0.59±0.07	0.39±0.04	0.21±0.04	0.48±0.18	0.44±0.25	0.23±0.23	0.31±0.03
Ferritin levels >300 μg/liter (men) or >200 μg/liter (women), and transferrin saturation >50% (men) or >45% (women)	1958	1.24±0.44	4.84±0.19	1.44±0.07	1.34±0.10	1.87±0.36	3.05±0.65	1.36±0.55	1.68±0.06

* An elevated ferritin level was defined in this study as greater than 300 μg per liter in men and greater than 200 μg per liter in women. An elevated transferrin saturation was higher than 45 percent in women and higher than 50 percent in men. A serum ferritin level greater than 1000 μg per liter has previously been reported to be associated with liver disease in C282Y homozygotes.[13,14] Participants who joined the study only because they heard about it from a participating family member, or who reported a previous diagnosis of hemochromatosis or iron overload, were excluded. The data are presented as prevalence estimates (percentages) with standard errors.

el greater than 1000 μg per liter, and previous studies of C282Y homozygotes have shown that the risk of cirrhosis increases as the ferritin level increases to above 1000 μg per liter.[13,14] However, this subgroup with a serum ferritin level greater than 1000 μg per liter contained only 29 C282Y homozygotes. Our observation that Pacific Islanders are much more

likely to have serum ferritin levels greater than 1000 μg per liter than whites has not to our knowledge been previously reported (Table 2).

The increase in self-reported arthritis in male H63D homozygotes and heart disease in female H63D homozygotes (Table 3) cannot readily be explained by iron overload, since most participants

Table 3. Association between *HFE* Genotypes and Medical Conditions Related to Iron Overload.*

Self-Diagnosis and Genotype	Men (N=36,474)		Women (N=62,055)	
	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value
Arthritis		<0.001		<0.001
C282Y/C282Y	0.99 (0.59–1.66)		1.10 (0.75–1.62)	
C282Y/H63D	0.83 (0.64–1.08)		0.86 (0.71–1.03)	
H63D/H63D	1.28 (1.03–1.59)†		1.02 (0.86–1.21)	
C282Y/+	1.07 (0.96–1.20)		1.01 (0.93–1.10)	
H63D/+	1.02 (0.95–1.10)		1.01 (0.96–1.07)	
No C282Y or H63D mutation	1.00		1.00	
Diabetes		0.003		<0.001
C282Y/C282Y	1.06 (0.57–1.99)		0.80 (0.43–1.50)	
C282Y/H63D	0.91 (0.67–1.25)		0.79 (0.59–1.06)	
H63D/H63D	0.89 (0.68–1.17)		1.13 (0.90–1.42)	
C282Y/+	0.81 (0.71–0.94)†		1.05 (0.93–1.17)	
H63D/+	0.98 (0.90–1.07)		0.97 (0.91–1.05)	
No C282Y or H63D mutation	1.00		1.00	
Liver disease‡		0.14		0.41
C282Y/C282Y	3.28 (1.49–7.22)†		0.60 (0.15–2.44)	
C282Y/H63D	1.65 (1.00–2.73)†		0.82 (0.45–1.49)	
H63D/H63D	0.71 (0.39–1.31)		1.13 (0.72–1.78)	
C282Y/+	1.12 (0.86–1.45)		1.23 (0.98–1.54)	
H63D/+	1.06 (0.90–1.25)		0.92 (0.79–1.07)	
No C282Y or H63D mutation	1.00		1.00	
Heart disease		0.74		0.02
C282Y/C282Y	0.62 (0.23–1.73)		0.26 (0.04–1.87)	
C282Y/H63D	1.08 (0.74–1.58)		0.90 (0.55–1.47)	
H63D/H63D	1.00 (0.71–1.41)		1.47 (1.04–2.07)†	
C282Y/+	0.88 (0.74–1.06)		1.10 (0.91–1.33)	
H63D/+	0.89 (0.79–1.01)		0.96 (0.85–1.09)	
No C282Y or H63D mutation	1.00		1.00	
Impotence or infertility		0.005		0.04
C282Y/C282Y	1.42 (0.69–2.91)		1.09 (0.53–2.24)	
C282Y/H63D	1.00 (0.68–1.48)		0.85 (0.57–1.26)	
H63D/H63D	1.13 (0.81–1.57)		0.99 (0.70–1.41)	
C282Y/+	0.85 (0.71–1.02)		0.93 (0.78–1.11)	
H63D/+	0.96 (0.86–1.08)		0.95 (0.85–1.07)	
No C282Y or H63D mutation	1.00		1.00	

* CI denotes confidence interval. P values for the genotype effect are from logistic-regression models and indicate the probability of observing these differences in prevalence if there is no effect of the C282Y or H63D allele. Odds ratios are adjusted for nonlinear age, field center, and race or ethnic group. Participants who joined the study only because they heard about it from a participating family member were excluded.
† This odds ratio is significant at P<0.05.
‡ Liver disease may include all types of liver disease.

with this genotype had normal serum ferritin levels. Arthritis is the symptom in hemochromatosis that has not shown a relationship with body iron stores.[24] These observations may be related to linkage disequilibrium in the HLA complex.

The observation that self-reported diabetes is not more common in C282Y homozygotes (Table 3) is surprising, since hemochromatosis was originally called bronze diabetes. However, the participants who were excluded from our study because their iron overload had previously been diagnosed may have had more advanced disease. Diabetes has also become more common in the general population with the high prevalence of obesity.

Our findings are similar to the findings of Beutler, who also found an increase in the prevalence of liver disease but no increase in diabetes, arthritis, or heart disease in C282Y homozygotes.[17] Our results also illustrate the nonspecific nature of many of the symptoms that have been attributed to hemochromatosis. In C282Y homozygotes with normal serum ferritin levels, iron accumulation may develop over time, but other studies have shown that a progressive rise in iron stores is not inevitable.[25-28] Elevated iron levels were more common in participants with the C282Y/H63D and H63D/H63D genotypes than in participants without *HFE* mutations, although the mean transferrin saturation and ferritin levels were below the upper limit of the reference range for those with all genotypes except C282Y homozygotes.

Nongenetic factors also contribute to the differences between ethnic and racial groups. Elevations in ferritin levels are commonly seen in patients who have obesity-related steatohepatitis,[29] chronic alcohol consumption, or hepatitis B or C.[30-32] Therefore, the results of our screening should not be considered indicative of the prevalence of iron overload in this primary care population.

APPENDIX

REFERENCES

1. Bacon BR. Hemochromatosis: diagnosis and management. Gastroenterology 2001;120:718-25.
2. Pietrangelo A. Hereditary hemochromatosis — a new look at an old disease. N Engl J Med 2004;350:2383-97.
3. Niederau C, Fischer R, Purschel A, Stremmel W, Haussinger D, Strohmeyer G. Long-term survival in patients with hereditary hemochromatosis. Gastroenterology 1996;110:1107-19.

4. Gordeuk VR, Boyd RD, Brittenham GM. Dietary iron overload persists in rural sub-Saharan Africa. Lancet 1986;1:1310-3.
5. Gordeuk V, Caleffi A, Corradini E, et al. Iron overload in Africans and African-Americans and a common mutation in the SCL40A1 (ferroportin 1) gene. Blood Cells Mol Dis 2003;31:299-304.
6. Barton JC, Acton RT, Rivers CA, et al. Genotypic and phenotypic heterogeneity of African Americans with primary iron

overload. Blood Cells Mol Dis 2003;31:310-9.
7. Eason RJ, Adams PC, Aston CE, Searle J. Familial iron overload with possible autosomal dominant inheritance. Aust N Z J Med 1990;20:226-30.
8. Oliver M, Scully L, Guiraudon C, Adams PC. Non-HLA-linked hemochromatosis in a Chinese woman. Dig Dis Sci 1995;40:1589-91.
9. Kng C, Ng FH, Ng WF, et al. A Chinese

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patient with non-HFE linked iron overload. J Clin Gastroenterol 2001;33:69-71.

10. McLaren CE, Barton JC, Adams PC, et al. Hemochromatosis and Iron Overload Screening (HEIRS) study design for an evaluation of 100,000 primary care-based adults. Am J Med Sci 2003;325:53-62.

11. Weir BS. Genetic data analysis II: methods for discrete population genetic data. Sunderland, Mass.: Sinauer, 1996.

12. Cox DR, Hinkley DV. Theoretical statistics. London: Chapman & Hall, 1974.

13. Beaton M, Guyader D, Deugnier Y, Moirand R, Chakrabarti S, Adams P. Noninvasive prediction of cirrhosis in C282Y-linked hemochromatosis. Hepatology 2002; 36:673-8.

14. Morrison ED, Brandhagen DJ, Phatak PD, et al. Serum ferritin level predicts advanced hepatic fibrosis among U.S. patients with phenotypic hemochromatosis. Ann Intern Med 2003;138:627-33. [Erratum, Ann Intern Med 2003;139:235.]

15. Ioannou GN, Dominitz JA, Weiss NS, Heagerty PJ, Kowdley KV. The effect of alcohol consumption on the prevalence of iron overload, iron deficiency, and iron deficiency anemia. Gastroenterology 2004;126: 1293-301.

16. Asberg A, Hveem K, Thorstensen K, et al. Screening for hemochromatosis: high prevalence and low morbidity in an unselected population of 65,238 persons. Scand J Gastroenterol 2001;36:1108-15.

17. Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T. Penetrance of the 845G to A (C282Y) HFE hereditary haemochromatosis mutation in the USA. Lancet 2002;359:211-8.

18. Steinberg KK, Cogswell ME, Chang JC, et al. Prevalence of C282Y and H63D mutations in the hemochromatosis (HFE) gene in the United States. JAMA 2001;285:2216-22.

19. Lucotte G, Dieterlen F. A European allele map of the C282Y mutation of hemochromatosis: Celtic versus Viking origin of the mutation? Blood Cells Mol Dis 2003;31: 262-7.

20. Merryweather-Clarke AT, Pointon JJ, Jouanolle AM, Rochette J, Robson KJ. Geography of HFE C282Y and H63D mutations. Genet Test 2000;4:183-98.

21. Rochette J, Pointon JJ, Fisher CA, et al. Multicentric origin of hemochromatosis gene (HFE) mutations. Am J Hum Genet 1999;64:1056-62. [Erratum, Am J Hum Genet 1999;64:1491.]

22. Adams PC, Agnew S. Alcoholism in hereditary hemochromatosis revisited: prevalence and clinical consequences among homozygous siblings. Hepatology 1996;23: 724-7.

23. Bacon BR, Olynyk JK, Brunt EM, Britton RS, Wolff RK. HFE genotype in patients with hemochromatosis and other liver diseases. Ann Intern Med 1999;130:953-62.

24. Adams PC, Deugnier Y, Moirand R, Brissot P. The relationship between iron overload, clinical symptoms, and age in 410 patients with genetic hemochromatosis. Hepatology 1997;25:162-6.

25. Yamashita C, Adams PC. Natural history of the C282Y homozygote for the hemochromatosis gene (HFE) with a normal serum ferritin level. Clin Gastroenterol Hepatol 2003;1:388-91.

26. Olynyk JK, Hagan SE, Cullen DJ, Beilby J, Whittall DE. Evolution of untreated hereditary hemochromatosis in the Busselton population: a 17-year study. Mayo Clin Proc 2004;79:309-13.

27. Andersen RV, Tybjaerg-Hansen A, Appleyard M, Birgens H, Nordestgaard BG. Hemochromatosis mutations in the general population: iron overload progression rate. Blood 2004;103:2914-9.

28. Olynyk JK, Cullen DJ, Aquilia S, Rossi E, Summerville L, Powell LW. A population-based study of the clinical expression of the hemochromatosis gene. N Engl J Med 1999; 341:718-24.

29. Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology 1999;30:1356-62.

30. Di Bisceglie AM, Axiotis CA, Hoofnagle JH, Bacon BR. Measurements of iron status in patients with chronic hepatitis. Gastroenterology 1992;102:2108-13.

31. Riggio O, Montagnese F, Fiore P, et al. Iron overload in patients with chronic viral hepatitis: how common is it? Am J Gastroenterol 1997;92:1298-301.

32. Tung BY, Emond MJ, Bronner MP, Raaka SD, Cotler SJ, Kowdley KV. Hepatitis C, iron status, and disease severity: relationship with HFE mutations. Gastroenterology 2003;124:318-26.

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