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Serum ferritin concentrations and body iron stores in a multicenter, multiethnic primary-care population

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Abstract

How often elevated serum ferritin in primary-care patients reflects increased iron stores (normally 0.8 g in men, 0.4 g in women) is not known. The Hereditary Hemochromatosis and Iron Overload Screening (HEIRS) study screened 101,168 primary-care participants (44% Caucasians, 27% African-Americans, 14% Asians/Pacific Islanders, 13% Hispanics, 2% others). Follow-up clinical evaluation was performed in 302 of 333 *HFE* C282Y homozygotes regardless of iron measures and 1,375 of 1,920 nonhomozygotes with serum ferritin >300 μg/L (men), >200 μg/L (women) and transferrin saturation >50% (men), >45% (women). Quantitative phlebotomy was conducted in 122 of 175 C282Y homozygotes and 122 of 1,102 nonhomozygotes with non-transfusional serum ferritin elevation at evaluation. The estimated prevalence in the Caucasian population of C282Y homozygotes with serum ferritin >900 μg/L at evaluation was 20 per 10,000 men and 4 per 10,000 women; this constellation was predictive of iron stores >4 g in men and >2 g in women. The estimated prevalence per 10,000 of non-C282Y homozygotes with serum ferritin >900 μg/L at evaluation was 7 among Caucasians, 13 among Hispanics, 20 among African Americans, and 38 among Asians and Pacific Islanders, and this constellation was predictive of iron stores >2 g but <4 g. In conclusion, serum ferritin >900 μg/L after initial elevations of both serum ferritin and transferrin saturation is predictive of mildly increased iron stores in multiple

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ethnic populations regardless of *HFE* genotype. Serum ferritin >900 μg/L in male C282Y homozygotes is predictive of moderately increased iron stores.

Introduction

The body does not have a physiologic mechanism to excrete excess iron. Therefore, increased iron stores develop in individuals who receive repeated blood transfusions in the absence of blood loss or who absorb from the diet more iron than is needed to replace the small obligatory losses of 1–1.5 mg/day [1]. Excessive iron absorption occurs as a result of systemic conditions characterized by ineffective erythropoiesis, or increased production and death within the bone marrow of red blood cell precursors [2]. Excessive absorption also occurs because of mutations in a variety of genes whose products transport or regulate the transport of iron across the intestinal mucosa [3,4]. *HFE* C282Y homozygosity is the most common hereditary basis for increased body iron stores in individuals of northern European ancestry [5–7]. Despite recent advances in understanding the molecular basis of iron-loading disorders, the prevalence and consequences of increased iron stores in the population are not known.

Serum ferritin concentration reflects body storage iron, [8] and increased iron stores are often suspected when the serum ferritin concentration is elevated. Among *HFE* C282Y homozygotes, serum ferritin concentrations have been shown to correlate fairly well with the amount of storage iron present as determined by quantitative phlebotomy [9]. However, by far most serum ferritin elevations occur among non-C282Y homozygotes, especially in multiethnic populations [10], and the correlation of serum ferritin with iron stores is less clear among such patients even when the clinical suspicion of iron overload is high [9]. Infectious and other inflammatory processes and hepatic disorders such as alcoholic and viral hepatitis and nonalcoholic steatohepatitis are potential causes of elevated serum ferritin despite normal iron stores [11–18]. Nonalcoholic steatohepatitis is a consequence of insulin resistance with histological findings ranging from fat deposition in hepatocytes plus balloon degeneration to fat plus sinusoidal fibrosis and polymorphonuclear infiltrates, with or without Mallory hyaline [19]. Therefore, among primary-care patients in general it is not known how often an elevated serum ferritin represents increased iron stores.

The Hereditary Hemochromatosis and Iron Overload Screening (HEIRS) study is a multicenter study designed to determine the prevalence of primary iron overload in adult primary-care patients of various ethnicities in the United States and Canada [20]. A total of 101,168 participants were screened by testing for *HFE* C282Y and H63D mutations and by measuring serum ferritin and transferrin saturation. The prevalences of C282Y homozygosity and of elevated serum ferritin and transferrin saturation at initial screening have been reported [10]. Participants with C282Y homozygosity and/or combined elevations of serum ferritin and transferrin saturation at initial screening were invited to return for a clinical evaluation to determine whether these findings indicated increased iron stores. This report addresses the prevalence of elevated serum ferritin at this clinical evaluation, the estimation of iron stores by phlebotomy in patients with elevated serum ferritin at this evaluation, and the associations of serum ferritin and iron stores with liver function tests and markers of inflammation. These findings are analyzed to determine whether they differ by ethnicity and by *HFE* genotype, Normal body iron stores have been generally regarded to be about 1.0 g in adult men and about 0.3 g in adult women of child-bearing age [1]. The HEIRS study prospectively defined increased iron stores as >2.0 g (2–4 g, mildly increased; 4–10 g, moderately increased; 10–20 g, substantially increased; and >20 g, severely increased).

Results

Participation in clinical evaluation and confirmed elevation of serum ferritin (Table I)

Of 101,168 multiethnic primary-care participants screened at five centers in the US and Canada, 333 (0.3%) were C282Y homozygotes, two-thirds of whom had serum ferritin elevation (>200 μg/L in women, >300 μg/L in men) at initial screen, and 1,920 (1.9%) were non-C282Y homo-zygotes with combined elevations of serum ferritin and transferrin saturation (>45% women, >50% men). Patients with elevated iron stores (and also patients with hepatic disorders) tend to have elevated transferrin saturation in association with increased serum ferritin [17,18], whereas patients with inflammatory processes typically have reduced serum iron concentration and transferrin saturation in association with increased serum ferritin [16]. Therefore, 15,619 non-C282Y homozygotes who had elevated serum ferritin but nonelevated transferrin saturation at initial screen were not eligible for a clinical evaluation. Ninety-one percent of the C282Y homozygotes and 72% of the nonhomozygotes with elevations in both serum ferritin and trans-ferrin saturation returned for a clinical evaluation. At this evaluation, 59% of the C282Y homozygotes versus 82% of the nonhomozygotes had elevated serum ferritin, but all of the nonhomozygotes had had elevated serum ferritin at the initial screening per study design. Rates of persistent serum ferritin elevation in nonhomozygotes were similar among various ethnic groups and *HFE* genotypes.

Blood transfusions and elevated serum ferritin

Two of 177 C282Y homozygotes (1.1%) versus 32 of 1,134 non-C282Y homozygotes (2.8%) with elevated serum ferritin at clinical evaluation had a history of more than 10 blood transfusions ($P = 0.3$). One of 43 C282Y homozygotes (2.3%) versus 24 of 142 non-C282Y homozygotes (16.9%) with serum ferritin > 900 μg/L at clinical evaluation had a history of more than 10 blood transfusions ($P = 0.028$). Participants with a history of more than 10 blood transfusions were classified as having elevated serum ferritin on the basis of blood transfusions.

Clinical findings in participants with nontransfusional elevation of serum ferritin (Table II)

Participants with nontransfusional elevation in serum ferritin at the clinical evaluation were further classified according the degree of elevation of serum ferritin as well as the results of liver function tests, C-reactive protein (CRP) concentrations, and hemoglobin levels. Although the criteria for eligibility for clinical evaluation for non-C282Y homozygotes were designed to exclude patients with inflammation as the cause of elevated serum ferritin, CRP was determined at the clinical evaluation to gauge the effectiveness of this approach. Liver enzymes were increased in 24% of the C282Y homozygotes and 44% of the nonhomozygotes and these increases were strongly associated with serum ferritin >900 μg/L in both groups. Serum CRP concentration was increased and/or anemia was present in the setting of normal liver enzyme levels in 30% of the C282Y homozygotes and 15% of the nonhomozygotes. Isolated elevation in serum ferritin (i.e., normal values for serum ferritin, CRP and hemoglobin) were found in 46% of the C282Y homozygotes and 41% of the nonhomozygotes at the clinical evaluation. Among non-C282Y homozygotes, the highest prevalence of elevated hepatic enzymes in association with increased serum ferritin occurred among African Americans and Hispanics (62%) and the lowest prevalence among Caucasians (37%). The highest prevalence of elevated CRP or anemia occurred among African Americans (21%) and the lowest prevalence among Asians and Pacific Islanders (8%). The highest prevalence of isolated elevation of serum ferritin occurred among Asians and Pacific Islanders (54%) and the lowest prevalence among African Americans (18%).

In multivariate analyses among the C282Y homozygotes, elevation of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) significantly associated with serum ferritin concentration >900 μg/L ($P < 0.001$) but not with estimated alcohol consumption. Serum ferritin concentration >900 μg/L significantly associated with elevation of ALT or AST and male sex ($P \leq 0.003$ for each variable) but not with estimated alcohol consumption. In multivariate analyses among the nonhomozygotes, elevated ALT or AST significantly associated with female sex, younger age, greater estimated daily alcohol consumption, serum ferritin concentration >900 μg/L, and higher hemoglobin concentration ($P \leq 0.007$ for each variable). Serum ferritin concentration >900 μg/L significantly associated with male sex, lower hemoglobin concentration, and the presence of elevated ALT or AST ($P < 0.001$ for each variable) but not estimated alcohol consumption.

Estimated population prevalence of elevated serum ferritin (Table III)

Based on observed rates of elevated serum ferritin at the clinical evaluation and model-derived weights to account for differential participation rates, the prevalence of nontransfusional elevations of serum ferritin in the population screened by HEIRS could be estimated. The prevalence of serum ferritin >900 μg/L per 10,000 population in association with *HFE* C282Y homozygosity was estimated to be 20 for Caucasian men and 4 for Caucasian women. The prevalence per 10,000 population of serum ferritin >900 μg/L following combined elevations of serum ferritin and transferrin saturation in association with non-C282Y homozygosity was estimated to be 38 among Asians and Pacific Islanders, 20 among African Americans, 13 among His-panics, and 7 among Caucasians.

Participation in quantitative phlebotomy

Of clinical evaluation participants who were potentially eligible for quantitative phlebotomy based on elevated serum ferritin at this evaluation, C282Y homozygotes participated in phlebotomy more than non-C282Y homozygotes (70% versus 11%; $P < 0.0001$). Among non-C282Y homozygotes, participation rates differed significantly by ethnicity ($P = 0.013$): 14% for African Americans and Caucasians, 8% for Asians and Pacific Islanders, 7% for Hispanics. Overall, serum ferritin was significantly greater in C282Y homozygotes and in non-C282Y homozygotes who participated in quantitative phlebotomy compared with those who did not ($P < 0.0001$) (Table IV). However, among African-Americans and Hispanic non-C282Y homozygotes, serum ferritin did not differ significantly according to participation in quantitative phlebotomy. Overall, individuals with serum ferritin >900 μg/L participated in phlebotomy more than those with serum ferritin 200/300–900 μg/L: 84% versus 68% for male C282Y homozygotes ($P = 0.162$), 90% versus 62% for female C282Y homozygotes ($P = 0.155$), 24% versus 10% for male nonhomozygotes ($P = 0.0002$) and 26% versus 9% for female nonhomozygotes ($P = 0.006$).

Quantification of iron stores by phlebotomy

Relationship of iron stores at quantitative phlebotomy to serum ferritin at clinical evaluation among participants who completed quantitative phlebotomy—

Figure 1 shows the relationships of serum ferritin concentration at clinical evaluation to iron stores estimated by quantitative phlebotomy among *HFE* C282Y homozygotes who completed the quantitative phlebotomy program, stratified by men and women. The relationships were highly significant ($P < 0.0001$ for men and $P = 0.0006$ for women) with coefficient of determination of 0.35 for men and 0.29 for women. Sixteen of 39 (41%) male C282Y homozygotes who completed phlebotomy therapy were observed to have mobilizable iron stores >4 g versus three of 37 (8%) female C282Y homozygotes ($P = 0.002$). Substantial proportions of both men (33%) and women (51%) had stores < 2g. In

addition to the results depicted in Fig. 1, 10 of 27 men (37%) but none of 19 women who did not complete phlebotomy had stores >4 g.

Significant relationships of serum ferritin concentration at clinical evaluation to iron stores estimated by quantitative phlebotomy were also observed among non-C282Y homozygotes of both sexes who completed the quantitative phlebotomy program. The coefficient of variation was 0.50 among 34 Caucasians ($P < 0.0001$), 0.51 among 7 African Americans ($P = 0.070$), 0.43 among 4 Hispanics ($P = 0.35$), and 0.20 among 20 Asians and Pacific Islanders ($P = 0.047$). None of the non-C282Y homozygote Caucasians, African Americans, Hispanics or Asians, and Pacific Islanders who completed phlebotomy were observed to have >4 g of mobilizable iron. Overall, 31% of these non-C282Y homozygotes were observed to have >2 g mobilizable iron, and according to ethnicity this ranged from 0 of 4 Hispanics to 9 of 34 Caucasians (26%), 2 of 7 African Americans (29%), and 6 of 20 Asians and Pacific Islanders (30%). In addition, among participants who did not complete the phlebotomy program, 4 of 19 Caucasians (21%), 2 of 24 African Americans (8%), 0 of 2 Hispanics, and 0 of 11 Asians and Pacific Islanders had >2 g of iron removed before the program ended.

Kaplan–Meier estimates of proportions with elevated iron stores among all phlebotomy participants, whether or not phlebotomy completed (Tables V and VI)—Iron stores > 2 g were significantly more common among 66 male C282Y homozygotes than among 56 female C282Y homozygotes, 75 male non-C282Y homozygotes, and 47 female non-C282Y homozygotes who underwent quantitative phlebotomy ($P < 0.001$). More iron was mobilized in participants with serum ferritin >900 μg/L than those with serum ferritin 200/300–900 μg/L. Iron stores > 2 g determined by phlebotomy in participants with serum ferritin >900 μg/L versus <900 μg/L were estimated to be 100% versus 63% among male C282Y homozygotes ($P < 0.001$), 85% versus 54% among female C282Y homozygotes ($P = 0.01$), 80% versus 20% among male non-C282Y homozygotes ($P < 0.001$), and 67% versus 34% among female nonhomozygotes ($P = 0.02$). The presence of liver enzyme elevations, serum CRP elevation, decreased hemoglobin concentration, and/or (for non-C282Y homozygotes) the presence of an *HFE* mutation did not have a significant effect on the amount of iron mobilized by phlebotomy.

Discussion

Whether an elevated serum ferritin concentration represents increased iron stores versus inflammation or abnormal liver function is a common question that confronts both primary-care physicians and specialists. Our results suggest that, among multiethnic primary-care patients in the United States and Canada, serum ferritin >900 μg/L in C282Y homozygotes or following initial elevations of serum ferritin and transferrin saturation in nonhomozygotes is highly predictive of body iron stores >2 g regardless of *HFE* genotype, ethnicity, gender, or elevations of ALT, AST, or CRP. Serum ferritin >900 μg/L is highly predictive of body iron stores >4 g in male C282Y homozygotes but not in female C282Y homozygotes and male or female nonhomozygotes. Our results further suggest that serum ferritin 200–900 μg/L (women) or 300–900 μg/L (men) in C282Y homozygotes or following initial elevations of serum ferritin and transferrin saturation is associated with iron stores >2 g in about one-half of C282Y homozygotes and about one-third of nonhomozygotes. Interestingly, our results predict that about 40% of primary-care male C282Y homo-zygotes with serum ferritin 300–900 μg/L have iron stores >4 g.

Among participants in the clinical evaluation, serum ferri-tin concentrations >900 μg/L strongly associated with elevated concentrations of ALT or AST and with male sex in both C282Y homozygotes and nonhomozygotes but not with estimated alcohol consumption. An

important feature of our study is that we attempted to clarify the meaning of the indirect iron status measure, elevated serum ferritin, with the objective measure of quantitative phlebotomy in the context of a large population-based investigation. Similar to the phlebotomy study of Beutler et al. published in 2002 [9], we found fairly robust correlations between serum ferritin and mobilizable iron stores among male and female *HFE* C282Y homozygotes (see Fig. 1) and among Caucasian, African-American, and Hispanic nonhomozygotes, although the numbers completing phlebotomy were small for the African Americans and Hispanics. The weakest but still statistically significant correlation was found among the Asians and Pacific Islanders.

Limitations to our results include the following: (1) The design of this study stipulated that non-C282Y homozygote participants would only be evaluated for a potential primary increase in body iron stores if they had elevation of both serum ferritin and transferrin saturation at the initial screening stage. This approach therefore excluded any individuals with increased body iron who had a decrease in transferrin saturation because of inflammation, diurnal variation, or other causes at the initial screening and any individuals with an iron-loading process not accompanied by the degree of transferrin saturation elevation specified. (2) Larger proportions of C282Y homozygotes participated in phlebotomy than nonhomozygotes. (3) Proportions of participants continuing phlebotomy to iron depletion out of those starting phlebotomy (62% for C282Y homozygotes and 53% for nonhomozygotes) were lower than compliance rates reported in hemochromatosis patients in routine medical care (93% and 100%, respectively) [21,22]. Nevertheless, by using Kaplan–Meier statistical procedures, all participants contributed to the estimates of iron stores, whether or not they completed phlebotomy.

The quantitative phlebotomy results suggest that elevated serum ferritin in primary-care C282Y homozygotes or after combined elevations of serum ferritin and transferrin saturation in nonhomozygotes raises the possibility of increased iron stores whether or not there are elevations in serum levels of liver enzymes or CRP or concomitant anemia. In particular, the presence or absence of these conditions that may elevate serum ferritin independently of iron stores did not affect the probability that elevated serum ferritin is associated with increased iron stores (Tables V and VI).

Historical estimates of iron stores, using quantitative phlebotomy with iron deficiency anemia as the endpoint and adjusting for decline in hemoglobin in calculations, indicated that median storage iron was 0.8 g (5 percentile to 95 percentile range of 0.6–1.2 g) among 39 normal men predominantly in the third and fourth decades of life and 0.4 g (0.2–0.6 g) among 20 normal women of similar age [23–27]. This study did not did not adjust for decline in hemoglobin concentration in calculating iron stores, but the participants were not phlebotomized to iron deficiency anemia but rather to serum ferritin <50 μg/L, suggesting that there was a minimal drop in hemoglobin concentration and that substantial iron stores remained in many of the subjects at the end of the phlebotomy program. Therefore, our definition of increased body iron stores as >2.0 g for the HEIRS study represents a substantial increase above the normal range, and our individual estimates of iron stores are probably conservative.

Given the foregoing considerations and the fact that present participants were more likely to undergo phlebotomy therapy if serum ferritin was >900 μg/L, the population prevalences of serum ferritin >900 μg/L in Table III may represent minimum point estimates for the prevalences of increased iron stores in the primary-care population. The estimated prevalence of serum ferritin >900 μg/L in association with C282Y homozygosity was 20 per 10,000 among Caucasian men and 4 per 10,000 among Caucasian women. The estimated prevalence of serum ferritin >900 μg/dL in association with non-C282Y homozygosity

ranged from 38 per 10,000 among Asians and Pacific Islanders to 7 per 10,000 among Caucasians. While only male C282Y homozygotes were observed to have a substantial prevalence of more than a mild degree of increased iron stores, many of these individuals came to light as a result of genotyping all screening participants for C282Yand high participation in clinical evaluation among C282Y homozygotes. We therefore cannot rule out the possibility that undiagnosed iron-loading mutations, perhaps ethnic-specific, also cause moderate to marked increases in iron stores.

A mild increase in iron stores of the magnitude seen in this study in female C282Y homozygotes and male and female non-C282Y homozygotes with elevated serum ferritin and transferrin saturation on screening has uncertain clinical significance. Such elevations of body iron stores are associated with symptomatic porphyria cutanea tarda [28] and some studies suggest such elevations may be associated with a general increased risk of cancer [29], the development of hepatocellular carcinoma in the absence of cirrhosis [30], and an increased risk of diabetes mellitus [31,32]. The causes of the nontransfusional increased iron stores in the non-C282Y homozygotes in our study are not clear. It seems possible that the increases are at least sometimes primary in nature, because of mutations in iron metabolism-related genes other than the *HFE* gene.

Methods

Initial screening

Descriptions of the design [10,20,33] of the HEIRS study have been published. Participants >25 years of age were screened over a 2-year period (February 2001 to March 2003) at five Field Centers (Washington, DC; Birmingham, AL; Irvine, CA; Portland, OR – Honolulu, HI; London, Ontario, Canada) at primary-care clinics and medical blood drawing laboratories. Race/ethnicity was determined by self-reported answers to two questions: one asking about Hispanic background and one for nonexclusive choice of five racial groups: Caucasian, African-American, Asian, Pacific Islander, and American Indian. Serum ferritin concentration, transferrin saturation and *HFE* C282Y and H63D alleles were determined as described [10]. The HEIRS study defined elevated serum measurements of iron status as serum ferritin >300 μg/L for men and >200 μg/L for women and transferrin saturation >50% for men and >45% for women.

Clinical evaluation

All C282Y homozygotes (n = 333) and non-C282Y homozygotes with elevations in initial screening serum ferritin and transferrin saturation (n = 1,920) were invited to participate in a clinical evaluation. Participants in the clinical evaluation gave written informed consent, in addition to the consent obtained in the initial screening part of the study. Medical history was recorded on a standardized form. In addition to repeat testing of serum ferritin and transferrin saturation (usually on fasting blood samples), participants were tested for ALT, AST, and CRP with a Hitachi 911 analyzer (Roche Diagnostics/Boehringer Mannheim Corp., Indianapolis, IN). To quantify average daily alcohol consumption over the preceding year, participants filled out the University of Hawaii Multiethnic Dietary Questionnaire [34]. The questionnaire was analyzed at the University of Hawaii. The mean ± SD number of days between screening evaluation and the clinical evaluation was 257 ± 204.

Follow-up with quantitative phlebotomy

At the discretion of evaluating physicians at each field center, C282Y homozygotes with elevated serum ferritin at clinical evaluation regardless of screening serum ferritin and nonhomozygotes with persistently elevated serum ferritin at clinical evaluation were advised to consider a quantitative phlebotomy program; in some cases the patients' personal

physicians were also so advised. The recommended phlebotomy program consisted of the weekly removal of one unit of blood until the serum ferritin concentration decreased to less than 50 μg/L. Phlebotomy was done at the discretion of the personal physicians and at the patient's or their third party payer's expense. For some eligible participants enrolled at the University of California, Irvine, quantitative phlebotomy was performed at the General Clinical Research Center under an approved research protocol. The HEIRS study monitored compliance with these recommendations with informed consent from participants.

The total amount of iron mobilized by a patient's phlebotomy program was calculated in the following manner. The amount of iron removed at each phlebotomy from the first phlebotomy until the phlebotomy that resulted in serum ferritin <50 μg/L was summed. Typically the phlebotomies were performed at weekly intervals; however the amount of iron removed was included in the sum as long as the phlebotomy was performed <56 days after the preceding phlebotomy. The amount of iron removed at a phlebotomy session was calculated on the basis of the volume of blood removed, the hemoglobin concentration, and an assumed 3.46 mg iron per gram of hemoglobin. If the hemoglobin concentration was not available, a unit of blood was assumed to contain 0.2 g of iron. For the present report, participants who had initiated phlebotomy therapy prior to participation in the HEIRS study were excluded from the analysis of quantitative phlebotomy.

Statistical methods

Serum ferritin values below the detection limit of 15 μg/L were imputed as 7.5 μg/L. Transferrin saturation values reported as less than 3% were imputed as 1.5%. If participants with elevated serum ferritin at clinical evaluation had received more than 10 lifetime blood transfusions, they were categorized as having increased body iron stores because of multiple blood transfusions. The remaining patients with elevated serum ferritin at clinical evaluation were placed in one of the following categories: (1) elevated liver function tests (ALT >40 U/L in men, >31 U/L in women, and/or AST >37 U/L in men, >31 U/L in women with any values for CRP and hemoglobin); (2) elevated CRP and/or anemia (CRP > 0.5 μg/dL and/or hemoglobin <13.2 g/dL in men or <11.6 g/dL in women but ALT and AST normal); or (3) isolated elevation in serum ferritin (CRP, hemoglobin, AST and ALT all within reference range). Participants were also categorized as to whether or not the serum ferritin was >900 μg/L at clinical evaluation, and, for non-C282Y homozygotes only, whether any *HFE* mutation was present. Proportions were compared with Yate's corrected Pearson χ^2.

The population prevalence of nontransfusional serum ferritin elevation was estimated using data collected from participants who attended a clinical evaluation visit. A semiparametric estimate of the population prevalence was derived from the probability of attending the clinical evaluation and the probability of elevated serum ferritin at the clinical evaluation to produce an estimate of the prevalence among all those screened [35].

The amount of iron removed to achieve serum ferritin < 50 μg/L was analyzed by Kaplan–Meier procedures. The proportions of patients undergoing phlebotomy who required removal of ≥2.0 g iron was estimated according to the presence or absence of C282Y homozygosity and also according to three categories of increased serum ferritin defined earlier. For C282Y homozygotes the proportion requiring removal of ≥4.0 g was also estimated. Log rank statistics were used to test for differences between groups defined by gender, liver enzyme elevation, presence of CRP elevation or anemia, serum ferritin >900 μg/L, and, in non-C282Y-homzogotes, presence of *HFE* mutation on the estimated amount of iron mobilized by phlebotomy.

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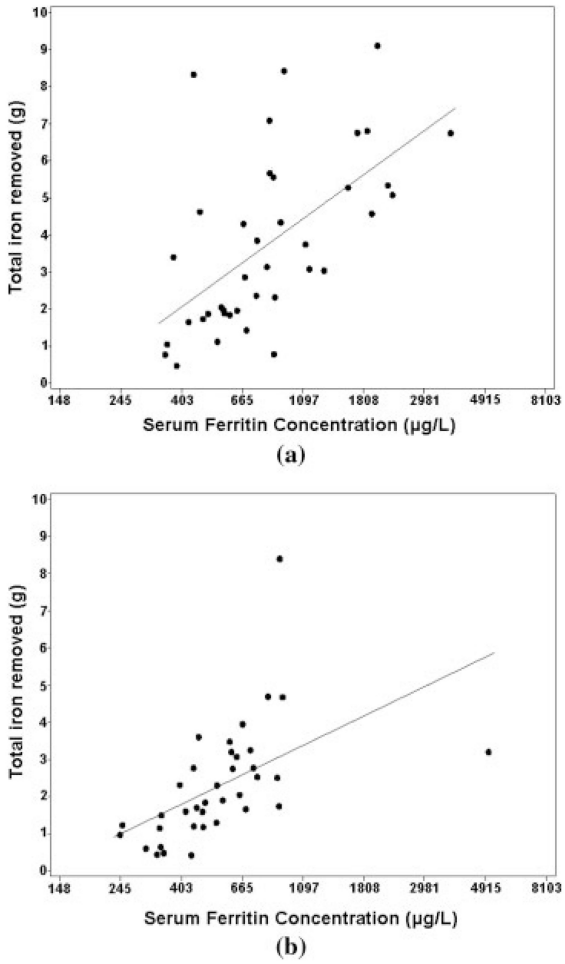

Figure 1.

Scatter plots of total iron removed versus serum ferritin concentration at clinical evaluation with linear regression line among (a) the C282Y homozygote men who completed the quantitative phlebotomy program ($N = 39$, R^2 5 0.35, $P < 0.0001$) and (b) the C282Y homozygote women who completed the quantitative phlebotomy program ($N = 37$, R^2 5 0.29, $P = 0.0006$).

Table I

Participation in and Biochemical Markers of Iron Status at Clinical Evaluation

	No. in population screened	No. eligible for clinical evaluation[3]	No. participating in clinical evaluation	Both serum ferritin and transferrin saturation elevated (no., row %)	Serum ferritin elevated but transferrin saturation not elevated (no., row %)	Transferrin saturation elevated but serum ferritin not elevated (no., row %)	Neither serum ferritin nor transferrin saturation elevated (no., row %)
A. C282Y homozygotes[a]							
Overall	101,168	333	302	155 (51.3)	22 (7.3)	75 (24.8)	50 (16.6)
Caucasian	44,808	315	285	147 (51.6)	21 (7.4)	69 (24.2)	48 (16.8)
African American	27,224	4	4	1 (25.0)	0	2 (50.0)	1 (25.0)
Hispanic	12,696	7	6	3 (50.0)	1 (16.7)	2 (33.3)	0
Asian and Pacific Islander	13,836	0	0	0	0	0	0
Native American	652	1	1	0	0	0	1 (100)
Other	1,952	6	6	4 (66.7)	0	2 (33.3)	0
B. Non-C282Y homozygotes[a]							
Overall	101,168	1,920	1,375	529 (38.5)	605 (44.0)	54 (3.9)	187 (13.6)
(1) By ethnicity							
Caucasian	44,808	643	476	184 (38.7)	202 (42.4)	27 (5.7)	63 (13.2)
African American	27,224	405	284	105 (37.0)	126 (44.4)	6(2.1)	47 (16.6)
Hispanic	12,696	168	120	45 (37.5)	39 (32.5)	13 (10.8)	23 (19.2)
Asian and Pacific Islander	13,836	664	466	182 (39.1)	227 (48.7)	7(1.5)	50 (10.7)
Native American	652	8	4	2 (50.0)	2 (50.0)	0	0
Other	1,952	32	25	11 (44.0)	9 (36.0)	1 (4.0)	4 (16.0)
(2) By *HFE* genotype[b]							
C282Y/H63D	1,063	104	87	49 (56.3)	27 (31.0)	8 (9.2)	3 (3.5)
H63D/H63D	1,294	64	55	20 (36.4)	22 (40.0)	2 (3.6)	11 (20.0)
C282Y/+	5,835	143	110	51 (46.4)	38 (34.6)	1 (0.9)	20 (18.2)
H63D/+	16,062	335	231	86 (37.2)	102 (44.2)	12 (5.2)	31 (13.4)
+/+	76,581	1,274	892	323 (36.2)	416 (46.6)	31 (3.5)	122 (13.7)

[a]For C282Y homozygotes, serum ferritin and transferrin saturation may or may not be elevated on screening; for non-C282Y homozygotes, both serum ferritin and transferrin saturation were elevated on screening,

[b] indicates *HFE* wildtype.

Table II

Clinical Findings in Participants With Non-Transfusional Elevation in Serum Ferritin at Clinical Evaluation

	All patients	Serum ferritin 200/300 to 900 μg/L	Serum ferritin >900 μg/L	*p*[a]
A. C282Y homozygotes				
N	175	133	42	
Elevated hepatic enzymes[b]	42 (24.0)	21 (15.8)	21 (50.0)	<0.0001
↑ CRP[c] and/or anemia[d] but normal liver enzymes	53 (30.3)	47 (35.3)	6 (14.3)	0.017
Isolated serum ferritin elevation[e]	80 (45.7)	65 (48.9)	15 (35.7)	0.166
B. Non-C282Y homozygotes				
All ethnicities				
N	1,102	984	118	
Elevated hepatic enzymes[b]	489 (44.4)	405 (41.2)	84 (71.2)	<0.0001
↑ CRP[c] and/or anemia[d] but normal liver enzymes	165 (15.0)	151 (15.3)	14 (11.9)	0.387
Isolated serum ferritin elevation[e]	448 (40.7)	428 (43.5)	20 (16.9)	<0.0001
Caucasians				
N	381	356	25	
Elevated hepatic enzymes[b]	141 (37.0)	125 (35.1)	16 (64.0)	0.007
↑ CRP[c] and/or anemia[d] but normal liver enzymes	75 (19.7)	70 (19.7)	5 (20.0)	1.000[f]
Isolated serum ferritin elevation[e]	165 (43.3)	161 (45.2)	4 (16.0)	0.008
African Americans				
N	213	174	39	
Elevated hepatic enzymes[b]	131 (61.5)	101 (58.1)	30 (76.9)	0.045
↑ CRP[c] and/or anemia[d] but normal liver enzymes	44 (20.7)	38 (21.8)	6 (15.4)	0.496
Isolated serum ferritin elevation[e]	38 (17.8)	35 (20.1)	3 (7.7)	0.110
Hispanics				
N	82	70	12	
Elevated hepatic enzymes[b]	51 (62.2)	41 (58.6)	10 (83.3)	0.189
↑ CRP[c] and/or anemia[d] but normal liver enzymes	12 (14.6)	11 (15.7)	1 (8.3)	0.684[f]
Isolated serum ferritin elevation[e]	19 (23.2)	18 (25.7)	1 (8.3)	0.278[f]
Asians and Pacific Islanders				
N	402	364	38	
Elevated hepatic enzymes[b]	155 (38.6)	130 (35.7)	25 (65.8)	0.001
↑ CRP[c] and/or anemia[d] but normal liver enzymes	31 (7.7)	29 (8.0)	2 (5.3)	0.755[f]
Isolated serum ferritin elevation[e]	216 (53.7)	205 (56.3)	11 (29.0)	0.002
Others				
N	24	20	4	
Elevated hepatic enzymes[b]	11 (45.8)	8 (40.0)	3 (75.0)	0.300[f]

	All patients	Serum ferritin 200/300 to 900 μg/L	Serum ferritin >900 μg/L	P[a]
↑ CRP[c] and/or anemia[d] but normal liver enzymes	3 (12.5)	3 (15.0)	0	1.000[f]
Isolated serum ferritin elevation[e]	10 (41.7)	9 (45.0)	1 (25.0)	0.615[f]

Results given as no. (column %). *P* value for comparison of serum ferritin 200–900 μg/L for women or 300–900 μg/L for men versus >900 μg/L for women and men.

[a] The continuity adjusted χ^2 test.

[b] Elevated hepatic enzymes: men, ALT >40 U/L or AST >37; women, ALT or AST >31 U/L.

[c] ↑ CRP: >0.5 mg/dL

[d] Anemia: hemoglobin ≤ 13.2 g/dL in men; ≤11.6 g/dL in women.

[e] Isolated serum ferritin elevation: normal CRP, hemoglobin, and hepatic enzyme concentrations.

[f] The Fisher exact test.

Table III

Estimated Population Prevalences of Non-Transfusional Elevation of Serum Ferritin at Clinical Evaluation

	No. screened	Estimated prevalence of serum ferritin >200/300 (per 10,000)[a]	Estimated prevalence of serum ferritin >900 μg/L (per 10,000)[a]
A. Elevated serum ferritin associated with C282Y homozygosity			
Caucasian men	17,323	54 (42, 65)	20 (13, 27)
Caucasian women	27,485	32 (25, 40)	4 (1, 6)
B. Elevated serum ferritin associated with non-C282Y homozygotes (initial screening showed elevations of both serum ferritin and transferrin saturation)			
Caucasians	44,808	115 (104, 126)	7 (4, 11)
African Americans	27,224	112 (97, 126)	20 (13, 28)
Hispanics	12,696	90 (71, 109)	13 (5, 21)
Asians and Pacific Islanders	13,130	419 (382, 457)	38 (24, 52)

[a] 95% confidence interval is given in parentheses.

Table IV

Serum Ferritin (μg/L) According to Participation in Quantitative Phlebotomy of Those Potentially Eligible

	N	Did not participate in quantitative phlebotomy	*N*	Participated in quantitative phlebotomy	*P*
C282Y homozygotes					
All	53	493 (281–863)	122	735 (395–1,366)	<0.0001
Non-C282Y homozygotes[a]					
All	980	493 (296–821)	122	665 (380–1,164)	<0.0001
Caucasians	328	446 (276–721)	53	545 (337–880)	0.002
African Americans	183	545 (299–992)	30	735 (392–1,380)	0.059
Hispanics	76	545 (293–1,012)	6	446 (324–614)	0.594
Asians and Pacific Islanders	371	446 (284–699)	31	812 (508–1,300)	<0.0001

Results in geometric mean and SD range. Those potentially eligible had elevated serum ferritin at the clinical evaluation. The natural logarithm transformation was applied to serum ferritin values. The SD range is defined as the antilog of mean ± 1 standard deviation (SD) of the transformed data.

[a]Of 24 non-C282Y homozygote participants of other ethnic groupings who were potentially eligible for phlebotomy, only two participated in quantitative phlebotomy; they are not included in the table to protect the identity of individual participants.

Table V

Assessment of Iron Stores by Quantitative Phlebotomy in C282Y Homozygotes With Non-Transfusional Elevation in Serum Ferritin

	Started quantitative phlebotomy	Completed quantitative phlebotomy	Est. Prop. (SE) > 2 g	Est. Prop. (SE) > 4 g
Men				
Overall (N = 89)	66	39	0.79 (0.05)	0.59 (0.07)
By serum ferritin (P < 0.001)				
300–900 μg/L (N = 57)	39	26	0.63 (0.08)	0.40 (0.09)
>900 μg/L (N = 32)	27	13	1.00 (0.00)	0.86 (0.08)
By liver enzymes (P = 0.14)				
Normal (N = 62)	45	28	0.70 (0.07)	0.49 (0.08)
Elevated (N = 27)	21	11	0.95 (0.05)	0.78 (0.10)
By increased CRP or anemia (P = 0.99)				
Absent (N = 64)	49	29	0.83 (0.06)	0.61 (0.08)
Present (N = 25)	17	10	0.66 (0.12)	0.51 (0.13)
Women				
Overall (N = 86)	56	37	0.59 (0.07)	
By serum ferritin (P = 0.01)				
200-900 μg/L (N = 76)	47	33	0.54 (0.08)	
>900 μg/L (N = 10)	9	4	0.85 (0.14)	
By liver enzymes (P = 0.98)				
Normal (N = 71)	47	31	0.55 (0.08)	
Elevated (N = 15)	9	6	0.75 (0.16)	
By increased CRP or anemia (P = 0.25)				
Absent (N = 46)	29	21	0.56 (0.10)	
Present (N = 40)	27	16	0.62 (0.11)	

Table VI

Assessment of Iron Stores by Quantitative Phlebotomy in Non-C282Y Homozygotes With Persistent Non-Transfusional Elevation in Serum Ferritin

	Started qph	Completed qph	Est. Prop. (SE) > 2 g
Men			
Overall (N = 653)	75	47	0.42 (0.07)
By serum ferritin (P < 0.001)			
300–900 μg/L (N = 570)	55	36	0.27 (0.07)
>900 μg/L (N = 83)	20	11	0.80 (0.10)
By liver enzymes (P = 0.53)			
Normal (N = 369)	31	21	0.41 (0.10)
Elevated (N = 284)	44	26	0.42 (0.09)
By increased CRP or anemia (P = 0.65)			
Absent (N = 510)	61	43	0.41 (0.07)
Present (N = 143)	14	4	0.45 (0.21)
By *HFE* mutation (P = 0.24)			
Absent (N = 419)	36	22	0.42 (0.10)
Present (N = 234)	39	25	0.42 (0.09)
Women			
Overall (N = 449)	47	18	0.42 (0.10)
By serum ferritin (P = 0.02)			
<900 μg/L (N = 414)	38	16	0.34 (0.11)
>900 μg/L (N = 35)	9	2	0.67 (0.19)
By liver enzymes (P = 0.20)			
Normal (N = 244)	21	9	0.31 (0.15)
Elevated (N = 205)	26	9	0.50 (0.13)
By increased CRP or anemia (P = 0.69)			
Absent (N = 298)	35	12	0.46 (0.12)
Present (N = 205)	12	6	0.32 (0.16)
By *HFE* mutation (P = 0.08)			
Absent (N = 292)	24	11	0.26 (0.13)
Present (N = 157)	23	7	0.58 (0.14)