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Characteristics of participants with self-reported hemochromatosis or iron overload at HEIRS Study initial screening

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Abstract

There are few descriptions of young adults with self-reported hemochromatosis or iron overload (H/IO). We analyzed initial screening data in 7,343 HEmochromatosis and IRon Overload Screening (HEIRS) Study participants ages 25–29 years, including race/ethnicity and health information; transferrin saturation (TS) and ferritin (SF) measurements; and *HFE* C282Y and H63D genotypes. We used denaturing high-pressure liquid chromatography and sequencing to

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detect mutations in *HJV*, *TFR2*, *HAMP*, *SLC40A1*, and *FTL*. Fifty-one participants reported previous H/IO; 23 (45%) reported medical conditions associated with H/IO. Prevalences of reports of arthritis, diabetes, liver disease or liver cancer, heart failure, fertility problems or impotence, and blood relatives with H/IO were significantly greater in participants with previous H/IO reports than in those without. Only 7.8% of the 51 participants with previous H/IO reports had elevated TS; 13.7% had elevated SF. Only one participant had C282Y homozygosity. Three participants aged 25–29 years were heterozygous for potentially deleterious mutations in *HFE2*, *TFR2*, and *HAMP* promoter, respectively. Prevalences of self-reported conditions, screening iron phenotypes, and C282Y homozygosity were similar in 1,165 participants aged 30 years or greater who reported previous H/IO. We conclude that persons who report previous H/IO diagnoses in screening programs are unlikely to have H/IO phenotypes or genotypes. Previous H/IO reports in some participants could be explained by treatment that induced iron depletion before initial screening, misdiagnosis, or participant misunderstanding of their physician or the initial screening questionnaire.

Introduction

Iron overload in some adults less than age 30 years is associated with increased iron absorption and mutations in genes that encode proteins of iron absorption and metabolism, including *HFE*, hemojuvelin (*HJV*), hepcidin (*HAMP*), transferrin receptor-2 (*TFR2*), and ferroportin (*SLC40A1*) [1–10]. Other young adults have iron overload associated with heritable types of anemia that require chronic erythrocyte transfusion [11–17]. Mutations in the 5′ untranslated region (UTR) of the ferritin light chain gene (*FTL*) can mimic hemochromatosis or iron overload (H/IO) phenotypes in young persons [18,19]. Although few *HFE* C282Y homozygotes under the age of 30 years have severe H/IO phenotypes [20,21], there are no reports of the phenotype and H/IO genotype characteristics of young adults in H/IO screening programs who report previous diagnoses of H/IO.

The HEmochromatosis and IRon Overload Screening (HEIRS) Study [22] is a cross-sectional study of attendees of primary care clinics. Initial screening data included responses to a health questionnaire; transferrin saturation (TS); serum ferritin concentration (SF); and *HFE* mutation analysis to detect C282Y and H63D alleles [22]. There were 7,343 evaluable participants aged 25–29 years of all race/ethnicity groups, 51 of whom reported previous diagnoses of H/IO. In the present analysis, we (a) tabulated medical and family history reports, iron phenotypes, and *HFE* genotypes at initial screening of the 51 participants with previous H/IO reports; (b) compared self-reported medical conditions and iron phenotypes of these 51 participants with those of participants without previous H/IO reports; (c) used denaturing high-performance liquid chromatography (DHPLC) and sequencing to detect mutations in H/IO-associated genes other than *HFE* in 40 of the 51 participants aged 25–29 years with previous H/IO reports to assess self-reports and future risk of H/IO; and (d) compared the prevalences of self-reported medical conditions, screening iron phenotypes, and C282Y homozygosity in participants 25–29 years with those of 1,165 participants aged 30 years or older who also reported a previous diagnosis of H/IO. The present results and their implications for H/IO diagnosis and screening are discussed.

Results

General characteristics of 7,343 HEIRS Study participants aged 25–29 years

There were 7,343 participants ages 25–29 years: 1,685 participants (468 men, 1,217 women) reported Hispanic race/ethnicity, 845 of whom (50.2%) did not submit additional responses that they had non-Hispanic race/ethnicity; 5,658 participants (1,706 men, 3,952 women) reported no Hispanic race/ethnicity, including 2,323 whites; 2,291 blacks; 815 Asians; 49

Native Americans; 37 Hawaiians or other Pacific Islanders; and 104 participants who reported multiple race/ethnicity. Thirty-nine non-Hispanic participants did not provide more specific race/ethnicity responses. The predominance of female participants in the present study is representative of the entire HEIRS Study cohort of 99,771 participants [23]. Details of the 16 *HFE* C282Y homozygotes ages 25–29 years identified in the Study are described elsewhere [21].

Initial screening reports of participants aged 25–29 years who reported previous diagnoses of hemochromatosis or iron overload

This cohort comprised 51 participants (11 men, 40 women) (Table I). The proportion of women with previous H/IO reports was significantly greater than the proportion of women in the 7,343 participants aged 25–29 years (78.4% vs. 60.2%, respectively; $P = 0.008$). Sixteen of 51 participants reported white, 17 reported black, and 13 reported Hispanic race/ethnicity (Table I). Initial screening phenotypes of the 51 participants are summarized in Table I. The percentage of participants aged 25–29 years with previous H/IO reports was 0.73% (51/6,980); 363 participants did not respond to this question. This percentage (0.73%) was significantly lower than the percentage of participants >30 years old with previous H/IO reports (1.30%; 1,165/89,688) ($P < 0.0001$); 4,137 participants ≥30 years old did not respond to this question.

Twenty-three of 51 participants (45.1%) aged 25–29 years reported that they had abnormalities associated with iron overload; three men (27.3%) and six women (15.0%) reported that they had three or more of these abnormalities. Seven men (63.6%) and 21 women (52.5%) did not report having any of these abnormalities. Twelve participants (23.5%) reported that they had one or more blood relatives with H/IO (Table I). The prevalences of reports of the following abnormalities were significantly greater in participants aged 25–29 years with reports of previous H/IO than in participants aged 25–29 years without reports of previous H/IO: arthritis; diabetes; liver disease or liver cancer; heart failure; and fertility problems or impotence (Table II). The greatest OR values were observed in association with reports of liver disease or liver cancer, and with reports of heart failure (41.1 and 40.0, respectively). OR values greater than unity were also observed with reports of arthritis, diabetes, and fertility problems or impotence. The prevalence of reports of blood relatives with H/IO was significantly greater in participants aged 25–29 years with reports of previous H/IO than in participants aged 25–29 years without reports of previous H/IO ($P < 0.0001$; OR = 7.9; CI 4.0, 15.8) (Table II).

Initial screening iron phenotypes of participants aged 25–29 years who reported previous diagnoses of hemochromatosis or iron overload

Mean TS and SF values in these 51 participants aged 25–29 years (Table I) were within the respective reference ranges for the HEIRS Study. One of the 11 men (9.1%) had elevated TS; three men (27.3%) had elevated SF. Three of the 40 women (7.5%) had elevated TS; four women (10.0%) had elevated SF (Table I). The proportions of men and women who had elevated TS or SF did not differ significantly. The mean SF levels were higher in men than in women (Table II), consistent with observations in the entire HEIRS Study cohort [23]. Participants aged 25–29 years had a greater risk of elevated SF (OR = 3.1; $P = 0.0109$) than did HEIRS Study participants without previous H/IO reports in an analysis stratified by age (nearest year), sex, and Field Center (Table II). Regardless, only 7.8% of the 51 participants with reports of previous H/IO had elevated TS and only 13.7% had elevated SF.

HFE genotypes of participants who reported previous diagnoses of hemochromatosis or iron overload

Fifty participants aged 25–29 years with reports of previous H/IO had *HFE* genotypes C282Y/H63D ($n = 2$), C282Y/wt ($n = 2$), H63D/wt ($n = 14$), and wt/wt ($n = 32$). The remaining participant was a white man with *HFE* genotype C282Y/C282Y[21]. The prevalences of C282Y homozygosity in the 15 white participants with reports of previous H/IO and in the 2,323 white participants ages 25–29 years were not significantly different (0.0625 vs. 0.0065, respectively; $P = 0.1043$, Fisher exact test). Corresponding nonsignificant results were also observed in other race/ethnicity groups (data not shown).

HJV, *HAMP*, *TFR2*, *SLC40A1*, and *FTL* mutation analyses of participants who reported previous diagnoses of hemochromatosis or iron overload

Forty of 51 participants aged 25–29 years with reports of previous H/IO gave written consent for DNA analysis in addition to *HFE* genotyping performed for initial screening. We evaluated these 40 participants for mutations of non-*HFE* H/IO-associated genes using DHPLC technique; none had C282Y homozygosity. These 40 participants did not qualify for evaluation after initial screening, in accordance with the HEIRS Study design [22].

A black woman with TS 29%, SF 50 μg/L, and *HFE* genotype wt/wt was heterozygous for the potentially deleterious *HJV* missense mutation 929C→G (exon 4; Ala310Gly) [24]. An Asian man with TS of 45%, SF 428 μg/L, and *HFE* genotype wt/wt was heterozygous for *HJV* −202G→A (exon 1); this mutation appears to be undescribed previously. A woman who reported both Native American and white race/ethnicity had TS 16%, SF 17 μg/L, and *HFE* genotype wt/wt; she was a double heterozygote for the *HAMP* promoter mutation −443C→T [25] and the potentially deleterious *TFR2* missense mutation 565G→A (exon 4; Asp189Asn), neither of which has been reported previously. An Asian man with TS 71%, SF 430 μg/L, and *HFE* genotype wt/wt was heterozygous for a previously unreported, potentially deleterious +274C→T mutation in the 3′ UTR of *HAMP*. A Hispanic man with TS 25% and SF 178 μg/L was heterozygous for a previously unreported mutation in the intervening sequence of *FTL* (103 −36C→A).

Twenty-six of the 40 participants had the synonymous *SLC40A1* polymorphism Val221Val (exon 6, 663T→C); 15 were heterozygotes and 10 were homozygotes. Race/ethnicity reported by these 26 participants were: 10 white; 7 black; 4 Hispanic; 2 white and Native American; 2 white and Hispanic; and 1 Asian. This common SNP (rs2304704) has been previously reported to occur in persons of various racial backgrounds with and without H/IO phenotypes [25–29]. Three participants had a synonymous mutation in *FTL* exon 2 (163T→C; Leu55Leu) (rs8108882) [29]; a black woman and a white woman were heterozygous, and a Hispanic woman was homozygous for this allele. No mutations were detected in the 5′ UTR region of *FTL* which contains the iron regulatory element.

Comparisons of participants aged 25–29 years with those ages 30 years or greater who reported previous diagnoses of hemochromatosis or iron overload

Observations were available in 1,165 participants aged 30 years or greater who reported a previous diagnosis of H/IO (Table I). Liver disease or liver cancer, diabetes mellitus, arthritis, heart failure, and fertility problems or impotence were reported by many participants with previous diagnoses of H/IO in participants of both age groups (Table I). Significant odds ratios were greatest for liver disease or liver cancer and lowest for diabetes mellitus; other reported conditions were associated with intermediate odds ratios in both age groups (Table II). Odds ratios for elevated transferrin saturation values were significant only in participants aged 30 years or greater, whereas odds ratios of elevated serum ferritin concentrations were significant in both age groups (Table II). The proportions of younger

and older participants who reported a previous history of H/IO who had C282Y homozygosity did not differ significantly (1/51 vs. 81/1,165, respectively; $P = 0.1277$, Fisher's exact test). The proportions of younger and older C282Y homozygotes who reported having a previous history of H/IO did not differ significantly (1/16 vs. 81/317, respectively; $P = 0.0630$, Fisher's exact test).

Discussion

Many of the 51 participants aged 25–29 years with previous H/IO reports also reported previous diagnoses of arthritis, diabetes mellitus, elevated body mass index, liver disease, or liver cancer, heart failure, or fertility problems or impotence, but these common conditions are not caused exclusively by H/IO. With the exception of liver disorders [30], the prevalences of these conditions are not significantly increased in persons with C282Y homozygosity identified in H/IO screening [31–33]. Herein, participants with reports of previous H/IO reported a significantly higher prevalence of blood relatives with H/IO than did participants without reports of previous H/IO. In contrast, family histories of hemochromatosis reported by hemochromatosis probands diagnosed in medical care were no more discriminative for the diagnosis of hemochromatosis than were the family histories of patients with chronic liver disorders [34]. The patterns of self-reported conditions, iron phenotypes at initial screening, and prevalence of C282Y homozygosity in participants 30 years of age or older were similar to those of participants aged 25–29 years. Altogether, our observations suggest that some medical conditions or family histories reported by the present participants are mistaken, or are not due to H/IO. It was beyond the scope of the HEIRS Study initial screening to review preexisting medical records or otherwise evaluate participants for self-reported medical conditions, or to evaluate their family members to ascertain the possible occurrence of heritable conditions [22].

Few of the 51 HEIRS Study participants aged 25–29 years who reported previous diagnoses of H/IO had elevated TS and SF at initial screening, although their aggregate risk of elevated SF (but not TS) was significantly increased. Diagnosis and treatment of H/IO before initial screening could partly explain these observations. Many common medical conditions such as liver disorders or diabetes also cause elevated TS or SF [30,31,33,35–47] which can lead to misdiagnosis of H/IO [30,45,48]. The HEIRS Study initial screening form did not permit reporting of factors needed to assess iron nutrition and balance, including diet, iron intake, reproductive and menstrual history, anemia, receipt of erythrocyte transfusion, therapeutic phlebotomy, liver or bone marrow biopsy, blood donation, or illness associated with blood loss [22]. The initial screening iron phenotypes of the 51 participants aged 25–29 years who reported previous diagnoses of H/IO are not characteristic of persons with heritable types of anemia that require chronic erythrocyte transfusion [11–17]. Participants with reports of previous H/IO were not invited to undergo HEIRS Study post-initial screening examinations unless they had elevated values of both TS and SF, or were *HFE* C282Y homozygotes [22]. Accordingly, there are no additional substantive observations pertinent to these 51 participants.

HFE genotypes other than C282Y homozygosity were observed in 98% of the 51 participants aged 25–29 years with previous H/IO reports. Two participants aged 25–29 years had *HFE* C282Y/H63D, but the risk of iron overload in persons with this genotype is low, and the severity of iron overload, if present, is usually markedly less than that in C282Y homozygotes [49–51]. Although digenic inheritance of *HFE* C282Y/H63D and *TFR*2 Q317X was associated with a severe early-onset H/IO phenotype [52], we did not identify any additional H/IO mutations in the two present participants aged 25–29 years with C282Y/H63D. Some participants aged 25–29 years had *HFE* C282Y or H63D heterozygosity, genotypes that are common in many race/ethnicity groups [23,53]. Because

these genotypes are not associated with significantly increased risk for H/IO [23,50], their occurrence in most persons with H/IO phenotypes is probably coincidental.

We detected potentially deleterious mutations in *HJV*, *HAMP* promoter, and *TFR*2 in only three participants aged 25–29 years. Each mutation occurred in a heterozygous configuration, and each of these three participants had *HFE* genotype wt/wt. One African American had *HJV* 929C→G (A310G), the allele frequency of which is similar in African Americans with and without iron overload (0.02 and 0.07, respectively) [24]. Further, most heterozygotes for *HFE*2 (*HJV*) missense mutations, including A310G, have normal iron phenotypes [3,4,24]. One woman was a double heterozygote for *HAMP* −443C→T and *TFR*2 565G→A. Digenic inheritance of mutations in H/IO-associated genes can result in a severe H/IO phenotype [52,54], but simple heterozygosity for *HAMP* −443C→T alone did not appear to modify expression of TS or SF [25]. An Asian man was heterozygous for a potentially deleterious +274C→T mutation in the 3′ UTR of *HAMP*. In contrast, most persons with early age-of onset H/IO have two deleterious mutations of *HJV*, *HAMP*, or *TFR*2, respectively [3,4,6–9,55–57]. Mutations in the 5′ UTR of *FTL* were not detected in any of 40 participants and were uncommon in another large population survey [58]. Although 5′ UTR *FTL* mutations cause hyperferritinemia (and cataract), they do not cause iron overload [58,59]. It was not possible to test any participant for all possible mutations of *HJV*, *HAMP*, *TFR*2, or *SLC40A*1. Likewise, we cannot exclude the occurrence of rare *HFE* or ALAS2 alleles in the present participants, although such mutations have been reported in some young adults with H/IO phenotypes [1,60–63]. Taken together, these observations demonstrate that mutations in the regions of *HFE*2 (*HJV*), *TFR*2, *HAMP*, and *SLC40A*1 (*FPN1*) that we analyzed, or in 5′ UTR of *FTL*, do not account for H/IO diagnoses reported by most HEIRS Study participants ages 25–29 years without C282Y homozygosity. This is consistent with previous, largely negative results of analyses of multiple H/IO-associated genes in adults without C282Y homozygosity who have unexplained H/IO phenotypes [2,24,25,64].

Physicians in practice who encounter patients with a history of previous H/IO typically review their medical records and interview and evaluate them further, as appropriate. These opportunities are crucial for informed diagnosis and management decisions, but were unavailable to the HEIRS Study and the present analyses. Nonetheless, our observations infer that practicing physicians should be skeptical about such H/IO reports in patients without evidence of iron overload proven by techniques such as liver biopsy or quantitative phlebotomy, especially in young adults without a history of heritable anemia that requires chronic erythrocyte transfusion or otherwise causes iron overload. Recommending phlebotomy management should depend on a demonstration of iron overload, and not on an elevated TS or SF alone. Evaluating non-*HFE* H/IO genes in such patients using DHPLC analysis or sequencing is unlikely to identify potentially deleterious mutations.

We conclude that persons who report previous H/IO diagnoses in screening programs are unlikely to have H/IO phenotypes or genotypes. Previous H/IO reports in some participants could be explained by treatment that induced iron depletion before initial screening, misdiagnosis, or participant misunderstanding of their physician or the initial screening questionnaire.

Methods

Study approval

The local Institutional Review Board of each Field Center approved the Study protocol [22,23]. The HEIRS Study Field Centers recruited participants ≥ 25 years of age who gave informed consent [22,23].

Selection of study subjects

Participants were recruited during the interval February 2001 – April 2003 from a health maintenance organization, diagnostic blood collection centers, and public and private primary care offices and ambulatory clinics [22,23]. All HEIRS Study participants at the time of recruitment were included in the present analyses. Participants completed an initial screening form that included questions about race/ethnicity; we categorized participants' race/ethnicity as previously described [22]. Participants were asked to respond to the questions, “Has a doctor ever told you that you have: too much iron in your body, iron overload, or hemochromatosis?; arthritis?; diabetes?; liver disease or liver cancer?; heart failure?; fertility problems or impotence?” [22].

Analyses of medical conditions and family histories reported by participants aged 25–29 years

We compared observations on participants with reports of previous H/IO diagnoses to those who did not report previous H/IO; data were stratified by age (nearest year), sex, and Field Center.

Phenotype measurements

Methodology and quality control used for measurements of serum iron and unsaturated iron-binding capacity, calculated total iron-binding capacity and TS, and SF are described elsewhere [65]. The HEIRS Study defined these initial screening phenotypes to be elevated: TS > 50% for men and TS >45% for women; and SF >300 μg/L for men and SF >200 μg/L for women [22]. The Study defined these screening phenotypes to be subnormal: TS <10% or SF <15 ng/mL [22].

HFE genotype analyses

HFE C282Y and H63D were detected using buffy coat samples from whole-blood EDTA samples and a modification of the Invader assay (Third Wave Technologies, Madison, WI) [65]. Participants without C282Yor H63D were defined by the HEIRS Study as having *HFE* wild-type genotype (wt/wt), although it was acknowledged at the outset of the Study in 1999–2000 that certain novel *HFE* mutations, many of which are rare or “private” alleles, occur in some persons with H/IO phenotypes [60,61]. Analyses to detect *HFE* mutations other than C282Yand H63D were not performed.

Mutation analyses of HJV, HAMP, TFR2, SLC40A1, and FTL

Analysis of non-*HFE* genes pertinent to H/IO was performed in participants aged 25–29 years who reported a previous diagnosis of H/IO, and who also gave express written consent for this aspect of the study. DHPLC technique is described in detail elsewhere [21,66]. We selected exons for DHPLC screening that correspond to most or all of the H/IO-associated mutations previously reported in the respective genes [1– 10]. Amplicons screened by DHPLC analysis included: hemojuvelin (*HJV*) exons 1, 2, 3, and 4; hepcidin (*HAMP*) promoter region and exons 1, 2, and 3; transferrin receptor-2 (*TFR*2) exons 2, 4, and 6; and ferroportin (*SLC40A*1) exons 3, 5, and 6. Sequencing was performed to detect any DNA variations in exon 2 of the ferritin light chain gene (*FTL*) [18,19]. Analysis to detect mutations in *ALAS2* or other non-*HFE* H/IO genes was not performed. Analysis of non-*HFE* genes was not performed in participants ages 30 years or greater.

We used an on-line tool to predict the effect of an amino acid substitution on the structure and function of human proteins using straightforward physical and comparative considerations [67], and thus to determine if a mutation were potentially deleterious. We searched on-line sources including the Single Nucleotide Polymorphism Database (dbSNP)

[29] to identify previous reports of alleles that we detected; these are recognized herein by dbSNP “rs” notations and literature citations, as appropriate.

Statistical considerations

The primary data set consisted of observations of all 7,343 participants aged 25–29 years for whom initial screening TS, SF, and *HFE* genotype were available. We also compiled demographic characteristics, self-reported conditions, initial screening phenotypes, and *HFE* genotypes in the 1,165 of 93,825 participants ages 30 years of age or greater who reported previous diagnoses of H/IO, and compared these data to corresponding observations in younger participants. Statistical analyses were performed using SAS [68]. TS values <3% were imputed as 1.5%. SF values <15 μg/L were imputed as 7.5 μg/L. SF measurements were normalized by natural log transformation for analysis [69]. Most descriptive data are displayed as enumerations, percentages, prevalences (as simple proportions), mean of transformed data, or mean ± 1 S.D., ranges, and 95% confidence intervals. Analyses of medical conditions were stratified by age, sex, and Field Center. The greatest odds ratios and associated confidence intervals were calculated using the method of Wald [70]. Frequency values were compared using chi-square analysis or Fisher exact test, as appropriate. Mean values were compared using student's *t* test; odds ratios were computed for some comparisons. Values of $P < 0.05$ were defined as significant.

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Appendix

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Table I

Initial Screening Characteristics of HEIRS Study Participants Who Reported a Previous Diagnosis of Hemochromatosis or Iron Overload[a]

	Ages 25–29 years		Ages 30 years or greater	
Questionnaire responses	**Men (*n* = 11)**	**Women (*n* = 40)**	**Men (*n* = 492)**	**Women (*n* = 673)**
Mean age ± 1 S.D., y (range)	27 ± 2 (25–29)	28 ± 1 (25–29)	55 ± 13 (30–91)	53 ± 13 (30–90)
Hispanic race/ethnicity, % (*n*)	27.3 (3)	25.0 (10)	8.3% (41)	10.3% (69)
Black race/ethnicity, % (*n*)	36.4 (4)	32.5 (13)	18.5% (91)	25.1% (169)
Asian race/ethnicity, % (*n*)	18.2 (2)	0 (0)	12.6% (62)	5.7% (38)
White race/ethnicity, % (*n*)	18.2 (2)	35.0 (14)	56.5% (278)	54.2% (365)
Hawaiian/Pacific Islander race/ethnicity, % (*n*)	0 (0)	2.5 (1)	0.8% (4)	0.5% (4)
Multiple race/ethnicity, % (*n*)	0 (0)	5.0 (2)	3.3% (16)	4.2% (28)
Arthritis, % (*n*)	18.2 (2)	37.5 (15)	36.8% (181)	44.9% (302)
Diabetes, % (*n*)	27.3 (3)	17.5 (7)	23.6% (116)	21.4% (144)
Liver disease or liver cancer, % (*n*)	27.3 (3)	15.0 (6)	16.5% (81)	10.7% (72)
Heart failure, % (*n*)	36.4 (4)	17.5 (7)	11.2% (55)	11.7% (79)
Fertility problems or impotence, % (*n*)	36.4 (4)	17.5 (7)	17.3% (85)	10.4% (70)
Three or more abnormalities[b], % (*n*)	27.3 (3)	15.0 (6)	25.4% (125)	23.8% (160)
Blood relatives with hemochromatosis or iron overload, % (*n*)	45.5 (5)	17.5 (7)	13.6% (67)	15.5% (104)
Initial screening phenotypes				
Mean transferrin saturation ± 1 S.D., % (range)	36 ± 14 (22–71)	27 ± 12 (7–61)	38 ± 20 (4, 100)	29 ± 16 (undetectable, 100)
Mean serum ferritin, lg/L (95% CI)	157 (103, 238)	50 (36, 70)	227 (48, 984)	88 (18, 415)

[a]The question answered affirmatively by all of these participants was: “Has a doctor ever told you that you have too much iron in your body, iron overload, or hemochromatosis?” [22]. All characteristics were reported or measured at the time of initial screening; prevalences of initial screening characteristics of men and women displayed above are similar. TS or SF values at the time of diagnosis of self-reported hemochromatosis or iron overload were not available, nor were results of liver biopsy analysis or therapeutic phlebotomy that were performed before participation in the HEIRS Study. Mean and S.D. serum ferritin (SF) data were computed from natural log-transformed values. No participant failed to report race/ethnicity, or reported Native American race/ethnicity. The percentages of Asian men and women ages 25–29 years were significantly different ($P = 0.04$; Fisher exact test). Mean SF was significantly greater in men ages 25–29 years than women ages 25–29 years ($P = 0.0003$). Other characteristics of men and women ages 25–29 years were not significantly different.

[b]Three men and six women ages 25–29 years reported that they had three or more of these abnormalities: arthritis; diabetes; liver disease or liver cancer; heart failure; or fertility problems or impotence. Seven men (63.6%) and 21 women (52.5%) ages 25–29 years reported that they did not have any of these abnormalities.

Table II

Odds Ratios in HEIRS Study Participants Who Reported That They Had Hemochromatosis or Iron Overload[a]

	Odds ratio (lower, upper 95% Wald confidence limits)[b]	
Questionnaire responses	**Ages 25–29 years**	**Ages 30 years or greater**
Heart failure	40.0 (18.2, 87.5)	2.5 (2.1, 3.1)
Arthritis	8.4 (4.5, 15.7)	1.9 (1.6, 2.1)
Diabetes	5.8 (2.8, 11.9)	1.8 (1.6, 2.1)
Liver disease or liver cancer	41.1 (17.4, 97.4)	5.9 (4.9, 7.0)
Fertility problems or impotence	9.4 (4.6, 19.1)	3.2 (2.7, 3.9)
Blood relatives with hemochromatosis or iron overload	7.9 (4.0, 15.8)	4.9 (4.1, 5.8)
Phenotypes		
Transferrin saturation elevation[c]	1.2 (0.4, 3.4)	2.8 (2.3, 3.3)
Serum ferritin elevation[c]	3.1 (1.3, 7.3)	2.1 (1.8, 2.4)

[a] Participants aged 25–29 years or ages 30 years or greater who reported a previous diagnosis of hemochromatosis or iron overload (H/IO) were compared to all available HEIRS Study participants of the corresponding ages who did not report a previous diagnosis of H/IO, stratified by age (by nearest year for ages 25–29 years and by decades for participants ages 30 years or greater), sex, and Field Center. The question answered affirmatively by participants classified as having reported a previous diagnosis of H/IO was: “Has a doctor ever told you that you have too much iron in your body, iron overload, or hemochromatosis?” [22].

[b] Values of p for all odds ratios were <0.0001, except transferrin saturation in participants ages 25–29 years ($P = 0.7197$), and serum ferritin elevation in participants ages 25–29 years ($P = 0.0109$).

[c] The HEIRS Study defined these initial screening phenotypes to be elevated: TS >50% for men and TS >45% for women; and SF >300 μg/L for men and SF >200 μg/L for women [22].