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Original Article

Initial screening transferrin saturation values, serum ferritin concentrations, and *HFE* genotypes in Native Americans and whites in the Hemochromatosis and Iron Overload Screening Study

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We compared initial screening transferrin saturation (TfSat) and serum ferritin (SF) phenotypes and *HFE* C282Y and H63D genotypes of 645 Native American and 43,453 white Hemochromatosis and Iron Overload Screening Study participants who did not report a previous diagnosis of hemochromatosis or iron overload. Elevated measurements were defined as TfSat >50% in men and >45% in women and SF >300 ng/ml in men and >200 ng/ml in women. Mean TfSat was 31% in Native American men and 32% in white men ($p = 0.0337$) and 25% in Native American women and 27% in white women ($p < 0.0001$). Mean SF was 153 μg/l in Native American and 151 μg/l in white men ($p = 0.8256$); mean SF was 55 μg/l in Native American women and 63 μg/l in white women ($p = 0.0015$). The C282Y allele frequency was 0.0340 in Native Americans and 0.0683 in whites ($p < 0.0001$). The H63D allele frequency was 0.1150 in Native Americans and 0.1532 in whites ($p = 0.0001$). We conclude that the screening TfSat and SF phenotypes of Native Americans are similar to those of whites. The allele frequencies of *HFE* C282Y and H63D are significantly lower in Native Americans than in whites.

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Transferrin saturation (TfSat) and serum ferritin (SF) values have been characterized in few cohorts of Native American adults who reside in North America (1–3). The results of nutrition surveys and hemochromatosis screening programs in the US include few or no observations on Native Americans (2, 4–7). TfSat phenotype screening of two Native American populations in Canada revealed no subject with evidence of iron overload (3). There is evidence that Native Americans are descendants of Asians who migrated to the Americas through a Bering land bridge during the last ice age (8–10). Furthermore, Asian adults appear to have lower mean TfSat and higher mean SF than white adults (11), suggesting that a similar pattern of serum iron measures could occur in Native Americans. Common missense mutations of the *HFE* gene (C282Y, H63D) were detected in various cohorts that included Native Americans, but the numbers of Native American subjects who were evaluated were small (12). The phenotypes associated with *HFE* genotypes in Native Americans have not been reported.

The Hemochromatosis and Iron Overload Screening (HEIRS) Study performed initial screening on 101,168 participants using phenotyping (TfSat and SF measurements) and *HFE* genotyping (C282Y and H63D alleles) (13). We analyzed the initial screening data of participants who identified themselves as Native American or white. We postulated that 1) prevalences of elevated TfSat are higher in whites and prevalences of elevated SF are higher in Native Americans; 2) there is a positive association of SF with age in Native American and white participants grouped by gender; 3) *HFE* C282Y and H63D are less prevalent in Native Americans than in whites; 4) Native Americans and whites with *HFE* genotypes that include C282Y or H63D are more likely to have elevated TfSat and SF than those without these genotypes; and 5) elevated SF as defined by initial screening in the HEIRS Study is not significantly associated with inheritance of C282Y- or H63D-containing *HFE* genotypes in Native Americans.

Materials and methods

Study approval

The local Institutional Review Board of each field center approved the study protocol (13, 14). The HEIRS Study field centers recruited participants $\geq$25 years of age who gave informed consent.

Selection of study subjects

Participants were recruited during the interval February 2001 to February 2003 from a health maintenance organization, diagnostic blood collection centers, and public and private primary care offices and ambulatory clinics as described elsewhere (13, 14).

At the initial screening visit, participants completed a questionnaire (13, 14). For the present analyses, whites are defined as participants who reported on the questionnaire that they were only 'White or Caucasian'. Native Americans are defined as participants who reported that they were only 'American Indian or Alaska Native' at field centers in the US or 'North American Indian, Metis, or Inuit' at the Canadian field center. Participants who identified themselves as Black or African-American at field centers in the US or Black (African, Haitian, Jamaican, and Somal) at the Canadian field center (27,124), Asian (13,130), Hispanic (12,696), Native Hawaiian or other Pacific Islander (706), or as having multiple or unknown race/ethnicity (1952) were excluded. For phenotype analyses, we excluded participants who reported that they had been previously diagnosed to have hemochromatosis or iron overload. Blood samples were obtained without regard for state of fasting for measurement of TfSat and SF, and for *HFE* mutation analysis (13, 14).

Laboratory methods for screening

Phenotype measurements

Methodology and quality control used for measurements of serum iron and unsaturated iron-binding capacity, SF, and calculated total iron-binding capacity and TfSat are described elsewhere (14). The HEIRS Study defined these initial screening phenotypes to be elevated: TfSat $>$50% for men and TfSat $>$45% for women; and SF $>$300 μg/l for men and SF $>$200 μg/l for women (13).

Genotype analyses

HFE C282Y and H63D were detected using buffy coat samples from whole-blood ethylenediaminetetraacetic acid samples and a modification of the Invader assay (Third Wave Technologies, Inc., Madison, WI) as described in detail elsewhere (14). Participants without C282Y or H63D are designated as having *HFE* wild-type genotype (wt/wt).

Statistical considerations

Observations on 648 Native Americans and 44,083 whites were used to compute frequencies

of *HFE* genotypes. Iron phenotype frequencies based on initial screening TfSat and SF measurements were computed after exclusion of participants who reported that they had been previously diagnosed to have hemochromatosis or iron overload. In preliminary analyses, we observed that many whites who reported that they had been previously diagnosed had also been treated with phlebotomy, and thus this exclusion criterion had no significant effect on mean TfSat and SF values in whites. *HFE* allele frequencies in whites met Hardy–Weinberg expectations only after removal of previously diagnosed participants, a large proportion of whom were C282Y homozygotes (11). Because very few Native Americans reported that they had been previously diagnosed to have hemochromatosis or iron overload, exclusion of these participants from the present analyses had no significant effect on mean TfSat and SF values or *HFE* allele frequency estimates for Native Americans. The data set excluded observations on participants for whom data on initial screening TfSat, SF, or *HFE* genotype were not available.

HFE C282Y and H63D observed allele frequencies were defined as: (total of respective alleles detected by genotype screening) ÷ (numbers of screened participants × 2); allele frequencies were computed using data from all Native American and white participants who did not report a previous diagnosis of hemochromatosis or iron overload. Estimated frequencies of C282Y and H63D were also computed as described elsewhere (15).

Statistical analyses were performed using SAS (16). SF measurements were normalized by natural logarithmic ($\log_e$) transformation for analysis (17). Most descriptive data are displayed as enumerations, percentages, mean ± 1 SD, antilogs of mean log-transformed SF data, ranges, or 95% confidence intervals (CIs). Frequency values were compared using Pearson's χ^2 analysis or Fisher's exact tests, as appropriate. Mean values were compared using Student's *t*-test, with Satterthwaite's adjustment for unequal variances, when appropriate. Odds ratios were computed for some comparisons. Multiple regression models were also used to estimate the significance of the independent variables age, sex, race/ethnicity, and *HFE* genotype on the dependent variables TfSat and SF. Values of $p < 0.05$ were defined as significant.

Results

General characteristics of participants

There were observations on 645 Native American participants (206 men and 439 women). Seventy-one percent of Native Americans were recruited from field centers in Ontario, Alabama, and Washington, D.C. The mean age (±1 SD) of Native American participants was 49 years ± 13 years (range 25–94 years). There were observations on 43,453 white participants (16,715 men and 26,737 women). Seventy-three percent of whites were recruited from field centers in Ontario, Alabama, and Washington, D.C. The mean age (±1 SD) of white participants was 53 years ± 14 years (range 25–100 years).

Transferrin saturation phenotypes

The mean TfSat in 206 Native American men was 31% (95% CI 29%, 32%); the mean TfSat in 16,716 white men was 32% (95% CI 32%, 33%) ($p = 0.0460$) (Table 1). In participants grouped by decade, mean TfSat values were significantly lower in Native American men than in white men in age groups of 65 years or older. In other age groups, mean TfSat values were also lower in Native American men than in white men, but the differences were not statistically significant (Table 1). The percentage of Native American men with elevated TfSat was similar to that of white men (6.8 *vs* 7.0%, respectively; $p = 0.9122$).

The mean TfSat in 439 Native American women was 25% (95% CI 24%, 26%); the mean TfSat in 26,737 white women was 27% (95% CI 27%, 28%) ($p < 0.0002$). Mean TfSat values were significantly lower in Native American women than in white women in the age group of 25–34 years (Table 1). In other age groups, mean TfSat values in Native American women did not differ significantly from those of white women (Table 1). The percentage of Native American women with elevated TfSat was similar to that of white women with elevated TfSat (5.2 *vs* 6.6%, respectively; $p = 0.2559$).

Serum ferritin phenotypes

The mean SF in 206 Native American men was 153 μg/l (95% CI 135 μg/l, 173 μg/l); the mean SF in 16,716 white men was 151 μg/l (95% CI 148 μg/l, 153 μg/l) ($p = 0.8256$). Mean SF values were greater in Native American men than in white men aged 25–54 years and were greater in white men than in Native American men aged 55 years or older. However, these differences were not statistically significant (Table 2). The percentage of Native American men with elevated SF was similar to that of white men with elevated SF (21.4 *vs* 19.4%, respectively; $p = 0.4773$).

Table 1. Serum transferrin saturation (TfSat) values in Hemochromatosis and Iron Overload Screening Study participants by age[a]

Age (years)	Native American men	White men	p value	Native American women	White women	p value
Overall	31 ± 13 (206)	32 ± 12 (16,716)	0.0460	25 ± 12 (439)	27 ± 12 (26,737)	0.0002
25–34	30 ± 13 (33)	34 ± 13 (1589)	0.0953	22 ± 13 (81)	28 ± 14 (3557)	0.0003
35–44	34 ± 16 (53)	33 ± 13 (2673)	0.5898	25 ± 15 (119)	27 ± 13 (5042)	0.2111
45–54	30 ± 11 (46)	32 ± 12 (4033)	0.3236	26 ± 10 (112)	27 ± 12 (6948)	0.1670
55–64	30 ± 13 (44)	32 ± 12 (3987)	0.3861	27 ± 14 (71)	27 ± 10 (5864)	0.8122
65–74	27 ± 8 (19)	33 ± 12 (2939)	0.0050	25 ± 8 (38)	28 ± 10 (3523)	0.1488
75+	25 ± 10 (11)	32 ± 12 (1488)	0.0461	26 ± 6 (18)	28 ± 11 (1783)	0.0819

[a]Data at the time of initial screening expressed as percentage TfSat (mean ± 1 SD (n)); values were rounded to the nearest integer. Totals of participants in each column above differ from totals of participants reported in the *Results* (*General characteristics of participants*) due to missing age data.

The mean SF in 439 Native American women was 55 μg/l (95% CI 50 μg/l, 60 μg/l). The mean SF in 26,737 white women was significantly greater (63 μg/l) (95% CI 62 μg/l, 64 μg/l) ($p = 0.0059$). The mean SF of Native American women aged 25–34 years was significantly lower than the mean SF of white women aged 25–34 years, but otherwise the mean SF in women grouped by ages did not reveal a consistent pattern of greater values in either Native Americans or whites (Table 2). The percentage of Native American women with elevated SF was similar to that of white women with elevated SF (8.4 *vs* 8.7%, respectively; $p = 0.8329$).

HFE allele and genotype frequencies

The observed and estimated frequencies of C282Y and H63D were significantly lower in Native Americans than in whites (Table 3). The prevalence of each *HFE* genotype that included either C282Y or H63D was significantly lower in Native American participants than in white participants, except C282Y/C282Y and C282Y/H63D (Table 4). The prevalence of genotype wt/wt was significantly greater in Native Americans than in whites (72 *vs* 61%, respectively) (Table 4). Genotype frequencies for the HEIRS Study participants that were adjusted for the observed lack of conformity to Hardy–Weinberg predictions are reported elsewhere (11).

Associations of initial screening phenotypes and *HFE* genotypes

The *HFE* genotype C282Y/C282Y was not detected in Native American participants who did not report a previous diagnosis of hemochromatosis or iron overload (Table 5). No Native Americans had elevated values of both TfSat and SF and either genotypes C282Y/C282Y, C282Y/H63D, or H63D/H63D. The prevalence of white participants who had elevated values of both TfSat and SF and genotypes C282Y/C282Y, C282Y/H63D, or H63D/H63D was 0.1201 (251/2089) (Table 5). The prevalence of elevated TfSat, elevated SF, or both was similar in Native Americans and in whites who had the *HFE* genotype wt/wt (Table 5).

We computed the mean TfSat and SF in participants not previously diagnosed to have hemochromatosis or iron overload who had the *HFE* genotype wt/wt. The mean TfSat values in 146 Native American men and in 10,213 white men were similar (29 ± 12% *vs* 31 ± 11%, respectively; $p = 0.3106$). In the same men, the mean SF values in Native Americans and in whites were also similar (239 ± 489 μg/l *vs* 193 ± 173 μg/l, respectively;

Table 2. Serum ferritin (SF) concentrations in Hemochromatosis and Iron Overload Screening Study participants[a]

Age (years)	Native American men	White men	p value	Native American women	White women	p value
Overall	153 (135, 173) [206]	151 (149, 153) [17,716]	0.8256	55 (50, 60) [439]	63 (62, 64) [26,737]	0.0059
25–34	154 (118, 201) [33]	153 (148, 158) [1589]	0.9143	29 (23, 37) [81]	42 (41, 43) [3557]	0.0002
35–44	179 (140, 228) [53]	164 (159, 169) [2673]	0.3963	42 (35, 51) [119]	46 (45, 47) [5042]	0.4463
45–54	171 (140, 209) [46]	161 (157, 165) [4033]	0.6035	67 (56, 80) [112]	58 (57, 60) [6948]	0.0897
55–64	140 (102, 192) [44]	151 (147, 155) [3987]	0.7074	90 (74, 110) [71]	86 (84, 88) [5864]	0.4528
65–74	107 (55, 208) [19]	140 (135, 144) [2939]	0.4200	84 (65, 108) [38]	88 (86, 91) [3523]	0.7112
75+	108 (66, 174) [11]	127 (122, 133) [1488]	0.4692	70 (43, 114) [18]	91 (88, 94) [1783]	0.1574

[a]Data at the time of initial screening represent antilogs of $\log_e$-transformed participant mean SF as μg/l (95% confidence interval values) rounded to the nearest integer [number of participants]. Totals of participants in each column above differ from totals of participants reported in the *Results* (*General characteristics of participants*) due to missing age data.

Table 3. *HFE* C282Y and H63D alleles in Hemochromatosis and Iron Overload Screening Study participants[a]

HFE allele	Race/ethnicity	Observed allele frequency (95% CI)	p value[b]	Estimated allele frequency (95% CI)	p value[b]
C282Y	Native American	0.034 (0.025, 0.045)		0.034 (0.025, 0.045)	
	White	0.068 (0.067, 0.070)	$p < 0.0001$	0.066 (0.064, 0.069)	$p < 0.0001$
H63D	Native American	0.115 (0.099, 0.134)		0.115 (0.099, 0.134)	
	White	0.153 (0.151, 0.156)	$p = 0.0001$	0.153 (0.151, 0.156)	$p = 0.0001$

CI, confidence interval.
[a]*HFE* C282Y and H63D observed allele frequencies were defined as: (total of respective alleles detected by genotype screening) ÷ (numbers of screened participants × 2); allele frequencies were computed using data from all Native American and white participants who did not report a previous diagnosis of hemochromatosis or iron overload. Estimated frequencies of C282Y and H63D were also computed (15).
[b]p values indicate comparisons of respective *HFE* allele frequencies determined by the same methods in Native Americans and whites.

$p = 0.4392$). The mean TfSat values in 324 Native American women and in 16,287 white women were similar ($24 \pm 12\%$ *vs* $26 \pm 11\%$, respectively; $p = 0.0064$). In the same women, the mean SF values in Native Americans and in whites were also similar (82 ± 83 μg/l *vs* 89 ± 95 μg/l, respectively; $p = 0.0074$).

Multiple regression models

Sex, race/ethnicity, and *HFE* genotype were significant independent determinants of TfSat evaluated as a continuous dependent variable ($p < 0.0001$ each variable). Age was not a significant independent variable that affected TfSat. When TfSat was evaluated as a dichotomous dependent variable (elevated or not elevated), age, race/ethnicity, and *HFE* genotype were significant independent determinants ($p < 0.0001$ each variable), but sex was not a significant independent variable.

Sex, age, and *HFE* genotype were significant independent determinants of SF evaluated as a continuous dependent variable ($p < 0.0001$ each variable). Race/ethnicity was not a significant independent variable that affected SF. When SF was evaluated as a dichotomous dependent variable (elevated or not elevated), age, race/ethnicity, and *HFE* genotype were significant independent determinants ($p < 0.0001$ each variable), whereas sex was not a significant independent variable that affected SF.

Participants previously diagnosed to have hemochromatosis or iron overload

The three Native Americans who reported that they had been previously diagnosed to have hemochromatosis or iron overload had *HFE* genotypes C282Y/C282Y ($n = 1$) and H63D/wt ($n = 2$). The 629 whites who had been previously diagnosed to have hemochromatosis or iron overload had *HFE* genotypes C282Y/C282Y ($n = 68$), C282Y/H63D ($n = 34$), H63D/H63D ($n = 27$), C282Y/wt ($n = 85$), H63D/wt ($n = 136$), and wt/wt ($n = 279$). The proportion of Native Americans who were previously diagnosed to have hemochromatosis or iron overload was significantly lower than the proportion of

Table 4. Prevalence of *HFE* C282Y and H63D genotypes in Hemochromatosis and Iron Overload Screening (HEIRS) Study participants[a]

HFE genotype[b]	Native Americans ($n = 648$)	Whites ($n = 44{,}082$)	p value	Odds ratio (95% CI)
C282Y/C282Y	1, 0.11%, (0.061, 0.20)	281, 0.44%, (0.42, 0.47)	0.20	4.2 (0.6, 29.6)
C282Y/H63D	7, 0.77%, (0.56, 1.1)	908, 2.0%, (2.0, 2.1)	0.08	1.9 (0.91, 4.1)
H63D/H63D	7, 1.3%, (0.98, 1.8)	1029, 2.4%, (2.3, 2.4)	0.04	2.2 (1.04, 4.6)
C282Y/wt	35, 5.7%, (4.2, 7.7)	4548, 10%, (10, 11)	<0.0001	2.0 (1.4, 2.8)
H63D/wt	128, 20%, (17, 22)	10,537, 24%, (24, 24)	0.01	1.3 (1.05, 1.6)
wt/wt	470, 72%, (69, 76)	26,779, 61%, (60, 61)	<0.0001	0.6 (0.5, 0.7)

CI, confidence interval.
[a]These data are displayed as number of positive subjects, prevalence, and (95% CI). Data represent observations on all HEIRS Study participants for whom initial TfSat, SF, and *HFE* genotype data were available at initial screening, including those who reported that they had been previously diagnosed to have hemochromatosis or iron overload. p values and odds ratios are based on unadjusted frequency counts.
[b]*HFE* genotyping was performed to identify mutations C282Y and H63D; *HFE* genotype wt/wt indicates that C282Y or H63D alleles were not detected.

Table 5. Elevated transferrin saturation (TfSat) and serum ferritin (SF) phenotypes and *HFE* genotypes in Hemochromatosis and Iron Overload Screening (HEIRS) Study participants[a]

HFE genotype[b]	Native Americans (n = 645)	Whites (n = 43,453)	p value
C282Y/C282Y	(n = 0)	(n = 213)	
TfSat elevated	–	77.93%	–
SF elevated	–	69.01%	–
TfSat and SF elevated	–	59.62%	–
C282Y/H63D	(n = 7)	(n = 874)	
TfSat elevated	28.57%	26.89%	0.9999
SF elevated	14.29%	25.97%	0.6840
TfSat and SF elevated	0.00%	8.92%	–
H63D/H63D	(n = 7)	(n = 1002)	
TfSat elevated	28.57%	15.27%	0.2934
SF elevated	0.00%	20.26%	–
TfSat and SF elevated	0.00%	4.59%	–
C282Y/wt	(n = 35)	(n = 4463)	
TfSat elevated	11.43%	10.58%	0.7831
SF elevated	22.86%	13.40%	0.1293
TfSat and SF elevated	2.86%	2.22%	0.5461
H63D/wt	(n = 126)	(n = 10,401)	
TfSat elevated	3.97%	7.23%	0.1589
SF elevated	15.87%	13.02%	0.3443
TfSat and SF elevated	0.79%	1.50%	0.9999
wt/wt	(n = 470)	(n = 26,500)	
TfSat elevated	5.11%	4.35%	0.4293
SF elevated	11.06%	11.48%	0.7794
TfSat and SF elevated	1.28%	0.85%	0.3037

[a]The HEIRS Study defined these initial screening phenotypes to be elevated: TfSat >50% for men and TfSat >45% for women and SF >300 μg/l for men and SF >200 μg/l for women (13). These data exclude observations on participants who reported that they had been previously diagnosed to have hemochromatosis or iron overload.
[b]*HFE* genotyping was performed to identify mutations C282Y and H63D; *HFE* genotype wt/wt indicates that C282Y or H63D alleles were not detected.

whites who were previously diagnosed (0.46 *vs* 1.43%; p = 0.0178, Fisher exact test).

Discussion

The overall mean TfSat values in Native American men and women were similar to the respective values in white men and women; the mean TfSat values of Native American and white participants who had the *HFE* genotype wt/wt were also similar. The overall mean SF values in Native American men and women were similar to the respective values in white men and women; mean SF values of Native American and white participants who had the *HFE* genotype wt/wt were also similar. Thus, the present results indicate that our initial postulate was, in practicality, incorrect. The proportions of Native Americans who had *HFE* wt/wt and elevated TfSat or SF were similar to those of whites. Taken together, these observations suggest that the screening iron phenotypes of Native Americans are similar to those of whites.

The present HEIRS Study results and other cross-sectional studies (4, 6, 18) demonstrate that there are age-related variations in TfSat and SF, confirming our second postulate. In men, age-related increases in TfSat and SF may be due to net iron accumulation from dietary sources. In women, race/ethnicity-specific patterns of menstruation and volumes of menstrual blood loss could account for differences in TfSat and SF (19); age-related increases in TfSat and SF after the fifth decade of life are probably due largely to net iron accumulation from dietary sources after cessation of menstruation and childbearing. In men and women, the prevalence of inflammatory or other disorders that affect TfSat or SF is age related (4). Within-person, diurnal, and day-to-day variations in serum iron and total iron-binding capacity are due in part to genetic variants of transferrin, consumption of various foods, menstruation, pregnancy, iron deficiency, and inflammation (5, 20–23), but these sources of variation were not assessed in the present analyses. Sex was not a significant independent determinant of either TfSat or SF as a dichotomous dependent variable (either elevated or not elevated). This lack of significance could be explained partly by the use of sex-specific

cut-points to define elevated TfSat and SF in this study.

The HEIRS Study results confirm that the frequencies of *HFE* C282Y and H63D are significantly lower in Native Americans than in whites (6, 24, 25), thus substantiating our third postulate. Our fourth postulate indicated that Native Americans and whites with *HFE* genotypes that include C282Y or H63D would be more likely to have elevated TfSat and SF than those without these genotypes. The low proportions of Native Americans who inherited two common *HFE* mutations reduced the statistical power to show significant differences between Native Americans and whites with these genotypes, thus limiting confirmation of the fourth postulate by univariate analyses. However, *HFE* genotype was a significant independent variable associated with elevated TfSat and elevated SF in both Native Americans and whites in multiple regression analyses, confirming our fifth postulate. The occurrence of *HFE* C282Y in Native Americans may be due to admixture with Caucasians or to a *HFE* C282Y mutation of non-Caucasian origin (26), whereas the geographic and racial/ethnic distribution of *HFE* H63D is broad (12). *HFE* mutations other than C282Y or H63D, or mutations in other iron-pertinent genes, account for elevated TfSat, elevated SF, or iron overload in some whites (6, 27–29) but have not been reported in Native Americans. Hemoglobinopathy and thalassemia, some types of which increase the risk of developing iron overload, have been reported infrequently in Native Americans (30, 31).

Iron overload and elevated SF sometimes develop after repeated erythrocyte transfusion, but the prevalence of disorders treated with chronic transfusion is low. HEIRS Study participants who reported having hemochromatosis or iron overload were excluded from most of the present analyses. Some reports indicate that the dietary iron intake of adult Native Americans is normal or increased (2, 32–36). However, dietary iron intake does not explain differences in mean TfSat or SF (37) or account for differences in the prevalence of primary or *HFE*-associated iron overload (25, 37, 38), although these reports do not include observations on Native Americans. Total body iron excretion is similar across race/ethnicity groups (39). Thus, it is unlikely that iron from exogenous sources accounts for elevated TfSat or SF phenotypes or for phenotype–*HFE* genotype associations in this study.

Mean body mass index (BMI) is greater in Native Americans than in whites matched for age and sex (40–43). SF was positively correlated with BMI in elderly adults (44). In white adults with *HFE* wt/wt, age-adjusted BMI was negatively correlated with TfSat and positively correlated with SF (45, 46). Thus, greater mean BMI in Native Americans than in white HEIRS Study participants could partly account for the slightly lower mean TfSat we observed in Native Americans, although evaluation of this in the HEIRS Study was not possible because participant height and weight measurements were not obtained at the time of initial screening (13).

Non-alcoholic steatosis and steatohepatitis, chronic viral hepatitis C, and excessive alcohol consumption are common liver disorders that are sometimes associated with elevated TfSat or elevated SF. Risk factors of hepatitis C in Native Americans are similar to those of other persons living in North America (47). In one study, the prevalence of chronic hepatitis C was estimated to be two to three times greater in Native Americans than in whites (48) but was reported to be 'largely unknown' in another study (49). The prevalence of alcohol abuse varies widely across race/ethnicity groups (50, 51).

TfSat testing identifies many whites with typical *HFE*-associated hemochromatosis and some others who have less common disorders associated with iron overload (6, 52). The present results suggest that screening with TfSat or SF measurements will identify similar proportions of Native Americans and whites but that the proportion of Native Americans who have iron overload as defined elsewhere in detail (13), with or without typical hemochromatosis-associated *HFE* genotypes, will be much lower than that of whites. This is consistent with a previous report that the TfSat phenotype screening of two Native American populations in Canada (1407 'Native Americans', 310 Inuit) revealed no subject with evidence of iron overload (3). Furthermore, the Native American proportional ancestry of white adult hemochromatosis probands with *HFE* C282Y homozygosity in central Alabama was similar to that in control subjects (53).

The present results do not constitute recommendations that screening for hemochromatosis and iron overload be performed. However, race/ethnicity, age, sex, and disease associations of TfSat, SF, and *HFE* genotypes suggest that strategies can be formulated to increase the sensitivity and specificity of possible future screening to detect presumptive hemochromatosis and iron overload in target populations in which the predominant racial/ethnic groups are Native Americans and whites. In both race/ethnicity groups, the positive predictive value of phenotype testing alone may be reduced by the effects of increased BMI and common liver disorders.

HFE C282Y and H63D testing will likely identify few Native Americans and many whites who have or are at risk to develop iron overload, although *HFE* genotyping will probably identify some Native Americans and whites who would be missed by phenotype testing alone. Regardless of the approach to population testing, it is necessary to evaluate all screen-positive individuals adequately for iron overload and its complications.

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