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Thyroid-Stimulating Hormone and Free Thyroxine Levels in Persons with *HFE* C282Y Homozygosity, a Common Hemochromatosis Genotype: The HEIRS Study

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Background: Relationships of thyroid and iron measures in large cohorts are unreported. We evaluated thyroid-stimulating hormone (TSH) and free thyroxine (T4) in white participants of the primary care–based Hemochromatosis and Iron Overload Screening (HEIRS) Study.
Methods: We measured serum TSH and free T4 in 176 *HFE* C282Y homozygotes without previous hemochromatosis diagnoses and in 312 controls without *HFE* C282Y or H63D who had normal serum iron measures and were matched to C282Y homozygotes for Field Center, age group, and initial screening date. We defined hypothyroidism as having TSH >5.00 mIU/L and free T4 <0.70 ng/dL, and hyperthyroidism as having TSH <0.400 mIU/L and free T4 >1.85 ng/dL. Multivariate analyses were performed using age, sex, Field Center, $\log_{10}$ serum ferritin (SF), *HFE* genotype, $\log_{10}$ TSH, and $\log_{10}$ free T4.
Results: Prevalences of hypothyroidism in C282Y homozygotes and controls were 1.7% and 1.3%, respectively, and of hyperthyroidism 0% and 1.0%, respectively. Corresponding prevalences did not differ significantly. Correlations of $\log_{10}$ SF with $\log_{10}$ free T4 were positive ($p = 0.2368$, C282Y homozygotes; $p = 0.0492$, controls). Independent predictors of $\log_{10}$ free T4 were $\log_{10}$ TSH (negative association) and age (positive association); positive predictors of $\log_{10}$ SF were age, male sex, and C282Y homozygosity. Proportions of C282Y homozygotes and controls who took medications to supplement or suppress thyroid function did not differ significantly.
Conclusions: Prevalences of hypothyroidism and hyperthyroidism are similar in C282Y homozygotes without previous hemochromatosis diagnoses and controls. In controls, there is a significant positive association of SF with free T4. We conclude that there is no rationale for routine measurement of TSH or free T4 levels in hemochromatosis or iron overload screening programs.

Introduction

Hemochromatosis in Western European whites is typically associated with homozygosity for C282Y, a mutation in the *HFE* gene on chromosome 6p (1). Approximately 0.44–0.48% of non-Hispanic whites in North America are C282Y homozygotes (2,3). Some C282Y homozygotes develop iron overload and complications such as liver disease, diabetes mellitus, arthropathy, and hypogonadotrophic hypogonadism (1–4). Among 391 patients with hemochromatosis diagnosed in medical care combined from five studies, 4.1% had primary hypothyroidism and 0.3% had hyperthyroidism (5–9). To date, there is no report of thyroid-related laboratory measures in participants of large population or workplace hemochromatosis screening programs (2,10–12).

The Hemochromatosis and Iron Overload Screening (HEIRS) Study is a cross-sectional, multi-center, multi-ethnic study of primary care clinic attendees (13). The HEIRS Study performed initial screening for hemochromatosis and iron overload using transferrin saturation (TS) and serum ferritin (SF) measurements and *HFE* genotyping (C282Y and H63D alleles) in 101,168 participants (13). In this HEIRS substudy,

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we measured and compared serum concentrations of thyroid-stimulating hormone (TSH), free thyroxine (T4), and SF at a post-initial screening examination in 176 white participants with C282Y homozygosity and in 312 white participants without *HFE* C282Y or H63D designated as control subjects. The implications of the present results for understanding the prevalence and pathogenesis of thyroid abnormalities in whites with hemochromatosis and C282Y homozygosity are discussed.

Materials and Methods

Study approval

The local Institutional Review Boards of the HEIRS Study Coordinating Center, Central Laboratory, and each Field Center approved the study protocol that is described in detail elsewhere (13). The Field Centers recruited participants ≥25 years of age who gave informed consent. It is the privacy policy of the HEIRS Study to display the age of individual subjects by decade.

Initial screening of HEIRS Study subjects

Participants were recruited during the interval February 2001–February 2003 from HEIRS Study Field Centers (13). These clinics serve ethnically and socioeconomically diverse primary care patients. At initial screening, blood samples were obtained from participants for measurement of TS and SF without regard for state of fasting, and for *HFE* mutation analysis (13). The dataset excluded observations on participants for whom TS, SF, or *HFE* genotype data on initial screening were not available. All adult volunteers were eligible to participate in the HEIRS Study, but those who participated only because a family member participated or those who reported on their screening questionnaire that they had been previously diagnosed to have hemochromatosis or iron overload were excluded from the present analysis (3).

Post-initial screening clinical examination and subject selection

All C282Y homozygotes diagnosed in the HEIRS Study were invited to participate in a clinical examination that included listing of participants' medications and collection of blood samples for additional testing (13). Control subjects invited to attend a clinical examination were selected from initial screening participants who had (i) *HFE* genotype wt/wt (defined as absence of alleles C282Y and H63D) and (ii) both TS and SF in the eligible range. Eligible ranges for men were TS 20–34% and SF 87–247 ng/dL. Eligible ranges for women were TS 16–28% and SF 19–121 ng/dL. Among participants eligible to be control subjects, a subset was selected that was frequency matched in a 1:1 ratio to the group of C282Y homozygotes and provisional iron overload cases (defined as participants with confirmed elevations of both SF and TS without evidence of elevated C-reactive protein or elevated serum levels of hepatic transaminases). Variables used for the frequency matching were Field Center (UAB, UCI, Howard, KP-Portland, LHSC, and Toronto), age group (24–44, 45–64, and 65+ years), and date of initial screening visit. Potential control participants who could not be contacted or who declined to participate were replaced. New potential control subjects were selected using frequency matching in a 1:1 ratio to the group of the potential control subjects who declined. Variables used for this frequency matching were Field Center (as above), age group (as above), date of initial screening, gender, and race/ethnicity. Most C282Y homozygotes in the HEIRS Study were white (3). Therefore, we included only those participants and control subjects who reported that they were only "white or Caucasian" (13).

Laboratory methods for serum thyroid-related and SF measures

Serum concentrations of TSH and free T4 were measured at the HEIRS Study Central Laboratory at the University of Minnesota Medical Center-Fairview (13) using a Siemens/Bayer ADVIA Centaur® immunoassay analyzer (Siemens, New York, NY). The coefficients of variation for the TSH (TSH-3) and free T4 (FrT4) assays in the normal range were 5.0% and 4.0%, respectively. The TSH assay has a functional sensitivity of approximately 0.019 mIU/L. Reference ranges were TSH 0.400–5.00 mIU/L and free T4 0.70–1.85 ng/dL. These ranges represent the central 95% confidence intervals (CIs) of serum TSH and free T4 measurements in ostensibly healthy young adults. We defined hypothyroidism as having TSH >5.00 mIU/L and free T4 <0.70 ng/dL, and hyperthyroidism as having TSH <0.400 mIU/L and free T4 >1.85 ng/dL, regardless of cause. SF levels were measured using a turbidometric immunoassay (Roche Diagnostics/Hitachi 911, Indianapolis, IN) (3,14). The SF reference ranges defined by the HEIRS Study were 15–200 ng/mL (women) and 15–300 ng/mL (men) (3,14). We defined severe hyperferritinemia as SF >1000 ng/mL.

Statistical considerations

We evaluated observations on 176 C282Y homozygotes and 312 wt/wt control subjects. Statistical analyses were performed using SAS (15), Excel 2000® (Microsoft, Redmond, WA), and GB-Stat® (v. 10.0, 2003; Dynamic Microsystems, Silver Spring, MD). Initial evaluations revealed that TSH and free T4 data were not normally distributed. This is characteristic of thyroid screening programs, largely due to the relatively high prevalence of hypothyroidism (16–20). Similarly, SF values are not normally distributed (3,14). Therefore, TSH, free T4, and SF data were normalized using $\log_{10}$ transformation for analysis; some results were reported as antilog values, as appropriate. For display of TSH values, we used three significant figures. Descriptive data are displayed as enumerations, percentages, or mean ± 1 SD (or 95% CIs, as appropriate). Frequency values were compared using chi-square analysis or Fisher exact test, as indicated. Mean values were compared using Student's *t*-test. We determined the correlations of $\log_{10}$ SF as a dependent variable with $\log_{10}$ free T4 and with $\log_{10}$ TSH as independent variables. Multivariate analyses were performed using age, sex (male or female), Field Center, *HFE* genotype (either C282Y/C282Y or wt/wt), $\log_{10}$ SF, and $\log_{10}$ TSH as independent variables, and $\log_{10}$ free T4 as the dependent variable. In other multivariate analyses, we evaluated the determinants of $\log_{10}$ SF as the dependent variable. For correlation and multivariate analyses, we included only those observations from the 437 study participants who reported taking neither thyroid

supplements nor medications to suppress thyroid function. Values of $p < 0.05$ were defined as significant.

Results

General characteristics of study subjects

There was a predominance of women among the C282Y homozygotes and wt/wt control subjects, consistent with the overall greater participation of women than men in the HEIRS Study (Table 1) (3). C282Y homozygotes were significantly younger and had a significantly higher mean free T4 than control subjects in univariate analyses, although the magnitudes of these respective differences were small (Table 1). The prevalence of abnormal values of TSH, free T4, or both did not differ significantly between *HFE* C282Y homozygotes and control subjects (Table 1). Mean SF was significantly greater in C282Y homozygotes than in control subjects (Table 1).

Prevalence of hypothyroidism and hyperthyroidism

Three participants had TSH and free T4 levels defined as hypothyroidism; each was a control subject (Table 2). Seven participants had TSH and free T4 levels defined as hyperthyroidism: three were C282Y homozygotes and four were control subjects (Table 2). The mean SF in the three participants with hypothyroidism was significantly lower than the mean SF in the seven participants with hyperthyroidism (Table 2). The respective prevalence estimates for hypothyroidism and hyperthyroidism did not differ significantly between C282Y homozygotes and control subjects (Table 1), or between male and female C282Y homozygotes and control subjects of the corresponding sex (data not shown).

Participants with severe hyperferritinemia

Twenty-two *HFE* C282Y homozygotes (12.5%; 18 men and 4 women) had SF >1000 ng/mL. None had hypothyroidism as defined herein, although one man reported taking thyroid supplements. Another man had hyperthyroidism. By study design, no *HFE* wt/wt control subjects had hyperferritinemia.

Participants who reported taking thyroid supplements

Forty-nine participants (15 *HFE* C282Y homozygotes, 34 *HFE* wt/wt control subjects) reported that they took thyroid supplements (Table 3). These respective proportions of these participants were 8.5% and 10.9% ($p = 0.4019$). Mean values of age, TSH, free T4, proportions of participants with elevated or subnormal TSH or free T4, or those who had hypothyroidism or hyperthyroidism as defined herein did not differ significantly between C282Y homozygotes and control subjects (Table 3). Mean SF was significantly higher in C282Y homozygotes than in control subjects (Table 3). Two control participants who reported taking thyroid supplements met the present definition for having hyperthyroidism, suggesting that their elevated T4 and subnormal TSH values were due to thyroid supplements (Table 2).

Participants who reported taking medications used to suppress thyroid function

A man in his 70s with *HFE* C282Y homozygosity and SF 1462 ng/mL reported taking propylthiouracil; he met the present definition for having hyperthyroidism. A woman in her 30s with *HFE* wt/wt and SF 22 ng/mL reported that she took methimazole; her values of TSH and free T4 were within the respective reference limits. The proportions of C282Y homozygotes and control subjects who reported taking medications to suppress thyroid function did not differ significantly ($p = 0.5883$).

Correlations of $\log_{10}$ SF with $\log_{10}$ free T4

The correlation of $\log_{10}$ SF with $\log_{10}$ free T4 in all 437 study participants who reported taking neither thyroid supplements nor medications to suppress thyroid function was significant (Pearson correlation coefficient 0.1148; $p = 0.0166$) (data not shown). To determine if the relationship of $\log_{10}$ SF with $\log_{10}$ free T4 were significant in both C282Y homozygotes and in control subjects, we computed correlations in each group of subjects. In 160 C282Y homozygotes, the correlation was positive but not significant (Pearson correlation

Table 1. Characteristics of 488 White HEIRS Study Participants

Characteristic[a]	*HFE C282Y homozygotes* (n = *176; 64.2% F)*	*HFE wt/wt controls* (n = *312; 60.6% F)*	p-*value*[b]
Mean age ± 1 SD (range), years	50 ± 13	55 ± 14	<0.0001
Mean TSH, mIU/L (95% CI)	1.67 (1.42, 1.96)	2.00 (1.80, 2.22)	0.0652
TSH <0.400 mIU/L, % (*n*)	5.1 (9)	2.9 (9)	0.2097
TSH >5.00 mIU/L, % (*n*)	8.0 (14)	8.3 (26)	0.8835
Mean free T4, ng/dL[c] (95% CI)	1.12 (1.09, 1.15)	1.01 (1.05, 1.10)	0.0492
Free T4 <0.70 ng/dL, % (*n*)	1.7 (3)	1.0 (3)	0.3741
Free T4 >1.85 ng/dL, % (*n*)	1.7 (3)	1.3 (4)	0.4925
TSH <0.400 mIU/L and free T4 >1.85 ng/dL, % (*n*)	0	1.0 (3)	0.2604
TSH >5.00 mIU/L and free T4 <0.70 ng/dL, % (*n*)	1.7 (3)	1.3 (4)	0.4925
Mean SF, ng/mL (95% CI)	308 (252, 376)	77 (71, 84)	<0.0001

[a]TSH, thyroid-stimulating hormone; T4, thyroxine; SF, serum ferritin. For display of TSH values, we used three significant figures.
[b]Comparisons were made between *HFE* C282Y homozygotes and *HFE* wt/wt controls using Student's *t*-tests, Fisher exact tests, or chi-square tests, as appropriate.
[c]When mean $\log_{10}$ free T4 was computed in C282Y homozygotes and in controls after all participants with free T4 values outside the reference range of 0.70–1.85 ng/dL were removed, a slightly higher mean was still observed in C282Y homozygotes ($p = 0.0423$).

Table 2. Characteristics of 10 White HEIRS Study Participants with Abnormal Thyroid-Related Measures

Condition[a]	*Age, sex*[b]	*HFE genotype*	*Thyroid-stimulating hormone (TSH), mIU/L*	*Free thyroxine (T4), ng/dL*	*Serum ferritin, ng/mL*[c]
Hypothyroidism	20s F	wt/wt	291	0.16	23
Hypothyroidism	50s F	wt/wt	88.9	0.36	90
Hypothyroidism	50s F	wt/wt	19.4	0.69	27
Hyperthyroidism	50s M	C282Y/C282Y	0.014	2.04	24
Hyperthyroidism	20s F	C282Y/C282Y	0.009	1.98	124
Hyperthyroidism	70s M	C282Y/C282Y	0.007	4.59	1,462
Hyperthyroidism	40s M	wt/wt	0.064	2.11	144
Hyperthyroidism[d]	50s M	wt/wt	0.054	2.07	230
Hyperthyroidism	80s M	wt/wt	0.015	2.35	199
Hyperthyroidism[d]	40s F	wt/wt	0.011	2.73	124

[a]We defined hypothyroidism as the concurrence of TSH >5.00 mIU/L and free T4 <0.70 ng/dL, and hyperthyroidism as the concurrence of TSH <0.400 mIU/L and free T4 >1.85 ng/dL. These measurements were performed at the time of post-initial screening examination. For display of TSH values, we used three significant figures.
[b]It is the privacy policy of the HEIRS Study to display the age of individual subjects by decade.
[c]The mean serum ferritin level was lower in participants with hypothyroidism than in those with hyperthyroidism (38 ng/mL vs. 167 ng/mL, respectively; $p = 0.0446$).
[d]These participants reported that they took thyroid supplements.

coefficient 0.0947; $p = 0.2368$) (Fig. 1). In 277 control subjects, the correlation was positive and significant (Pearson correlation coefficient 0.1183; $p = 0.0492$) (Fig. 1).

Predictors of $\log_{10}$ free T4 in multivariate analyses

We used age, sex, Field Center, *HFE* genotype, $\log_{10}$ SF, and $\log_{10}$ TSH as independent variables, and $\log_{10}$ free T4 as the dependent variable. Values of p for these analyses were as follows: Field Center, 0.4687; age, 0.0142; gender, 0.3238; *HFE* genotype, 0.9689; $\log_{10}$ SF, 0.2102; and $\log_{10}$ TSH, <0.0001. These analyses reveal significant direct (positive) relationships of age and $\log_{10}$ TSH with $\log_{10}$ free T4.

Predictors of $\log_{10}$ SF in multivariate analyses

We used age, sex, Field Center, *HFE* genotype, $\log_{10}$ TSH, and $\log_{10}$ free T4 as independent variables, and $\log_{10}$ SF as the dependent variable. Values of p for these analyses were as follows: Field Center, 0.8192; age, 0.0006; gender, <0.0001; *HFE* genotype, <0.0001; $\log_{10}$ TSH, 0.1571; and $\log_{10}$ free T4, 0.2102. These analyses reveal significant direct (positive) relationships of age, male gender, and *HFE* C282Y homozygosity with SF.

Discussion

The prevalence of hypothyroidism or hyperthyroidism as defined in the present HEIRS substudy did not differ significantly between white *HFE* C282Y homozygotes and white *HFE* wt/wt control subjects, and is similar to that reported in other large population studies in which screening of whites for thyroid disorders was performed (16–20). Multivariate analyses demonstrated that there is a significant inverse relationship of age with $\log_{10}$ free T4 levels, consistent with other population studies of thyroid-related measures (16–20).

Table 3. Characteristics of 49 White HEIRS Study Participants Who Reported Taking Thyroid Supplements[a]

Characteristic[b]	*HFE C282Y homozygotes (n = 15; 86.7% F)*	*HFE wt/wt controls (n = 34; 85.3% F)*	p-*value*[c]
Mean age ± 1 SD (range), years	53 ± 11	53 ± 15	0.9351
Mean TSH, mIU/L (95% CI)	1.51 (0.651, 3.51)	1.79 (0.875, 3.64)	0.7604
TSH <0.400 mIU/L, % (n)	20.0 (3)	14.7 (5)	0.4677
TSH >5.00 mIU/L, % (n)	20.0 (3)	17.6 (6)	0.5670
Mean free T4, ng/dL (95% CI)	1.28 (1.14, 1.43)	1.10 (0.93, 1.30)	0.1294
Free T4 <0.70 ng/dL, % (n)	0	5.9 (2)	0.4770
Free T4 >1.85 ng/dL, % (n)	0	5.9 (2)	0.4770
TSH <0.400 mIU/L and free T 4 >1.85 ng/dL, % (n)	0	5.9 (2)	0.4770
TSH >5.00 mIU/L and free T4 <0.70 ng/dL, % (n)	0	5.9 (2)	0.4770
Mean SF, ng/mL (95% CI)	301 (129, 705)	68 (54, 85)	0.0036

[a]Thyroid supplements reported in medication lists provided by participants, by descending numbers of reports, were Synthroid®, levothyroxine, dessicated thyroid, Levoxyl®, Thyrolar®, Liotrix®, liothyronine, Levothroid®, and Cytomel®.
[b]TSH, thyroid-stimulating hormone; T4, thyroxine; SF, serum ferritin. For display of TSH values, we used three significant figures.
[c]Comparisons were made between *HFE* C282Y homozygotes and *HFE* wt/wt controls using Student's *t*-tests, Fisher exact tests, or chi-square tests, as appropriate.

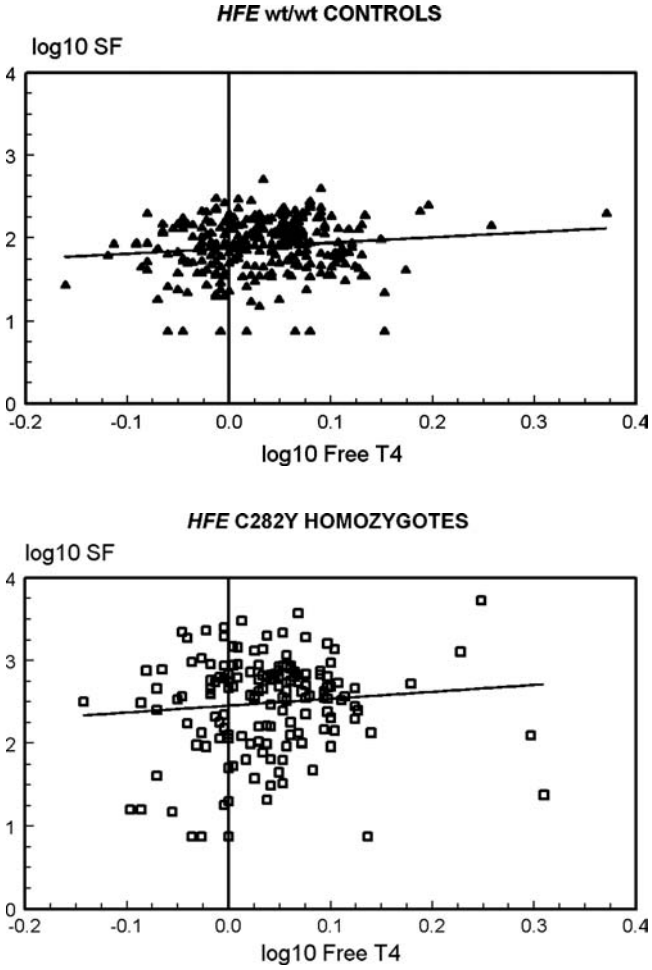

FIG. 1. Correlations of $\log_{10}$ serum ferritin (SF) and $\log_{10}$ free thyroxine (T4) in Hemochromatosis and Iron Overload Screening (HEIRS) Study participants who reported taking neither thyroid supplements nor medications to suppress thyroid function. In 160 C282Y homozygotes, the correlation was positive but not significant ($p = 0.2368$) (above). In 277 control subjects, the correlation was positive and significant ($p = 0.0492$) (below).

In a large population hemochromatosis screening program in Norway, 12.5% of women aged 20–49 years with C282Y homozygosity reported having hypothyroidism, whereas only 3.0% of the control participants reported having hypothyroidism (11). Thyroid-related laboratory measures were not quantified in participants in this study (11). By design, only study participants with elevated TS at initial screening underwent *HFE* genotyping, and therefore C282Y homozygotes without elevated TS would have been excluded from the Norway screening study (11). This difference of hypothyroidism reports between C282Y homozygotes and control subjects in the Norway screening study could be due to misunderstanding of the screening questionnaire by study participants, the higher proportion of thyroid peroxidase antibody positivity in younger than older women observed in other studies, or a consequence of multiple comparisons in any large study (11,13,16,20,21). The HEIRS Study identified all C282Y homozygotes at initial screening, and afterward obtained reports of thyroid supplement use and measurements of TSH and free T4 in selected participants. Therefore, it is not possible to compare the results of the present HEIRS substudy directly with the hypothyroidism reports in the Norway screening study.

There is evidence that iron deposits in the anterior pituitary gland or thyroid gland do not contribute to the pathogenesis of hypothyroidism or hyperthyroidism in most persons with hemochromatosis. In persons with severe iron overload due to hemochromatosis, iron deposits were visualized in a minority of thyrotrophs; the deposits were much less prominent than those in gonadotrophs (22,23). There are few well-documented hemochromatosis patients who had hypothyroidism due to impaired thyrotroph function; most of them also had hypogonadotrophic hypogonadism (24–29). In rare cases, secondary hypothyroidism resolves after therapeutic phlebotomy to achieve iron depletion (29). Iron deposits were detected in the thyroid gland in a majority of persons diagnosed to have hemochromatosis in medical care or at autopsy (23,30,31). Nonetheless, only 4.1% of 391 patients with hemochromatosis diagnosed in medical care had primary hypothyroidism and only 0.3% had hyperthyroidism (5–9). It has been suggested but remains unproven that iron deposits in the thyroid glands of persons with hemochromatosis cause Hashimoto thyroiditis or Graves disease to develop (6,23).

In persons with hemochromatosis, there is a significant positive correlation of SF with hepatic iron content and iron removed by phlebotomy to achieve iron depletion (32,33). Mean SF in the present *HFE* C282Y homozygotes was significantly greater than that of *HFE* wt/wt control subjects, but our prevalence estimates for hypothyroidism and hyperthyroidism did not differ significantly between these groups. The proportion of C282Y homozygotes who reported taking thyroid supplements did not differ significantly from that of control subjects, although two control subjects who reported that they took thyroid supplements met present criteria for having hyperthyroidism. Mean SF in participants with hyperthyroidism was significantly greater than mean SF in participants with hypothyroidism, but both means were within the reference range for SF. None of 22 C282Y homozygotes with severe hyperferritinemia had hypothyroidism; the only C282Y homozygote with hyperthyroidism did not report taking thyroid supplements. Although direct measures of hepatic iron content or quantitative phlebotomy in these substudy participants were not available, our SF observations agree with previous evidence that hypothyroidism or hyperthyroidism in persons with hemochromatosis is usually unrelated to the severity of iron overload (23).

We observed a significant positive correlation of $\log_{10}$ SF with $\log_{10}$ free T4 in control subjects, and a nonsignificant positive correlation of these variables in C282Y homozygotes. The mean SF in the three participants with hypothyroidism was significantly lower than the mean SF in the seven participants with hyperthyroidism. Nonetheless, multivariate analysis of observations from all subjects who reported taking neither thyroid supplements nor medications to suppress thyroid function did not reveal that $\log_{10}$ free T4 was a significant, independent determinant of $\log_{10}$ SF. Hashimoto reported that SF was significantly lower in patients with primary hypothyroidism than in those who were euthyroid, and that SF increased significantly in patients with primary hypothyroidism after normalization of thyroid function (34). In patients with hyperthyroidism due to Graves disease, SF was

significantly higher than in patients with primary hypothyroidism (34). In rats made hypothyroid with propylthiouracil or hyperthyroid with L-T4, the liver ferritin synthesis rate was reduced by 36% in hypothyroid rats, and elevated by 38% in hyperthyroid rats; a similar trend was observed in liver ferritin concentration (35). Thus, the present results confirm and extend previous reports that SF is positively correlated with free T4 in control subjects without *HFE* C282Y homozygosity. The present observations also suggest that increased absorption of iron characteristic of many persons with C282Y homozygosity has a greater influence on SF than free T4, and that increasing quantities of storage iron in C282Y homozygotes are not typically associated with decreasing levels of free T4.

It is unlikely that a non-*HFE* determinant of either TSH or free T4 on chromosome 6 could explain the occurrence of hypothyroidism or hyperthyroidism in most patients with hemochromatosis. *HFE* C282Y occurs in linkage disequilibrium with class I alleles at human leukocyte antigen (HLA)-A and -B loci and with the hemochromatosis ancestral haplotype HLA-A*03,B*07 on chromosome 6p (1,36). In Western European whites, Hashimoto thyroiditis and Graves' disease have been associated with HLA class II loci (37–39), but there are few reports of these disorders in whites with hemochromatosis phenotypes or C282Y homozygosity (6,40,41). This suggests that linkage disequilibrium of *HFE* C282Y with HLA class II loci is not great, that the ancestral hemochromatosis haplotype does not include susceptibility alleles for common autoimmune thyroid disorders, or that there are other susceptibility factors for Hashimoto thyroiditis and Graves' disease. Thyroid-related abnormalities are not typical of chromosome 6p deletion syndromes that involve the region 6p24-pter (42). The *CGA* gene that encodes the alpha chain subunit common to TSH, gonadotrophin, luteinizing hormone, and follicle-stimulating hormone occurs on the long arm of chromosome 6 (6q12–q21) (43–45).

The HEIRS Study was not designed to determine the clinical severity of or need for treatment of thyroid dysfunction. Our review of medications reported by participants suggests that some were previously diagnosed and treated for thyroid dysfunction, causing their TSH and free T4 values to return to normal before they enrolled in this post-initial screening evaluation. Obtaining self-reports or family history of thyroid gland disease, examining the thyroid gland and related attributes, measuring thyroid peroxidase and thyroglobulin antibodies, and performing thyroid ultrasonography and biopsy were beyond the scope of the HEIRS Study. Long-term follow-up of participants that would have permitted estimation of incidence rates of thyroid conditions was not possible.

In routine medical care settings, some patients who report having fatigue, weakness, or malaise need evaluation for thyroid-related disease, iron overload, or both (8,23,46). In hemochromatosis and iron overload screening programs, however, the prevalence of these symptoms in participants with hemochromatosis phenotypes and C282Y homozygosity was similar to that in age- and sex-matched control subjects (11,47). In this HEIRS substudy, the prevalence of abnormal values of free T4, TSH, or both is similar in white persons with or without C282Y homozygosity. Based on these observations, we conclude that there is no rationale for routine measurement of TSH or free T4 levels in hemochromatosis or iron overload screening programs.

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