Effect of Hemochromatosis Genotype and Lifestyle Factors on Iron and Red Cell Indices in a Community Population

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Background: Heterozygotes for the C282Y mutation of the HFE gene may have altered hematology indices and higher iron stores than wild-type subjects.

Methods: We performed a cross-sectional analysis of 1488 females and 1522 males 20–79 years of age drawn from the Busselton (Australia) population study to assess the effects of HFE genotype, age, gender, and lifestyle on serum iron and hematology indices.

Results: Male C282Y heterozygotes had increased transferrin saturation compared with the wild-type genotype. Neither male nor female heterozygotes had significantly increased ferritin values compared with the wild-type genotype. Younger (20–29 years) wild-type males, but not heterozygous males, had significantly lower ferritin values than wild-type males in the older age groups. Compound heterozygous subjects had increased means for serum iron, transferrin saturation, corpuscular volume, and corpuscular hemoglobin compared with the wild-type genotype, and the males also had increased ferritin values (medians 323 vs 177 μg/L; P = 0.003). In both male and female wild-type subjects, an increased body mass index was associated with decreased serum iron and transferrin saturation and increased ferritin values. There was a significant increase in ferritin concentrations in both genders with increasing frequency of red meat consumption above a baseline of 1–2 times per week and alcohol intakes >10 g/day.

Conclusions: Male C282Y heterozygotes had significantly increased transferrin saturation values. Compound heterozygous (C282Y/H63D) subjects formed a separate category of C282Y heterozygotes in whom both iron and red cell indices were significantly increased compared with the wild-type genotype.

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Hereditary hemochromatosis is a common iron overload disease with autosomal recessive inheritance occurring predominantly in individuals of northern European origin. A novel candidate gene, termed HFE for hereditary hemochromatosis, containing two missense mutations was identified in 1996 (1), and homozygosity for the C282Y mutation has been observed in 85–90% of patients of northern European origin with typical hereditary hemochromatosis (2–4). The H63D mutation is heterozygous in 15–20% of the population and may contribute to increased hepatic iron concentrations, especially when combined with the C282Y mutation (5, 6). The proportion of heterozygotes carrying one C282Y mutated allele is high in Anglo-Celtic-based populations. Heterozygosity for the C282Y mutation can occur as either a C282Y wild-type heterozygous (C282Y/wt)7 or compound heterozygous (C282Y/H63D) HFE genotype. Our recent population study of asymptomatic Australians indicated prevalences of 11.9% for C282Y/wt heterozygosity, 2.2% for C282Y/H63D heterozygosity, and 0.53% (1 in 188) for C282Y homozygosity (7, 8).

Two recent prospective population-based studies have reported an association between heterozygosity for the C282Y mutation of the HFE gene for hereditary hemo-
chromatosis and vascular events (9, 10). These findings have led to speculation that C282Y/wt subjects had either increased serum ferritin compared with wild-type subjects or had reached the same ferritin concentrations at an earlier age (11). We therefore studied the effect of HFE genotype, age, gender, and lifestyle factors (obesity and consumption of alcohol and red meat) on iron indices.

**Materials and Methods**

**PATIENTS**

Busselton is a town in the southwest of Western Australia that has been prospectively studied since 1966 and is in many respects similar to the Framingham population (12). The population is essentially ethnically homogeneous, with 90% being of Anglo-Celtic descent. The most recent follow-up study of this population was in 1994. At this evaluation, clinical assessment was performed, and whole blood and serum samples were obtained from ~5000 Caucasian subjects. All blood tests were performed in the fasting state. From this group, we randomly selected 1488 female and 1522 male nonrelated subjects 20–79 years of age.

Permission was granted for this study by the Busselton Population Medical Research Foundation and The Committee for Human Rights at The University of Western Australia.

**MEASUREMENT OF SERUM INDICES**

Serum iron concentrations were measured using a standard colorimetric method, and the transferrin concentration was determined by rate immunoturbidimetry on a Hitachi 917 analyzer. Serum transferrin saturation was calculated from these results as follows: transferrin saturation (\%) = serum iron (\(\mu\)mol/L)/[2 \times transferrin (\(\mu\)mol/L)] \times 100. Serum ferritin concentrations were measured by chemiluminescence immunoassay on a Chiron ACS-180 analyzer.

**MEASUREMENT OF RED CELL INDICES**

Hemoglobin, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) measurements were performed on a Coulter STKS automated hematology analyzer.

**DETERMINATION OF THE C282Y AND H63D MUTATIONS**

Analysis was performed on DNA extracted from whole blood spotted onto neonatal screening cards as described by Walsh et al. (13). PCR amplification of the regions containing the missense mutations was performed using the published primer sequences of Feder et al. (1) (GenBank Accession No. U60319) and cycling conditions described by Cullen et al. (14). Mutations were identified using restriction enzyme digestion followed by analysis on a 2% agarose gel. The C282Y missense mutation leads to the formation of a unique SnaBI restriction site, whereas the H63D mutation leads to the loss of a DpnII site. The status of all C282Y homozygous subjects was confirmed by separate testing with the primer sequence described by Jeffrey et al. (15) to avoid possible false-positive results attributable to the G5569A polymorphism. The H63D mutation was determined only in subjects who were heterozygous for the C282Y mutation to ascertain the prevalence of C282Y wild-type heterozygous (C282Y/wt) and compound heterozygous (C282Y/H63D) genotypes. Wild-type refers to absence of the C282Y mutation.

**STATISTICAL ANALYSIS**

The Fisher exact test, the \(\chi^2\) test, and generalized linear models adjusted for multiple means testing using the least significant difference method were used. The normality test was carried out on all variables. Ferritin was highly skewed, and log transformation was used for all subsequent analyses. Statistical analyses were performed with SAS software (16).

**Results**

**GENOTYPE PREVALENCE**

The prevalences of wild types (wt/wt), heterozygotes (C282Y/wt), compound heterozygotes (C282Y/H63D), and homozygotes (C282Y/C282Y) for the C282Y mutation are shown in Table 1. The prevalences of the population with one mutated C282Y allele (C282Y/wt and C282Y/H63D genotypes combined) were 14.1% in females and 14.5% in males. These prevalences were consistent with those predicted with the Hardy-Weinberg equation, based on an allelic frequency of 7.6% for the C282Y mutation. There were no significant between-gender differences in genotype prevalence (\(\chi^2\) test, \(P = 0.95\)). Hemozygosity for the C282Y mutation occurred in 0.53% (1 in 188) of the subjects, nine females and seven males. Phenotypic presentation and clinical data for these subjects with hereditary hemochromatosis have been reported separately (7).

**IRON AND HEMATOLOGY STUDIES**

The data for serum iron, red cell indices, and body mass index (BMI) according to genotype in 1479 females are shown in Table 2. A threshold of 45% for transferrin saturation has been proposed for population screening for hereditary hemochromatosis (17), and the proportions exceeding this value are shown. Iron depletion was de-

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**Table 1. Genotype prevalence.**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt/wt</td>
<td>1268</td>
<td>1295</td>
</tr>
<tr>
<td>C282Y/wt</td>
<td>179</td>
<td>187</td>
</tr>
<tr>
<td>C282Y/H63D</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>C282Y/C282Y</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>1488</td>
<td>1522</td>
</tr>
</tbody>
</table>

*wt, wild-type (refers to absence of the C282Y mutation); C282Y/wt, heterozygote; C282Y/H63D, compound heterozygote; C282Y/C282Y, C282Y homozygote.*
fined by a ferritin concentration <20 μg/L (18) and iron deficiency by a ferritin concentration <12 μg/L and a transferrin saturation <15% (19). The significance values are shown for comparison of either C282Y/wt or C282Y/H63D genotypes to the wild type.

The iron indices in C282Y/wt females were not significantly different from those in the wild-type genotype; however, the mean MCV and MCH values were significantly increased. Compound heterozygous females had increased means for serum iron, transferrin saturation (21.9% exceeded a saturation of 45%), MCV, and MCH compared with the wild-type genotype. There were no significant differences between genotypes for the prevalence of iron depletion or deficiency.

Comparable data for serum iron, red cell indices, and BMI according to genotype in 1515 males are shown in Table 3. Compared with the wild-type genotype, C282Y/wt males had significantly increased means for serum iron, transferrin saturation, MCV, and MCH, and the C282Y/H63D males also had increased means for ferritin. The prevalences of both C282Y/wt and C282Y/H63D males with transferrin saturation exceeding a threshold of 45% were significantly higher than the prevalence for wild-type males. There were no significant differences between genotypes for the prevalence of iron depletion or deficiency.

**IRON INDICES AND AGE**

Box and whisker plots for ferritin according to deciles of age in wild-type and C282Y/wt genotype males are shown in Fig. 1. Initial univariate regression analyses showed that there was no age-related increase in ferritin. A significant overall decrease of ferritin with age occurred in men with the wild-type genotype (coefficient ± SE, \(-0.0029 ± 0.0014; P = 0.05\)) but not the C282Y/wt genotype. Subsequent multiple comparison tests of age deciles showed that ferritin values in wild-type males
significantly increased between the 20–29 years and 30–39 years deciles (geometric means, 126 and 181 μg/L, respectively; \( P < 0.0001 \)), and no subsequent changes occurred. Similar analysis by age deciles for C282Y/wt men confirmed the univariate regression analysis results and showed that there were no significant differences in ferritin values throughout the age range we examined (20–79 years). The results in Table 3 for the entire age range show that there were no significant differences between ferritin values in wild-type males and their C282Y/wt counterparts.

Box and whisker plots for ferritin according to deciles of age in wild-type and C282Y/wt females are shown in Fig. 2. For females 20–49 years of age, both univariate analysis and multiple comparison tests of age deciles showed no significant differences in mean ferritin values for either the wild-type or the C282Y/wt genotype. There was a significant increase in ferritin values for both wild-type and C282Y/wt genotypes occurring between the fourth and fifth decades, consistent with the average age of menopause for our population (51 years). There were no differences in the mean ferritin values between wild-type and C282Y/wt genotypes either before (geometric means for 20–49 years, 42 and 36 μg/L, respectively) or after menopause (geometric means for 50–79 years, 84 and 87 μg/L, respectively).

Serum iron and transferrin saturation values were not correlated with age in either gender.

**Iron Indices and Obesity**

A BMI between 20 and 25 kg/m\(^2\) is usually considered normal, and obesity is defined as a value >30. Obesity as assessed by BMI had highly significant effects on iron indices. Table 4 shows coefficients ± SE for univariate regression analyses of the relationship between age-adjusted BMI (dependent variable) and serum iron, transferrin saturation, and log ferritin (independent variables) according to HFE genotype. In wild-type subjects of both genders, the age-adjusted BMI was negatively correlated with serum iron and transferrin saturation and positively correlated with ferritin. Obesity tends to increase ferritin, whereas it decreases serum iron and transferrin saturation. In male C282Y/wt subjects, the age-adjusted BMI was not correlated with serum iron, transferrin saturation, or ferritin, but in female C282Y/wt subjects, it was correlated with serum iron. No correlations were detected between BMI and serum iron, transferrin saturation, or log ferritin in C282Y/H63D subjects.

**Alcohol Consumption and Ferritin**

Males admitted to a higher alcohol consumption than females, and 63% of males compared with 28% of females estimated their intake to be >10 g/day. Fig. 3 shows box and whisker plots for ferritin according to alcohol consumption and gives ferritin concentrations and levels of

Table 4. Linear regression analyses for the relationship between BMI and serum iron, transferrin saturation, and log ferritin adjusted for age according to HFE genotype.\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>wt/wt</th>
<th>C282Y/wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient ± SE</td>
<td>( P )</td>
</tr>
<tr>
<td>Females Iron, μmol/L</td>
<td>-0.1753 ± 0.0342</td>
<td>0.0001</td>
</tr>
<tr>
<td>Transferrin sat, %</td>
<td>-0.3012 ± 0.0948</td>
<td>0.001</td>
</tr>
<tr>
<td>Ferritin, μg/L</td>
<td>0.0211 ± 0.0057</td>
<td>0.0002</td>
</tr>
<tr>
<td>Males Iron, μmol/L</td>
<td>-0.0965 ± 0.0457</td>
<td>0.03</td>
</tr>
<tr>
<td>Transferrin sat, %</td>
<td>-0.3365 ± 0.0813</td>
<td>0.0001</td>
</tr>
<tr>
<td>Ferritin, μg/L</td>
<td>0.0397 ± 0.0066</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

\(^a\) No correlations were detected between BMI and serum iron, transferrin saturation, or log ferritin in C282Y/H63D subjects.

\(^b\) sat, saturation; NS, not significant.
significance compared with a baseline intake of 1–10 g/day. Ferritin concentrations (median, interquartile range) for both males and females showed a significant increase with increasing alcohol consumption. Ferritin values for males consuming either 11–50 g of alcohol/day or >50 g/day were both significantly higher than for those who consumed 1–10 g/day (P < 0.0007 and 0.0001, respectively). Similarly, females consuming either 11–50 g of alcohol/day or >50 g/day had significantly higher ferritin values than those who consumed 1–10 g/day (P = 0.0002 and 0.006, respectively).

MEAT CONSUMPTION AND FERRITIN
The frequency of red meat (beef) consumption was high, with 89% of men and 81% of women reporting eating red meat three or more times per week. Fig. 4 shows box and whisker plots for ferritin according to the frequency of red meat consumption and gives ferritin concentrations and levels of significance compared with a baseline intake of one to two times per week. Ferritin concentrations (median, interquartile range) for both males and females were significantly increased with increasing frequency of consumption. Ferritin values for males consuming red meat either three to six times per week or every day were significantly higher than for those who consumed one to two times per week (P = 0.0002 and 0.0001, respectively). Similarly, ferritin values for females consuming red meat either three to six times per week or every day were significantly higher than for those who consumed one to two times per week (P = 0.007 and 0.005, respectively).

We compared the log-ferritin values for C282Y/wt and wild-type subjects who consumed meat every day by two-sample t-test: although male C282Y/wt subjects had higher median ferritin values than wild-type subjects, the difference did not achieve significance (medians, 221 vs 190 μg/L; P = 0.098), and there was no difference in the females (medians, 75 vs 70 μg/L; P = 0.691).

Discussion
The prevalences of the C282Y/wt and C282Y/H63D genotypes were 12.0% and 2.1% in females and 12.3% and
2.2% in males, respectively (Table 1); 14.1% of the females and 14.5% of the males therefore had one mutated C282Y allele. These relatively high prevalence rates for the C282Y mutation make this population ideal for studying the effect of HFE genotype.

Significantly increased serum iron and transferrin saturation values were observed in females with the C282Y/H63D but not the C282Y/wt genotype (Table 2). Thus, 21.9% of female C282Y/H63D subjects exceeded a transferrin saturation value of 45%, which has been proposed as a threshold for the investigation of subjects for hereditary hemochromatosis (17). Previous studies have reported increased transferrin saturation values in female heterozygotes (20, 21), but these studies were conducted before the availability of genotyping for HFE mutations and putative heterozygotes were identified on the basis of HLA typing in family studies of hereditary hemochromatosis probands. Subjects with the C282Y/H63D genotype would have been included as heterozygotes, and we have shown that this group has significantly increased transferrin saturation values. No significant between-genotype differences were observed in females for ferritin or hemoglobin, although both C282Y/wt and C282Y/H63D genotypes had increased MCV and MCH indices.

Both C282Y/wt and C282Y/H63D males had significantly increased serum iron, transferrin saturation, MCV, and MCH values compared with wild-type subjects (Table 3). These results agree with previous studies of heterozygous males (20, 21).

The mean ferritin values for both female and male C282Y/wt subjects were not significantly different from wild-type subjects (Tables 2 and 3, respectively). The assertion that heterozygotes have significantly increased serum ferritin compared with wild-type subjects is based on studies conducted before the availability of genotyping for HFE mutations of heterozygotes identified in family studies of hereditary hemochromatosis patients (20, 21). Both studies identified putative heterozygotes on the basis of HLA typing in family studies of hereditary hemochromatosis probands. A US study reported significantly increased transferrin saturation values in C282Y/wt males and 260 female heterozygotes 31–60 years of age compared with healthy controls in the same age range (20). Another study of 255 heterozygotes in Canada (21) found that the mean ± SE for serum ferritin for heterozygotes was significantly higher than in control subjects (140 ± 10.2 vs 87 ± 8.5 μg/L; P <0.05). There may have been a selection bias resulting from studying hereditary hemochromatosis families rather than a community population. In the case of males, the inadvertent inclusion of the C282Y/H63D genotype as heterozygotes would have increased the mean ferritin values obtained in both studies (Table 3).

Our finding that ferritin did not differ significantly between C282Y/wt and wild-type subjects has been corroborated in a large community sample of 1233 complete pairs of Australian twins (22) in whom both HFE mutations were assessed. The latter study concluded that the effects of the HFE gene on serum ferritin are minor compared with the effects on serum iron and transferrin saturation. We found that the C282Y/wt genotype caused a significant increase in transferrin saturation in males, whereas no such effect was observed for ferritin in either gender. The mechanism for the effects of the C282Y/wt genotype on iron absorption and iron stores have not been fully studied. However, the main effect of C282Y heterozygosity seems to be to change the interactions between transferrin, its receptor, and the HFE protein (23), which may account for the increased transferrin saturation and implies that the effects on iron stores and hence ferritin may be secondary consequences.

Two recent prospective population-based studies have reported an association between heterozygosity for the C282Y mutation of the HFE gene for hereditary hemochromatosis and vascular events. A study of 12 239 Dutch women showed that C282Y/wt subjects were at significantly increased risk of death from either myocardial infarction or cerebrovascular disease compared with wild-type subjects (9). Similar findings were reported in a prospective study of 1150 Finnish men where C282Y/wt subjects were at a 2.3-fold increased risk of acute myocardial infarction (10). In an editorial accompanying these studies, it was stated that heterozygous subjects had higher mean serum ferritin concentrations than wild-type subjects and that it was likely that even heterozygotes with statistically normal ferritin concentrations achieved them at an earlier age (11). We have demonstrated that C282Y/wt subjects do not have higher ferritin concentrations than their wild-type equivalents. However, we present evidence that C282Y/wt males have achieved steady-state ferritin concentrations by their 20–29 years age decile, whereas wild-type males do not do so until the 30–39 years decile. Thus, there is a longer period of steady-state ferritin concentrations in C282Y/wt males than in wild-type subjects. This effect was not observed in females, and there were no significant increases of ferritin when the population was segregated into premenopausal (ages 20–49 years) and postmenopausal (ages 50–79 years) age groups.

We observed a striking correlation of age-adjusted BMI with iron indices for wild-type but not heterozygous subjects (Table 4). Obesity tended to increase ferritin, whereas it decreased serum iron and transferrin saturation in wild-type subjects of either gender. These results agree with findings in the study of Australian twins (22). The physiological mechanism responsible for these effects is unknown at this time.

We confirm a previous report showing that the frequency of meat intake and quantity of alcohol consumed are important lifestyle factors affecting serum ferritin concentrations for both genders (24). The heme content of red meat provides a dietary iron content of very high bioavailability. Intestinal absorption of heme iron is relatively unaffected by the status of body iron stores, whereas absorption of non-heme iron is regulated accord-
ing to the demands of iron stores (25). We observed significant increases in median ferritin concentrations with increasing frequency of red meat consumption above a baseline of one to two times per week, consistent with lack of regulation of iron uptake from dietary heme.

Median ferritin concentrations for both males and females showed a significant increase with increasing alcohol consumption above a baseline of 1–10 g/day. The mechanism for the effect of alcohol consumption on serum ferritin is poorly understood. Alcohol appears to have many modes of action that can affect serum ferritin concentrations. These include induction of an inflammatory response in the liver with resulting de novo ferritin synthesis, causing ferritin release from liver cells and changing gut permeability, thereby altering iron absorption (26).

In conclusion, we report iron and red cell indices on a large community population in whom the prevalence of heterozygosity for the C282Y mutation is relatively high. Previous studies found increased transferrin saturation and ferritin concentrations in putative heterozygotes; however, we confirmed significantly increased transferrin saturation only in male C282Y/wt subjects. Ferritin values for our C282Y/wt subjects were not significantly different from the wild-type genotype, although male C282Y/wt subjects achieved maximal ferritin concentrations in their second rather than their third decade. Compound heterozygous (C282Y/H63D) subjects formed a separate category of C282Y heterozygotes in whom both iron and red cell indices were significantly increased compared with the wild-type genotype.

We are indebted to the Busselton Population Medical Research Foundation for their invaluable cooperation.

References