Compound Heterozygous Hemochromatosis Genotype Predicts Increased Iron and Erythrocyte Indices in Women

ENRICO ROSSI,1* JOHN K. OLYNYK,2 DIGBY J. CULLEN,3,4 GEORGE PAPADOPOULOS,3 MAX BULSARA,5 LESA SUMMERVILLE,6 and LAWRIE W. POWELL6

Background: Women who inherit heterozygosity for the C282Y mutation of the HFE gene may have increased serum iron indices and hemoglobin and are less likely to develop iron deficiency compared with women with the wild-type genotype.

Methods: We performed a cross-sectional analysis of 497 women 20–44 years of age and 830 women >51 years of age drawn from the Busselton (Australia) population study to assess the effects of the HFE genotype on serum iron and hematologic indices.

Results: Heterozygosity for the C282Y mutation occurred in 13.8% of the study population, comprising 11.8% C282Y wild-type heterozygotes and 2.0% C282Y/H63D compound heterozygotes. In the younger age group, C282Y wild-type women did not have significantly increased serum iron, transferrin saturation, or hemoglobin values, and were not protected from developing iron deficiency, compared with women of the same age with the wild-type genotype. Young compound heterozygous women had higher means for serum iron (25.0 vs 16.9 μmol/L; P < 0.001), transferrin saturation (42.0% vs 25.6%; P < 0.05), hemoglobin (139.4 vs 132.3 g/L; P < 0.05), and corpuscular volume (91.1 vs 87.7 fL; P < 0.05), and a higher median ferritin (53 vs 44 μg/L; P < 0.05) compared with the wild-type genotype. Similar results were observed for compound heterozygotes in the >51 years age group.

Conclusions: Women with the compound heterozygous HFE genotype C282Y/H63D, but not the C282Y wild-type genotype, had increased values for serum iron and transferrin saturation, and the younger age group also had increased hemoglobin values. We conclude that the compound heterozygous genotype may have a beneficial effect in protecting women from iron deficiency.

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Hereditary hemochromatosis is a common iron overload disease with autosomal recessive inheritance that occurs predominantly in individuals of Northern European origin. Recently, a novel MHC class I-like candidate gene, termed HFE, for hereditary hemochromatosis containing two missense mutations was identified. 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 either as a C282Y wild-type heterozygous (C282Y/wt)7 or compound heterozygous (C282Y/H63D) HFE genotype. Our recent population study of asymptomatic Australians revealed a prevalence of 11.9% for C282Y/wt heterozygosity, 2.2% for C282Y/H63D heterozygosity, and 0.53% (1 in 188) for homozygosity of the C282Y mutation (7).

Women of childbearing age have a high prevalence of...
iron deficiency and associated anemia. A recent US survey showed that 9–11% of women in this age group were iron deficient and that 2–5% had iron-deficiency anemia (8). Iron deficiency has many negative effects on health, including impairments in immune response and work performance (9). Recent reports have suggested that heterozygosity for the C282Y mutation may confer a protective effect against iron deficiency in women of childbearing age. A study of young women in Austria reported significantly increased values for hemoglobin and transferrin saturation values in C282Y heterozygous women (10), and a US study found that heterozygous women had a significantly lower incidence of iron deficiency (11). However, these studies did not independently evaluate the contributions of the C282Y/wt and C282Y/H63D HFE genotypes.

We performed a cross-sectional analysis of 497 women 20–44 years of age and 830 women >51 years of age to determine the effect of the C282Y/wt and C282Y/H63D HFE genotypes on serum iron and hematology indices and the prevalence of iron deficiency.

Patients 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 1491 nonrelated female subjects 20 to 79 years of age and divided them into two age groups: 502 women between 20 and 44 years of age, and 833 women between 51 and 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.

Table 1. Genotype prevalence.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Overall group</th>
<th>20–44 years</th>
<th>&gt;51 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Prevalence, %</td>
<td>n</td>
</tr>
<tr>
<td>wt/wt&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1143</td>
<td>85.6</td>
<td>426</td>
</tr>
<tr>
<td>C282Y/wt&lt;sup&gt;b&lt;/sup&gt;</td>
<td>157</td>
<td>11.8</td>
<td>62</td>
</tr>
<tr>
<td>C282Y/H63D&lt;sup&gt;c&lt;/sup&gt;</td>
<td>27</td>
<td>2.0</td>
<td>9</td>
</tr>
<tr>
<td>C282Y/C282Y&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8</td>
<td>0.6</td>
<td>5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Wild type (refers to absence of the C282Y mutation).
<sup>b</sup> Heterozygote.
<sup>c</sup> Compound heterozygote.
<sup>d</sup> C282Y homozygote.

Measurement of serum indices

Serum iron concentrations were measured by a standard colorimetric method, and 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/2 × transferrin) × 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) estimations 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 (GenBank accession no. U60319) of Feder et al. (1) and cycling conditions described by Cullen et al. (14). Mutations were determined by 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 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, χ<sup>2</sup> test, and generalized linear models adjusted for multiple means test using the least significant difference method were used. The normality test was carried out on all indices. Ferritin was highly skewed, and log transformation was used for all subsequent analysis. Statistical analyses were performed with SAS software (15).
Table 2. Iron metabolism and hematology variables [given as mean (SD)]a in 497 women, ages 20–44 years, according to HFE genotype.

<table>
<thead>
<tr>
<th></th>
<th>wt/wt</th>
<th>C282Y/wt</th>
<th>C282Y/H63D</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>426</td>
<td>62</td>
<td>9</td>
</tr>
<tr>
<td>Age, years</td>
<td>35.6 (6.8)</td>
<td>34.9 (7.2)</td>
<td>34.2 (3.4)</td>
</tr>
<tr>
<td>Iron, μmol/L</td>
<td>16.9 (6.4)</td>
<td>17.8 (7.1)</td>
<td>25.0 (8.8)</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>25.6 (22.8)</td>
<td>28.3 (14.1)</td>
<td>42.0 (11.7)</td>
</tr>
<tr>
<td>Ferritin, μg/L</td>
<td>44 (9.1)</td>
<td>38 (1.02)</td>
<td>53 (0.87)</td>
</tr>
<tr>
<td>Hb, g/L</td>
<td>132.3 (9.5)</td>
<td>133.9 (8.3)</td>
<td>139.4 (11.9)</td>
</tr>
<tr>
<td>MCV, fL</td>
<td>87.7 (4.7)</td>
<td>89.2 (5.0)</td>
<td>91.1 (3.6)</td>
</tr>
<tr>
<td>MCH, pg</td>
<td>30.0 (1.8)</td>
<td>30.6 (1.9)</td>
<td>31.1 (1.4)</td>
</tr>
<tr>
<td>Ferritin &lt;20 μg/L</td>
<td>20.2%</td>
<td>29.0%</td>
<td>0%</td>
</tr>
<tr>
<td>Ferritin &lt;12 μg/L</td>
<td>3.0%</td>
<td>4.8%</td>
<td>0%</td>
</tr>
</tbody>
</table>

a Ferritin given as median (log SD).

Results

GENOTYPE PREVALENCE

The prevalences of the wild types (wt/wt), heterozygotes (C282Y/wt), compound heterozygotes (C282Y/H63D), and homozygotes (C282Y/C282Y) for the C282Y mutation in the overall study population and the two age groups are shown in Table 1. There were no significant differences in genotype prevalences between the two age groups (P = 0.46). For the overall study group, prevalence of the C282Y/wt genotype was 11.8% and of the C282Y/H63D genotype was 2.0%.

Homozygosity for the C282Y mutation occurred in 0.6% of the overall population (a ratio of 1:167), comprising five women between 20 and 44 years of age and three women >51 years of age. The phenotypic presentation and clinical data for these subjects with hereditary hemochromatosis are reported separately (7).

IRON AND HEMATOLOGY STUDIES

The mean and SD for serum iron and red cell indices according to genotype in the 20–44 years age group are shown in Table 2. Iron depletion was defined by a ferritin concentration <20 μg/L (16) and iron deficiency by a ferritin concentration <12 μg/L and a transferrin saturation <15% (8). The significance values are shown for comparison of either C282Y/wt or C282Y/H63D genotypes to the wild type.

When compared with the wild-type genotype, C282Y/wt women had significantly higher MCV (P = 0.02) and MCH (P = 0.03) values, but not serum iron (P = 0.33), transferrin saturation (P = 0.35), ferritin (P = 0.64), or hemoglobin values (P = 0.28). Iron depletion occurred in 20.2% of women with the wild-type genotype compared with 29.0% of C282Y/wt women (P = 0.11), and iron deficiency occurred in 5.4% and 4.8%, respectively.

C282Y/H63D women had significantly higher values for serum iron (P = 0.0003), transferrin saturation (P = 0.02), and ferritin (P = 0.01) when compared with the wild-type genotype. Hemoglobin (P = 0.03) and MCV (P = 0.03) were also higher, whereas the trend to a higher MCH did not achieve significance (P = 0.07). Neither iron depletion nor iron deficiency were present in any C282Y/H63D women, but this was not significantly different when compared with the wild-type genotype.

Comparisons of serum iron and red cell indices for the C282Y/wt and C282Y/H63D genotypes with the wild type in the group over 51 years of age are shown in Table 3. C282Y/wt women had significantly higher values for serum iron (P = 0.04) and transferrin saturation (P = 0.02) compared with the wild-type genotype, whereas the hemoglobin concentrations, red cell indices, and criteria for iron depletion or iron deficiency were not significantly different. We assessed ferritin data for both the wild-type and C282Y/wt genotype and found no trend to increased values with age. There was a slight negative trend toward lower ferritin (P = 0.017) with increasing age in the C282Y/wt women.

Compared with the wild-type genotype, women with the C282Y/H63D genotype had significantly higher values for serum iron (P = 0.002), transferrin saturation (P = 0.0001), MCV (P = 0.006), and MCH (P = 0.001) indices, but not hemoglobin (P = 0.45) or ferritin values. None of the C282Y/H63D women >51 years of age were iron deficient, but this was not significantly different when compared with women with the wild-type genotype.

Discussion

Recent reports have suggested that heterozygosity for the C282Y mutation may confer a protective effect against iron deficiency in women (10,11). We have performed a

Table 3. Iron metabolism and hematology variables [given as mean (SD)]a in 830 women >51 years according to HFE genotype.

<table>
<thead>
<tr>
<th></th>
<th>wt/wt</th>
<th>C282Y/wt</th>
<th>C282Y/H63D</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>717</td>
<td>95</td>
<td>18</td>
</tr>
<tr>
<td>Age, years</td>
<td>64.8 (7.9)</td>
<td>65.4 (8.8)</td>
<td>63.0 (6.8)</td>
</tr>
<tr>
<td>Iron, μmol/L</td>
<td>17.3 (4.9)</td>
<td>18.4 (5.1)</td>
<td>21.0 (5.9)</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>26.3 (9.1)</td>
<td>28.8 (9.3)</td>
<td>35.5 (10.1)</td>
</tr>
<tr>
<td>Ferritin, μg/L</td>
<td>90 (0.85)</td>
<td>92 (0.72)</td>
<td>82 (1.08)</td>
</tr>
<tr>
<td>Hb, g/L</td>
<td>135.5 (9.7)</td>
<td>136.8 (9.7)</td>
<td>137.3 (9.8)</td>
</tr>
<tr>
<td>MCV, fL</td>
<td>88.9 (4.3)</td>
<td>89.3 (3.9)</td>
<td>91.7 (2.8)</td>
</tr>
<tr>
<td>MCH, pg</td>
<td>30.3 (1.6)</td>
<td>30.5 (1.4)</td>
<td>31.6 (0.9)</td>
</tr>
<tr>
<td>Ferritin &lt;20 μg/L</td>
<td>4.6%</td>
<td>3.2%</td>
<td>5.6%</td>
</tr>
<tr>
<td>Ferritin &lt;12 μg/L</td>
<td>1.4%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

a Ferritin given as median (log SD).

b–d Significance when compared with the wt/wt genotype: b P <0.001; c P <0.05; d Hb, hemoglobin.
cross-sectional analysis of women in two age groups to define the effects of the heterozygous (C282Y/wt) and compound heterozygous (C282Y/H63D) HFE genotypes on serum iron and hematology indices and the prevalence of iron deficiency. When we compared each genotype to the wild type, we found significantly increased serum iron and transferrin saturation values in both age groups of C282Y/H63D women but not C282Y/wt women. Younger C282Y/H63D women also had higher hemoglobin and MCV indices than women with a wild-type genotype, consistent with a beneficial effect of this genotype in protecting against iron deficiency.

The prevalence of C282Y/wt in the overall study group was 11.8%, and the prevalence of C282Y/H63D was 2.0%; thus, a total of 13.8% of our population had one C282Y mutated allele. These values are among the highest reported globally. Population studies of both genders from the US (17) and Jersey, UK (18) reported prevalence rates of 9.7% and 11.4% for heterozygotes and 2.2% and 3.2% for compound heterozygotes, respectively. A previous study in young women from Austria studied only the C282Y mutation and reported that the frequency of heterozygosity for the C282Y mutation was 9.5% (10).

In women 20–44 years of age, no significant differences in serum iron, transferrin saturation, or ferritin were seen when wild-type and C282Y/wt genotypes were compared (Table 2). Datz et al. (10) recently reported that serum iron and transferrin saturation were significantly increased in young heterozygous women; however, only the C282Y mutation was tested, and subjects with the C282Y/H63D HFE genotype were also included in their heterozygote category. A US study of heterozygous women between 31 and 60 years of age reported significantly increased serum iron and transferrin saturation compared with wild-type subjects (11). However, these women were judged to be heterozygous on the basis of family studies and HLA typing rather than genotyping.

To facilitate comparison with other studies, we also assessed our data by combining the C282Y/wt and C282Y/H63D genotype women into one group, as in previous studies, to see if this would introduce significant differences when compared with the wild-type genotype. In the 20–44 years age group, serum iron was significantly higher in the total heterozygous group, but transferrin saturation and hemoglobin again failed to achieve the significantly increased values reported in the earlier literature. For the older age group, only MCH changed to become significantly higher. Therefore, only some of the discrepancies between the present work and previous studies (10, 11) could be related to the lack of differentiation between the C282Y/wt and C282Y/H63D genotypes. An alternative explanation for the discrepancies may be differences in the ethnic composition of our study population, which was 90% Anglo-Celtic, compared with populations studied in Austria (10) or the US (11).

Previous studies have indicated that heterozygosity for the C282Y mutation offers protection from iron deficiency. In a study of 350 heterozygous women between 12 and 50 years of age (11), 21% had ferritin values <12 µg/L, compared with 32% of wild-type subjects (P = 0.02). Datz et al. (10) reported that 6.7% of their young women with the wild-type genotype met criteria for iron deficiency compared with 4.5% of the C282Y heterozygous women (P, not significant). We observed a high prevalence for iron depletion (ferritin <20 µg/L) and iron deficiency (ferritin <10 µg/L) in young women. Iron depletion occurred in 20.2% of women with the wild-type genotype compared with 29.0% for the C282Y/wt genotype (P = 0.11).

We found significantly increased serum iron, transferrin saturation, and ferritin results in younger C282Y/H63D women compared with the wild-type genotype (Table 2). These results support the concept that subjects with the C282Y/H63D genotype are at increased risk of developing iron loading (5, 6). Young C282Y/H63D women also had higher hemoglobin and MCV indices than women with a wild-type genotype, but the trend to a higher MCH did not achieve significance. None of the younger C282Y/H63D women had either iron depletion or iron deficiency.

In summary, we have demonstrated that women with the C282Y/wt and C282Y/H63D women >51 years of age showed significantly increased serum iron and transferrin saturation (Table 3) compared with the wild-type genotype. This is in agreement with a study reporting on a postmenopausal group 61–90 years of age (11). There were no significant differences between C282Y/wt and wild-type genotypes for hematology indices, iron depletion, or iron deficiency criteria in the older age group. Previous studies have suggested that ferritin values increase with age in postmenopausal women (11, 19); however, neither the wild-type nor C282Y/wt genotype women in the >51 years age group demonstrated this trend.

In summary, we have demonstrated that women with the C282Y/H63D HFE genotype, but not the C282Y/wt genotype, had significantly increased values for serum iron and transferrin saturation. We conclude that the compound heterozygous genotype may have a beneficial effect in protecting women from iron deficiency.

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

References


