Heterozygosity for the C282Y mutation in the hemochromatosis gene is associated with increased serum iron, transferrin saturation, and hemoglobin in young women: a protective role against iron deficiency?

Christian Datz, Thomas Haas, Heinrich Rinner, Friedrich Sandhofer, Wolfgang Patsch, and Bernhard Paulweber

Genetic hemochromatosis (GH) is the most common autosomal-recessive disorder (1 in 300 in populations of Celtic origin). Homozygosity for a C282Y mutation in the hemochromatosis (HFE) gene is the underlying defect in ~80% of patients with GH, and 3.2–13% of Caucasians are heterozygous for this gene alteration. Because the high frequency of this mutation may result from a selection advantage, the hypothesis was tested that the C282Y mutation confers protection against iron deficiency in young women. To address this question the genotype of codon 282 was determined in a cohort of 468 unrelated female healthcare workers, ages 18–40 years. In all study participants, a complete blood count was obtained, and erythrocyte distribution width, serum iron, transferrin, transferrin saturation, and ferritin were measured. Two individuals were homozygous for the C282Y mutation, 44 were heterozygous, and 416 were homozygous for the wild-type allele. Heterozygous women had significantly higher values for hemoglobin (P = 0.006), serum iron (P = 0.013), and transferrin saturation (P = 0.006) than women homozygous for the wild-type allele. Our data provide evidence for a protective role of the C282Y mutation in the HFE gene against iron deficiency in young women and suggest that a more efficient utilization of nutritional iron may have contributed to the high prevalence of the mutation in Caucasian populations.

The high prevalence of iron deficiency and iron deficiency anemia (1, 2), especially in women of childbearing age, and its association with central nervous dysfunction (3–5), impaired work performance and response to exercise (6), impaired immune response (7), thermogenesis (8) and energy metabolism, and adverse outcome of pregnancy (9) represent a serious health problem. Worldwide, iron deficiency is the most common nutritional disorder. Women are more prone to suffer from the consequences of iron deficiency anemia because of menstrual blood loss and enhanced iron requirements during pregnancy and lactation, especially in developing countries (10).

Genetic hemochromatosis (GH), a disorder that causes iron overload, is the most common autosomal recessive disorder, affecting 1 in 300 individuals in Northern European populations (11). Recently, mutations in the hemochromatosis (HFE) gene that are responsible for GH have been identified (12). A G-to-A transition at cDNA position 845 produces a substitution of tyrosine for a highly conserved cysteine residue at position 282 of the HFE protein. Homozygosity for this mutation has been found in 67–100% of patients with GH (12–17), and 3.2–13% of Caucasians have been found to be heterozygous for this gene alteration (12–17). Such a high prevalence of a single mutation is unusual for a disorder with an autosomal-recessive mode of inheritance. Analysis of microsatellites at the HFE gene locus revealed that the 282 mutation occurs on chromosomes with the same haplotype in different populations (18, 19). This observation is a strong argument for a founder effect of the mutation arising on a single chromosome. The high frequency of this particular genetic defect may therefore be the result of a selection advantage for heterozygous carriers of this mutation. The metabolic consequences of the heterozygous state on iron
metabolism may confer such a biological advantage. In
the present study, the hypothesis was tested that the
C282Y mutation in the HFE gene represents a protective
factor against iron deficiency in young women.

Materials and Methods

Patients and Study Design
Blood was taken after an overnight fast from 468 unrelated,
nonpregnant female healthcare workers, ages 18–40 years.
Information about demographic variables and factors that
are known to potentially affect iron metabolism, such as age,
family history of liver disease, surgery or major trauma,
blood transfusions, blood donations (standard unit donation
of 400 mL), iron supplementation, time from menarche,
abnormal menstruation, metrorrhagia, number of pregnan-
cies, and meat and alcohol consumption was obtained by
questionnaires. In all subjects, various laboratory variables
were obtained, including a complete blood count, red cell
distribution width, C-reactive protein, alanine aminotrans-
ferase, serum creatinine, serum iron, transferrin, transferrin
saturation, and ferritin. Individuals showing evidence of
chronic inflammatory liver disease (n = 4) or signs of an
acute inflammatory process as judged by increases in C-re-
active protein (n = 2) were excluded from the analysis. Iron
deficiency was defined as a ferritin concentration <12 µg/L
and a serum transferrin saturation <15% (2). The hemoglo-
bin cutoff for anemia was calculated as the mean of the
study population minus 1.96 SD. Subjects were considered
to be anemic if their hemoglobin was <11.5 g/L.

Hematologic and Biochemical Assays
Complete blood counts were determined with a Sysmex
NE-1500 automated hematology analyzer (Toa Medical
Electronics, Ltd.). Serum iron was measured by the ferro-
zin method, and transferrin and C-reactive protein were
determined by turbidimetric procedures using a Hitachi
917 analyzer (Boehringer Mannheim Diagnostics) and the
respective kits (cat. nos. 1490869, 1552180, and 17300371;
Boehringer Mannheim). Transferrin saturation was cal-
culated by dividing the serum iron by the total iron-binding
capacity and multiplying by 100. Serum ferritin was
determined using the AxSYM microparticle enzyme im-
munoassay (Abbott Laboratories).

DNA Isolation and Amplification
Genomic DNA was prepared from blood, using the Isolate 1 DNA extraction kit (Crucachem Ltd.). DNA frag-
ments were amplified by PCR, using the primers de-
scribed (5′-ACATGGTTAAGGCTCTTGCC-3′ and 5′-GC-
CACATCTGGCTTGAAT-3′ for the C187G mutation
at codon 63; 5′-TGGCAAGGTTAACACATCC-3′ and 5′-
CTCGGACTCTCCTCACC-3′ for the G845A muta-
tion at codon 282 of the HFE gene) (12). Amplification of
both regions of the HFE gene was performed using the
same PCR conditions. Each 100-µL reaction contained 10
mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L
MgCl₂, 0.2 mmol/L each dNTP, 2.5 U of AmpliTaq DNA
Polymerase (Perkin-Elmer), 200 ng of genomic DNA, and
200 ng of each oligonucleotide primer. A “hot start” PCR
at 96 °C was followed by 35 cycles of denaturation at
96 °C for 30 s, annealing at 56 °C for 60 s, and extension at
72 °C for 60 s. PCR products were desalted using Ultrafree
R-MC (30 000 NMWL) filter units (Millipore Corp.).

Restriction Fragment Length Analysis
Mutations were detected by restriction fragment length analysis. The G-to-A transition at nucleotide 845 creates a
recognition site for the restriction enzyme SnaBI (New
England Biolabs); the C-to-G transversion at nucleotide 187
abolishes the DNA sequence recognition site for endonucle-
ase BclI (New England Biolabs). Restriction fragments were
separated on 2% NuSieve GTG agarose and 1% agarose
MP (Boehringer Mannheim) gels containing ethidium bro-
mide and visualized by ultraviolet transillumination.

Statistical Analysis
Statistical analysis was carried out using the S-PLUS 3.3 or SPSS
7.5 software for Windows. Univariate comparisons of demo-
graphic and biochemical variables between the two HFE geno-
type groups were performed with the Student t-test and the χ²
test, respectively. Continuous variables were checked for gauss-
ian distribution. Ferritin measures were log-transformed be-
cause their distribution was skewed. All statistical tests were
two-sided. To assess the effect of HFE genotype on hemoglobin
and other variables known to potentially affect hemoglobin,
data for these variables were analyzed using multiple linear
regression modeling.

Written informed consent was obtained from all study par-
ticipants. The study was approved by the local ethics
committee and was performed according to the guide-
lines of the Helsinki Declaration.

Results
The results of the demographic and genotype analyses are
summarized in Table 1. Of 462 subjects studied, two (0.4%)
were found to be homozygous for the C282Y mutation. The
first woman, a 19-year-old nurse, exhibited increased values
for serum iron (17.4 µg/L) and transferrin saturation (55%).
Her serum ferritin, hemoglobin, and red cell distribution
width were within the health-related reference range. The
second subject was a 40-year-old nurse with a history of
hysterectomy 2 years before investigation and who showed
moderate biochemical signs of iron overload as evidenced
by a serum iron of 22.1 µg/L, a transferrin saturation of 90%,
and a ferritin concentration of 403 µg/L. Her hemoglobin,
red cell distribution width, and serum transaminases were
within the health-related reference ranges.

Forty-four (9.5%) women were heterozygous for the
C282Y mutation, and 416 (90.1%) were homozygous for the
wild-type allele. Compared with the women heterozygous
for the C282Y mutation, wild-type subjects did not display
any differences in age, body mass index, and history of
surgery or major trauma. No difference was observed be-
tween the heterozygous and wild-type groups in the pro-
portion of subjects who had received blood transfusions, had a history of blood donation, or had used iron supplementation. Their histories of abnormal menstruation, number of pregnancies, and meat and alcohol consumption were similar, and no difference in time from menarche was observed. Significantly more subjects heterozygous for the C282Y mutation had a positive family history of liver disease ($P$, 0.001; Table 1). As shown in Table 2, analysis of the biochemical variables revealed significantly higher values for hemoglobin ($P$, 0.002), serum iron ($P$, 0.013), and transferrin saturation ($P$, 0.006) in individuals heterozygous for GH. The association between the $HFE$ genotype and hemoglobin remained highly significant ($P$, 0.01) in multivariate analysis, adjusting for variables such as family history of liver disease, history of surgery or major trauma, number of subjects receiving transfusions, medical iron supplementation, number of pregnancies, and alcohol consumption. We could not detect a difference in ferritin concentrations ($P$, 0.35) and red cell distribution width ($P$, 0.38) between heterozygous and wild-type homozygous individuals. Twenty-eight subjects (6.7%) homozygous for the wild-type allele presented with iron deficiency; nine of them fulfilled the criteria for iron deficiency anemia. Only two of the heterozygous individuals (4.5%) were iron deficient, and neither of them was anemic. These differences were not statistically significant. In the subgroup of women with two or more pregnancies, the difference in hemoglobin concentrations between subjects with different $HFE$ genotypes was more pronounced than among nulliparous women. Again, this difference was not statistically significant (data not shown).

**Discussion**

The data presented support the hypothesis of a biological advantage for young female carriers of the $HFE$ mutation. Several biochemical measures of iron status showed sig-

### Table 1. Demographic variables for women homozygous for the wild-type allele and women heterozygous for the Cys282Tyr mutation of the $HFE$ gene.

<table>
<thead>
<tr>
<th></th>
<th>Wild-type Cys/Cys</th>
<th>Heterozygous Cys/Tyr</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>416</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>27.83 (± 7.07)</td>
<td>28.05 (± 6.70)</td>
<td>0.85</td>
</tr>
<tr>
<td>Body mass index</td>
<td>22.50 (± 3.43)</td>
<td>21.82 (± 2.64)</td>
<td>0.21</td>
</tr>
<tr>
<td>Family history of liver disease, %</td>
<td>5</td>
<td>25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of surgery or major trauma, %</td>
<td>3</td>
<td>5</td>
<td>0.47</td>
</tr>
<tr>
<td>Subjects receiving transfusions, %</td>
<td>4</td>
<td>5</td>
<td>0.75</td>
</tr>
<tr>
<td>History of blood donations, %</td>
<td>21</td>
<td>18</td>
<td>0.70</td>
</tr>
<tr>
<td>History of iron supplementation, %</td>
<td>13</td>
<td>7</td>
<td>0.24</td>
</tr>
<tr>
<td>Time from menarche, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;7 years</td>
<td>14</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>&gt;14 years</td>
<td>39</td>
<td>39</td>
<td>0.86</td>
</tr>
<tr>
<td>&gt;28 years</td>
<td>47</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>History of abnormal menstruation, %</td>
<td>21</td>
<td>27</td>
<td>0.35</td>
</tr>
<tr>
<td>History of metrorrhagia, %</td>
<td>5</td>
<td>11</td>
<td>0.06</td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>307 (74%)</td>
<td>28 (64%)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>47 (11%)</td>
<td>7 (16%)</td>
<td>0.24</td>
</tr>
<tr>
<td>2</td>
<td>42 (10%)</td>
<td>8 (18%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>20 (5%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Meat consumption</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;three times weekly, %</td>
<td>33</td>
<td>36</td>
<td>0.69</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;twice weekly, %</td>
<td>19</td>
<td>18</td>
<td>0.87</td>
</tr>
</tbody>
</table>

### Table 2. $HFE$ genotype and biochemical variables related to iron metabolism in 460 young women.

<table>
<thead>
<tr>
<th></th>
<th>Wild-type Cys/Cys</th>
<th>Heterozygous Cys/Tyr</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>416</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Serum iron, μg/L</td>
<td>10.15 (± 4.43)</td>
<td>11.91 (± 4.54)</td>
<td>0.013</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>23.64 (± 11.84)</td>
<td>28.89 (± 12.43)</td>
<td>0.006</td>
</tr>
<tr>
<td>Ferritin, μg/L</td>
<td>42.56 (± 30.96)</td>
<td>50.73 (± 41.78)</td>
<td>0.35</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>13.44 (± 0.99)</td>
<td>13.93 (± 0.93)</td>
<td>0.002</td>
</tr>
<tr>
<td>Erythrocyte distribution width (fL)</td>
<td>41.38 (± 2.91)</td>
<td>40.99 (± 1.90)</td>
<td>0.38</td>
</tr>
<tr>
<td>Iron deficiency</td>
<td>28 (6.7%)</td>
<td>2 (4.5%)</td>
<td>0.58</td>
</tr>
<tr>
<td>Iron deficiency anemia</td>
<td>9 (2.2%)</td>
<td>0 (0.0%)</td>
<td>0.97</td>
</tr>
</tbody>
</table>
significant differences between women heterozygous for C282Y and those homozygous for the wild-type allele. These included serum iron and transferrin saturation, both of which were increased. Although serum ferritin values were also higher in heterozygotes, the range of ferritin concentrations was large, and differences were not significant. In a recently reported analysis of GH families, similar findings were obtained by Bulaj et al. (19) and Powell and Jazwinska (20) for a group of heterozygous females of comparable age, identified by HLA typing, whose serum iron and transferrin saturation were increased but whose ferritin concentrations were similar to those of controls. In contrast to this study, we investigated women, unrelated in reproductive age, who were most prone to develop iron deficiency. Furthermore, in our study a direct mutation detection assay was used.

Although it has been demonstrated recently that the HFE gene product complexes with the transferrin receptor and decreases the affinity of transferrin binding (21), full understanding of the observed changes in standard laboratory indices will not be possible until the function of the HFE gene product in iron metabolism is known. Nevertheless, we speculate that the C282Y mutation in the HFE gene is conducive to increased intestinal iron absorption, thereby conferring protection against iron deficiency and iron deficiency anemia.

The C282Y mutation is believed to have originated in a Celtic population. We suggest that the importance of its biological advantage decreased over time because iron deficiency is now less common because of reduced birth rates, the use of oral contraceptives, oxytocic drugs, medical iron supplementation, and improved nutrition. Notwithstanding a putative importance of the C282Y mutation in the past, its protective effect may still be relevant in present times for conditions of enhanced iron demand that may result from recurring pregnancies. Influences of the mutation on iron metabolism are supported by our study, which showed a difference in iron supplementation between the heterozygous and wild-type subjects. In addition, a history of metrorrhagia was reported by a larger percentage of heterozygous individuals, and this group also contained a higher proportion of multiparous women. These facts could have attenuated genotypic effects on some indicators reflecting iron metabolism. Interestingly, the prevalence of iron deficiency and iron deficiency anemia was considerably lower in our study population, which consisted exclusively of healthcare workers, than in other study cohorts of women with comparable age (2). Conceivably, such a sample bias could have attenuated the relevance of our results.

In conclusion, our data strongly suggest a role of the C282Y mutation in the HFE gene in preventing iron deficiency in young females. Protection against iron deficiency and its morbid consequences may have conferred a selection advantage for heterozygous carriers of the mutation in the past and may explain the high prevalence of this particular mutation, which accounts for the most frequent genetic disorder with an autosomal mode of inheritance.

This work was supported by grants from the Medizinische Forschungsgesellschaft Salzburg and by the Jubilaumsfonds Projekt No. 6041/2 of the Oesterreichische National Bank. We thank C. Talman, E. Meisl, and G. Sander for technical assistance.

References