Correlation between Iron Status and Genetic Hemochromatosis (Codon C282Y) in a Large German Population

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Abstract

Background: Genetic hemochromatosis leads to iron overload in many tissues and may lead to liver cirrhosis and hepatocellular carcinoma. Early diagnosis and therapy are crucial. Since 80–100% of hemochromatosis patients of European origin are homozygous for a cysteine to tyrosine exchange in the *HFE* gene at codon 282, genetic screening might be useful. Representative population studies are needed to evaluate the phenotype of people heterozygous and homozygous for the C282Y mutation.

Objective: To determine the correlation between parameters of iron metabolism and the hemochromatosis genotype in a large population-based study.

Methods: A representative population-based survey, the Diabetomobil study, analyzed 5,083 German probands. Serum transferrin saturation and ferritin levels were determined, and the C282Y mutation of the *HFE* gene was analyzed by restriction fragment length polymorphism-polymerase chain reaction analysis.

Results: Nine of 373 probands with a transferrin saturation > 55% (2.4%) and none of 264 randomly selected probands with a transferrin saturation ⩽ 55% (0%) were homozygous for the C282Y mutation. Three of the nine homozygous probands had ferritin values less than 250 μg/L. The frequency of the heterozygous genotype was 8.8%, and the percentage of heterozygous probands increased with increasing levels of transferrin saturation.

Conclusion: We propose a population screening strategy with an initial transferrin saturation test, followed by genotyping for the C282Y mutation if the transferrin saturation is above 55%, regardless of the ferritin level. Heterozygous individuals with higher transferrin saturation values may be protected against iron loss but may also be more susceptible for certain liver diseases, depending on the simultaneous prevalence of other diseases.

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Hereditary hemochromatosis is one of the most common inherited human diseases and leads to iron overload of many tissues. Clinical signs are fatigue, liver cirrhosis, diabetes, cardiac disease, pigmentation, hypogonadism and others. Early therapy can prevent organ damage and preserve normal life expectancy. Because of the high disease prevalence, potential serious manifestations, available screening methods and effective treatment possibilities, hemochromatosis is an excellent target for population screening [1].

The prevalence of hemochromatosis was previously estimated by pedigree analysis. These studies suggested a disease frequency of 0.3%–0.4% and a carrier frequency of 11% in the general population [2,3]. Based on pedigree analysis, screening for hemochromatosis by determining serum ferritin and transferrin saturation was recommended. However, the thresholds for TFS that were used varied between 45 and 62% [4,5].

Since the discovery of the *HFE* gene in 1996 [6], 80–100% of the patients with genetic hemochromatosis were found to be homozygous for the C282Y mutation of the *HFE* gene [7–9]. The high frequency of the C282Y mutation in patients with genetic hemochromatosis suggested that genetic testing be included in screening programs for hemochromatosis. However, at this time genetic testing in general screening programs is not recommended due to uncertainties regarding the prevalence and penetrance of the C282Y mutation [10,11]. The aim of the present study was to determine the correlation between parameters of iron metabolism and the hemochromatosis genotype in a large population-based study.

Subjects and Methods

Study population

The subjects in this study had originally participated in the Diabetomobil Study, a nationwide epidemiologic survey on metabolic disorders performed between 1993 and 1996 in the adult German population [12]. In that survey 5,083 subjects were investigated by a physician in a mobile survey unit in cities and rural communities in 5 of the 16 German states. Of these, 1,306 participants were recruited in a "street setting" (streets and house numbers were randomly chosen and the inhabitants were asked to participate in the study) and 3,777 subjects were investigated in a "marketplace setting" (where all bystanders could participate). All participants filled out a questionnaire including medical history, current health status and personal data (marital status, education, occupation, household size). Gender, age, body mass index and personal data were compared with statistical data on the total German population provided by the German Federal Statistic office

TFS = transferrin saturation

(Statistisches Bundesamt, Wiesbaden, Germany). The street setting cohort showed no significant difference in these parameters to the total German population and was therefore considered to be representative (data not shown) [12,13]. Gender was equally distributed between the "marketplace setting" and the "street setting." The subjects in the marketplace setting were older than those in the street setting (50.8 ± 14.4 years vs. 44.8 ± 14.3 years), but the means for TFS and ferritin in the age classes were not significantly different between the settings. The values for age group 18–30 years were TFS 34.6% (street setting) vs. 34.9% (marketplace setting), $P = 0.65$; for 31–40 years 36.0 vs. 36.0%, $P = 0.84$; for 41–50 years 34.8 vs. 35.6%, $P = 0.69$; for 51–60 years 36.0 vs. 36.6%, $P = 0.46$; and for 61–70 years 37.2 vs. 38.8%, $P = 0.12$.

Since there were no significant differences in the screening parameters TFS and ferritin between the cohorts, both groups were considered to be representative with respect to these parameters and were pooled for further analysis. All study subjects gave informed consent for the Diabetomobil Study. The participants remained anonymous throughout the study.

Biochemical measurements

Blood was taken by venipuncture. Transferrin and ferritin were measured in frozen serum samples by immunonephelometry (N antisera to human transferrin and N latex ferritin, Dade Behring, Marburg, Germany). Serum iron was measured with a photometric assay (No. 1553712, Boehringer Mannheim, Germany). TFS was calculated from transferrin and serum iron.

Genotyping

Genotyping for the C282Y mutation of the *HFE* gene was performed for all samples with a TFS above 55%. In addition, about 6% of the samples with a TFS of 0–5%, 6–10%, up to 51–55%, were selected by means of a random number generator (SPSS software) and genotyping was performed. For genetic testing DNA was prepared from whole-blood samples by standard procedures (QIAmp Blood Kit, Qiagen, Hilden, Germany). Polymerase chain reaction was performed with the primers previously described by Feder et al. [6], using an annealing temperature of 59°C. The mutation was detected by restriction digestion with SnaBI and agarose gel electrophoresis.

Statistical analysis

The mean values for the difference between the street setting and the marketplace setting were calculated with SPSS software (SPSS for Windows, Rel. 8.0.1, 1997). Significance tests were performed with the Mann-Whitney test, significance levels were set at $P < 0.05$. Linear correlation analyses were used to test the correlation between the TFS and the heterozygous genotype (SPSS 8.0.1 software). To estimate the frequency of heterozygous subjects in the whole study population the expected numbers of heterozygous subjects in each TFS class were calculated and added. For example, of 47 genotyped subjects in the TFS class 41–50%, 5 (10.6%) were found to be heterozygous. Of 1,001 participants in this TFS group 106 (10.6%) participants were therefore expected to be heterozygous.

Results

The study population consisted of 2,469 women (48.6%) and 2,614 men (51.4%). The characteristics of the study group are shown in Table 1. Women had mean (SD) ferritin values of 77.4 ± 96.2 µg/L, while the levels in men were significantly higher, 171.4 ± 175.9 µg/L ($P < 0.001$). The distribution curves of the TFS are shown in Figure 1. The 5th and 95th percentiles are located at 16% and 56% for women and 21% and 61% for men, respectively. The mean TFS in the study cohort was 34.4 ± 12.6% for women and 38.5 ± 13.3% for men ($P < 0.001$).

TFS was higher than 55% in 7.3% (n=373) of the participants and ⩽ 55% in 92.7% (n=4,710). Genotyping was performed for all samples with TFS above 55%. Below 55%, the samples were divided into TFS classes and about 6% of samples from each class were randomly selected and genotyped. Overall, 264 (5.6%) participants with a TFS ⩽ 55% were genotyped.

Nine of 373 subjects with a TFS above 55% were homozygous for the C282Y mutation (2.4%). Six of the nine homozygous subjects were male. All men had a TFS above 60% and a serum ferritin higher than 500 µg/L. The three women had a TFS of 66%, 56% and >95%, respectively. Corresponding serum ferritin values were 5, 19 and

Table 1. Characteristics of the study cohort (mean values)

	Women	Men	Total
No. (%)	2,469 (48.6%)	2,614 (51.4%)	5,083 (100%)
Age (yrs)	49.4	49.1	49.2
Weight (kg)	67.2	81.0	74.3
Body mass index (kg/m^2)	24.7	25.9	25.3
Ferritin (µg/L)	77.4	171.4	125.7
Transferrin saturation (%)	34.4	38.5	36.5

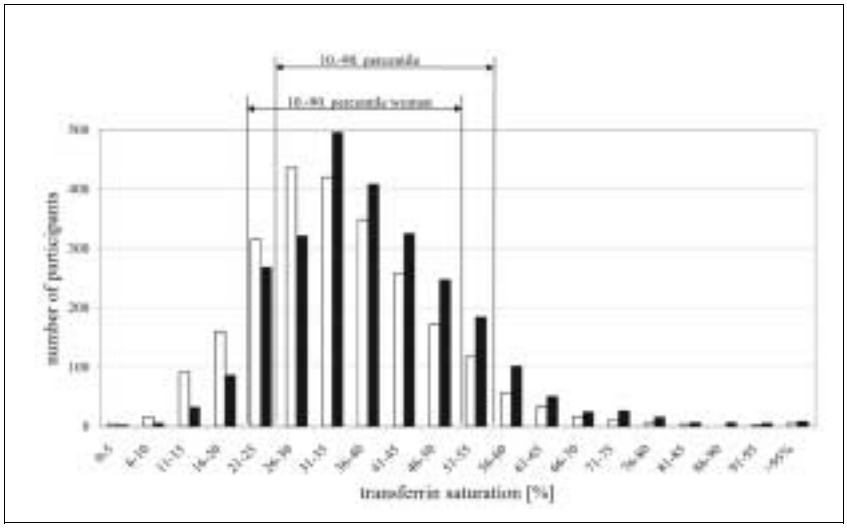

Figure 1. Distribution of the transferrin saturation for women (open bars) and men (solid bars) in the study population. The 5th and 95th percentiles are indicated (20% and 51% for women, 23% and 55% for men).

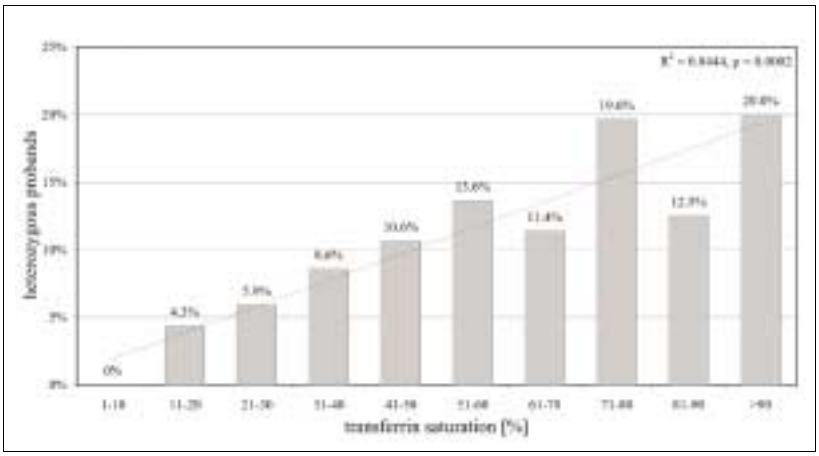

Figure 2. Probands heterozygous for the C282Y mutation in different transferrin saturation classes (n = 0, 1, 4, 6, 5, 29, 14, 11, 2, 4, respectively). The percentage of heterozygous probands increased with increasing transferrin saturation (P = 0.0002).

Table 2. Properties of subjects homozygous for the C282Y mutation

Subject	Gender	Age (yrs)	Transferrin saturation (%)	Ferritin (μg/L)
1	Female	57	66	5
2	Female	29	56	19
3	Female	34	>95	162
4	Male	52	66	504
5	Male	60	95	513
6	Male	66	>95	519
7	Male	36	>95	581
8	Male	66	72	605
9	Male	53	>95	1,108

162 μg/L, respectively [Table 2]. None of the 264 tested subjects with a TFS ⩽ 55% was homozygous for the C282Y mutation. Therefore, the estimated prevalence of the homozygous C282Y mutation in the cohort is 1.8/1000 (9/5,083).

Next we investigated whether the C282Y heterozygous genotype affected the TFS of the probands. Figure 2 shows that the percentage of heterozygous probands increased with increasing TFS (r^2=0.844, P = 0.0002). In addition, the mean TFS of the 76 genotyped heterozygous probands was significantly higher than the mean TFS of the 552 subjects in the wild-type group (P = 0.016). No significant differences were observed between the ferritin levels in these groups (P = 0.395).

The frequency of the heterozygous mutation in the study cohort was calculated from the expected numbers of heterozygous subjects in each TFS class. By using this method, the frequency of heterozygous subjects in the entire study population was 8.8%. Overall, 13.7% of the participants with a TFS above 55% and 8.4% with a TFS ⩽ 55% were heterozygous for the C282Y mutation.

Discussion

The discovery of the C282Y mutation in the *HFE* gene raised the question of genetic testing for hereditary hemochromatosis. We performed a large, representative population-based study to determine the C282Y genotype in correlation to the screening parameters TFS and ferritin. The frequency of 1.8/1,000 homozygous and 8.8% heterozygous participants in this study was slightly lower, as expected from other studies that found a disease frequency of 3–7/1,000 and a frequency of heterozygotes of approximately 11% [2,14–16]. However, some of these studies were pedigree studies, and the cohorts were different from that in this study.

Among the participants with TFS above 55%, 2.4% were homozygous and therefore at high risk to have hereditary hemochromatosis. Of the three homozygous women, two had a TFS of 55–60% and a low serum ferritin. We conclude that elevated ferritin values are not appropriate for hemochromatosis screening, particularly in women. This finding is supported by other studies where some of the homozygous subjects had low ferritin values [15]. It is unknown whether these subjects with low ferritin values will either develop iron overload later in life or have incomplete penetrance of the disease. Longitudinal studies will be necessary to address these questions. In addition, the possibility of secondary iron loss due to coexisting diseases cannot be ruled out and could not be further evaluated since the participants had to remain anonymous throughout the study.

It is still uncertain which TFS threshold should be used for hemochromatosis screening. In our study none of the randomly screened participants with a TFS ⩽ 55% was homozygous for the C282Y mutation. In another study 15 of 16 homozygous participants had a TFS above 55% in the initial test; the remaining participant had a TFS of 43% [17]. We propose a population screening strategy with an initial TFS test followed by determination of the *HFE* genotype if the TFS is above 55%, regardless of the ferritin level. In addition to hemochromatosis patients with mutations in the *HFE* gene and increased TFS, this strategy would allow the detection of patients with clinical hemochromatosis without known mutations in the *HFE* gene as well as several other diseases with iron overload. Since C282Y/H63D compound heterozygotes also develop significant iron overload [18,19], genetic testing for the H63D mutation should be included in further screening strategies.

In the present study we found a positive correlation between the C282Y heterozygous phenotype and TFS. Several published reports have related *HFE* genotypes to TFS, but most of them did not find significantly higher TFS values in C282Y heterozygotes [17,19]. However, in one of these studies, 10.6% (38/359) of the heterozygous subjects but only 4.9% (125/2,571) of the wild-type subjects had elevated TFS values in the initial screening test [17]. In pedigree studies, heterozygous probands had higher TFS values as compared to normal subjects [20]. Using a mathematical model, McLaren et al. [21] identified two distinct TFS populations which may represent the unaffected subjects and the subjects heterozygous for the C282Y mutation. The heterozygous phenotype may protect against iron deficiency and represent a selection advantage, thereby explaining the high prevalence of the mutation. In accordance with a protective role, higher serum iron values were found in celiac disease patients heterozygous for the C282Y

mutation [22]. However, the possibility has been raised that the heterozygous C282Y phenotype with elevated TFS values might be a susceptibility factor for diseases such as hepatitis C [23] or non-alcoholic steatohepatitis [24]. Therefore, the simultaneous prevalence of other diseases affecting iron homeostasis may predict the beneficial or adverse effects of the heterozygous C282Y mutation.

In this study, no information regarding liver function (liver enzymes, sonography or hepatic iron index) was available. Therefore, the possibility of secondary iron loss or overload cannot be ruled out. However, this situation where only scant clinical information is available may actually reflect the conditions present in many population screening settings.

The mean TFS in our population was 34.5% for women and 38.6% for men. These values were slightly higher than those reported by Merrywether-Clarke et al. [25]: namely, a mean TFS of 30.5% for females and 33.9% for males in 204 healthy blood donors in Jersey, UK. McLaren et al. [21] evaluated the distribution of TFS in 1,652 employees in Australia. They assumed two distinct populations: one heterozygous for hemochromatosis with TFS values of 37.3% for men and 37.6% for women, and one unaffected population with TFS values of 24.1% for men and 22.5% for women [21]. The existence of a distinct heterozygous population with higher TFS values concurs with our data but the TFS values were lower than in our study. A possible explanation could be a different distribution of TFS in Australia and Europe, especially since the TFS values reported in a study from the UK were much closer to our data [25]. The higher TFS values could contribute to the lower specificity of elevated TFS in hemochromatosis screening.

In conclusion, we favor a hemochromatosis screening strategy with a first-line TFS test and genotyping of subjects with a TFS above 55% regardless of the ferritin level. Since the number of heterozygous participants increased with rising TFS, heterozygosity for the C282Y mutation seems to influence the iron homeostasis. Longitudinal studies are required to elucidate the role of homozygous subjects with low serum ferritin.

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