The spectrum of premature atherosclerosis: from single gene to complex genetic disorder
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Prevalence of hereditary haemochromatosis in premature atherosclerotic vascular disease

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Summary

It has been proposed that iron accumulation may contribute to atherogenesis by increasing free radical formation and oxidative stress. Epidemiological studies in which the association of iron status with atherosclerosis was assessed raised conflicting results. To test whether genetic haemochromatosis is associated with increased atherosclerosis, we determined the prevalence of two mutations in the HFE gene related to haemochromatosis (845G→A : Cys282Tyr, and 187C→G, His63Asp) in 265 consecutive patients with premature (<50 years of age) angiographically proven atherosclerotic disease (coronary and/or peripheral) and in 272 healthy controls. PCR amplification followed by RsaI (Cys282Tyr analysis) and BclI (His63Asp analysis) restriction digestion was employed to define the genotypes. The mutant Cys282Tyr allele had a frequency of 0.07 among controls and 0.04 among patients (carrier frequency of 14.0% and 8.3%, respectively). The frequency of the His63Asp mutant allele was 0.14 (28.6% of carriers) in controls and 0.11 (22.2% of carriers) in patients. Five of 265 patients (1.1%) and 9/272 controls (3.3%) were compound heterozygotes. In conclusion, a lower prevalence of the Cys282Tyr mutation and a similar frequency of the His63Asp mutation was observed in patients with atherosclerotic disease in comparison with normal controls. These findings do not support an association between haemochromatosis and atherogenesis.

Introduction

The observation that iron increases free radical formation and oxidative stress lead to the hypothesis that iron accumulation might contribute to atherogenesis.1–2 However, the relationship between iron status and atherosclerotic disease remains a controversial issue, since several large epidemiological studies designed to assess the association yielded conflicting results.2–10

Hereditary haemochromatosis (HH) is an autosomal recessive disease of iron metabolism which leads to progressive iron accumulation and multi-organ dysfunction, and may be lethal when undetected.11 Recently, the recognition that two mutations in the HFE gene on chromosome 6 are associated with HH has significantly improved current knowledge concerning the molecular genetics of the disease, which is arguably the most common genetic disorder of Europeans (disease frequency is estimated at 1/300 in populations of Northern European origin).12–15
The Cys282Tyr mutation (845 G→M) is associated with haemochromatosis, and is considered a disease-causing mutation. In contrast, the association of the second mutation (His63Asp; 187 C→G) with HH is less evident, and it seems to cause haemochromatosis when inherited together with the Cys282Tyr mutation. Since both mutations may have a deleterious effect, it remains unknown why the haemochromatosis mutations have reached high frequencies in different Caucasian populations, and the possibility of a selective advantage for heterozygotes must be considered.

The identification of the Cys282Tyr and His63Asp mutations in the HFE gene as haemochromatosis-related mutations provides the unique opportunity to test the association between haemochromatosis and atherosclerosis by determining the prevalence of the two mutations in normal controls and in patients with atherosclerotic disease. In the present study we demonstrated that the Cys282Tyr mutation had a significantly lower prevalence in patients with premature arterial thrombotic disease than in healthy controls, a finding that diminishes the likelihood that genetic haemochromatosis is a risk factor for arterial thrombosis, at least in relatively young patients.

Materials and Methods

Patients and controls subjects.
265 consecutive patients (213 men, mean age 40, range 25-40 years, and 52 women, mean age 41, range 2-50 years) with premature atherosclerosis (coronary and/or peripheral disease) documented by angiography were recruited to the patient group. 215 patients suffered from coronary disease, 38 from peripheral disease and 12 from a combination of peripheral and coronary disease. 272 unmatched, apparently healthy, individuals composed the control group. Although unmatched for sex and age, patients and controls had the same ethnic background, i.e. Dutch Caucasians. All individuals gave their informed consent for the study.

Methods.
Genomic DNA was extracted from peripheral blood leucocytes employing standard methods. DNA analysis was carried out by PCR amplification followed by digestion with the restriction enzyme Rsal (Cys282Tyr analysis) and BclI (His63Asp analysis). The primers and PCR conditions employed have been previously reported. Allele frequencies were determined directly by counting genes from the observed
genotypes. The p-values were calculated according to standard techniques, employing the SPSS for Windows statistical package.

Results

The Cys282Tyr mutation was found to be heterozygous in 38/544 control chromosomes (allele frequency 0.07; carrier frequency 14.0%), and in 22/530 chromosomes (allele frequency 0.04; carrier frequency 8.3%) from patients with premature arterial disease (Table 1). This difference was statistically significant (p<0.05). No homozygote for the Cys282Tyr mutation was found. The frequency of the His63Asp mutant allele was 0.14 among controls and 0.11 among patients

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Controls</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cys 282Tyr*</td>
<td>38/544 (0.07)</td>
<td>22/530 (0.04)</td>
</tr>
<tr>
<td>His63Asp†</td>
<td>79/544 (0.14)</td>
<td>59/530 (0.11)</td>
</tr>
</tbody>
</table>

Number of mutated chromosomes/number of chromosomes analysed allele frequencies are given in parentheses.

* Difference between patients and controls was statically significant (p<0.05); odd ratio 1.7 (95% CI 1.0-2.8).
† Difference between patient and control group was not significant (p=0.07)

<table>
<thead>
<tr>
<th>genotype</th>
<th>Patients (n=265)</th>
<th>Controls (n=272)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C282Y +/- H63D -/-(1)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>C282Y +/- H63D -(2)</td>
<td>19 (7.1%)</td>
<td>29 (10.6%)</td>
</tr>
<tr>
<td>C282Y +/- H63D -(3)</td>
<td>4 (1.5%)</td>
<td>1 (0.4%)</td>
</tr>
<tr>
<td>C282Y +/- H63D -(4)</td>
<td>48 (18.1%)</td>
<td>68 (25.0%)</td>
</tr>
<tr>
<td>C282Y +/- H63D -(4)</td>
<td>3 (1.1%)</td>
<td>9 (3.3%)</td>
</tr>
<tr>
<td>C282Y +/- H63D -(4)</td>
<td>191 (71.7%)</td>
<td>165 (60.6%)</td>
</tr>
</tbody>
</table>

C282Y and H63D indicate Cys282Tyr and His63Asp mutations, respectively. Differences shown in (1), (2) and (4) were not statistically significant; p-values (1) p=0.15, (2) p=0.2, (4) p=0.3. Differences shown in (3) was marginally significant (p<0.05).
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(Table 1), which was not statistically significant (p=0.07). Compound heterozygosity for both mutations was observed in 9/265 controls (3.3%) and 3/265 patients (1.1%) (p=0.08). The frequency of the His63Asp mutation among Cys282Tyr heterozygotes was 3/22 (13.6%) among patients and 9/38 (23.6%) among controls. Haemochromatosis genotypes of controls and arterial disease patients are shown in Table 2.

Discussion

The prevalence of 14% for the Cys282Tyr mutation in the HFE gene in a normal Dutch population is in agreement with the high prevalence of this mutation among Caucasian populations of Northern European origin\textsuperscript{14,15} and shows that the Cys282Tyr mutation is also commonly found in the Dutch population. Interestingly, the Cys282Tyr mutation was found to be less prevalent among 265 patients with angiographically proven atherosclerotic disease. These data indicated that heterozygosity for the Cys282Tyr mutation was not associated with increased susceptibility for premature atherosclerotic vascular disease. Conversely, should our findings be further confirmed, a protective effect against atherosclerosis in carriers of the Cys282Tyr mutation might be suggested. In this respect, the identification of a possible protection of haemochromatosis for atherogenesis is intriguing, since iron overload would be expected to accelerate atherosclerosis progression, if one considers the hypothesis that iron increases free radical production and oxidative stress\textsuperscript{16}: which theoretically might contribute to atherogenesis.\textsuperscript{1,2} On the other hand, it must be emphasized that, although attractive, the 'iron hypothesis' was not confirmed in several epidemiological studies which have failed to demonstrate any association between iron status and cardiovascular disease.\textsuperscript{2} Moreover, a recent study employing a hypercholesterolaemic rabbit model to test the effects of both iron deficiency and iron overload on atherosclerosis reported a 56% reduction in aortic atherosclerosis formation in rabbits submitted to iron overload in comparison with controls, whereas iron deficiency did not seem to influence atherosclerosis.\textsuperscript{17} The authors suggested that iron excess decreased atherosclerosis by exerting a hypocholesterolaemic effect.\textsuperscript{17} Even though these data were obtained in an animal model, they support the present findings, which may also suggest a protective effect of haemochromatosis in atherosclerosis.

The prevalence of the His63Asp mutation in the HFE gene was not statistically different between controls and patients with arterial disease, suggesting that, in
isolation, this polymorphism does not influence atherosclerosis outcome. This mutation was found to be highly prevalent in both groups (allele frequency 0.14 and 0.11, respectively), and was similar to that of other Caucasian European populations. The His63Asp mutation was previously found at a higher rate in haemochromatosis patients who were heterozygous for the Cys282Tyr mutation, than in normal individuals. The frequency of the His63Asp was not increased in patients with porphyria cutanea tarda (PCT) in comparison with normal controls in one study, but, in contrast, a high prevalence of this mutation was recently observed among Italian patients with PCT. In our study the frequency of the His63Asp mutation among Cys282Tyr heterozygotes was similar among controls and patients.

The compound heterozygous state for Cys282Tyr and His63Asp mutations was associated with iron overload and genetic haemochromatosis, with a higher, but not significantly different, prevalence among controls than among patients with atherosclerosis in the present study (Table 2). This finding also suggests that haemochromatosis does not indicate an increased predisposition for atherosclerosis progression.

In conclusion, the present study represents the first extensive investigation of the Cys282Tyr and His63Asp mutations in the HFE gene in patients with premature arterial thrombotic disease. Our data do not support an association between iron accumulation and increased atherosclerosis.

**Acknowledgement**

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References
