Analysis of haemochromatosis gene mutations in a population from the Mediterranean Basin


Abstract: Background/Aims: The C282Y mutation in the haemochromatosis gene (HFE) located on chromosome 6 has been identified as the main genetic basis of hereditary haemochromatosis (HH). Two more mutations of that gene, H63D and S65C, appear to be associated with milder forms of HH. A high allele frequency for C282Y and H63D mutations was reported in populations from North Europe, while incomplete information is available for individuals from the Mediterranean Basin where C282Y homozygotes comprise a smaller percentage of HH cases. In this study we investigated the allele frequency of HFE mutations and the association between HFE mutations and cases of HH in a population from the South of Italy (Sicily and Calabria). In addition, we evaluated a possible association between HFE mutations and either chronic liver disease or type II diabetes. Patients and Methods: Three hundred and twenty-seven individuals (654 chromosomes) were tested for C282Y, H63D and S65C mutations of the HFE gene by restriction fragment length polymorphism. Four had HH, 23 had hepatocellular carcinoma, 100 had chronic liver disease, 100 had type II diabetes, and 100 were healthy controls. Results: Both C282Y and S65C mutations were each detected in one of the 654 chromosomes analysed (allele frequency \( \frac{1}{2} = 0.15\% \)), while H63D change was found in 122 chromosomes (allele frequency \( \frac{1}{2} = 18.6\% \)) and was equally distributed in all the categories examined. One healthy individual had compound heterozygosity for C282Y and H63D mutations. The frequency of C282Y in this Southern Italian sample was the lowest yet reported for a population of European origin. None of the four HH patients was either homozygous or heterozygous for C282Y. Conclusions: In Mediterranean populations from Southern Italy the C282Y mutation occurs sporadically and HFE polymorphisms seem to have little diagnostic relevance.

Hereditary haemochromatosis (HH) is an inherited disorder of iron metabolism common in northern European populations (1–4). Mutations of the haemochromatosis gene (HFE) located on chromosome 6 have been identified as the genetic basis of HH. In particular, homozygosity for the C282Y mutation and compound heterozygosity for C282Y and H63D mutations account for the vast majority of HH cases (5–9). More recently, an S65C substitution has been found to be associated with a significantly increased risk of developing a milder form of haemochromatosis (10). In addition, it has been suggested that HFE mutations may be involved both in cases of liver disease of different aetiology complicated by iron overload and in cases of type II diabetes (11–13).

Extensive epidemiological studies have shown a very high allele frequency for C282Y and H63D mutations among populations of Celtic origin from Northern Europe, while incomplete information is available for individuals of Southern European origin, and in particular in populations from the Mediterranean Basin where haemo-
chromatosis is reported to be uncommon (4, 14). In Italy, the percentage of haemochromatosis patients homozygous for C282Y was reported to be higher in patients from the north of the country (64%) than in central and southern regions (33%) (15).

In this study we investigated the frequency of HFE mutations in individuals from Sicily and the adjacent Calabria, two regions of Southern Italy that are in the centre of the Mediterranean Basin. We also aimed to study in the same population the association between the infrequent cases of haemochromatosis and HFE mutations, and the prevalence of such mutations in patients with chronic liver disease of different aetiology and in patients with type II diabetes.

Patients and methods

Patients

We studied DNA extracts from peripheral blood lymphocytes (PBL) of 327 unrelated individuals from Sicily and Calabria. One hundred of them had chronic liver disease, ranging from histologically proven mild chronic hepatitis to decompensated cirrhosis, 23 of them had hepatocellular carcinoma (HCC), four had haemochromatosis, 100 had type II diabetes and 100 were healthy volunteer blood donors (Table 1). Patients with chronic liver disease and those with HCC had been admitted consecutively to the Liver Unit of our Department as day patients from March to October 1997. Among the 529 patients who underwent liver biopsy from January 1992 to December 1999, only the four patients with haemochromatosis had the histological diagnosis of HH according to previously published criteria (16, 17). Two of these four subjects were men (20 years and 40 years old, respectively) and showed clinical, biochemical and histological responses to phlebotomy, while the other two patients were two sisters aged 30 and 32 years who refused to be followed up after diagnosis. The diabetic patients and the healthy control subjects were consecutively observed in, respectively, the Diabetic Unit and the Blood Bank of our Hospital.

Patients with liver disease, HCC and HH were tested retrospectively, using stored frozen PBL samples, while DNA extraction of lymphocytes from diabetic and healthy individuals was performed at the time of blood sampling.

Molecular studies

DNA was extracted from PBL of each individual, and the exons 4 and 2 of the HFE gene containing the three missense mutations to be checked were amplified by polymerase chain reaction (PCR) using the oligonucleotide primer sequences of Feder et al. (5). Aliquots of amplified products were digested with SnaB I, Bcl I and Hinf I to detect, respectively, C282Y, H63D and S65C mutations. Direct sequencing of HFE coding regions from the four haemochromatosis patients was performed as described elsewhere (18). In brief, genomic DNA was amplified by using oligonucleotide primers whose sequence has been previously reported (19). PCR products were purified with the use of a gel extraction kit (QIAquick Gel Extraction Kit, QIAGEN, Hilden, Germany) and were sequenced using a DNA Sequencing kit (Big Dye Terminator Cycle Sequencing Ready Reaction, Applied Biosystem, Warrington, UK). The fragments were then electrophoretically separated and analysed on an ABI PRISM 310 Genetic Analyzer.

Results

Three hundred and twenty-seven individuals (654 chromosomes) were studied for the presence of the C282Y, H63D and S65C mutations of the HFE gene by restriction fragment length polymorphism. C282Y substitution and S65C change were each detected in only one of the 654 chromosomes analysed (allele frequency 0.15%), H63D was found in 122 chromosomes (allele frequency 18.6%). The analysis of the HFE genotype distribution in the different categories of subjects examined revealed that: two haemochromatosis patients were mutation free, while the two sisters were heterozygous for the H63D mutation; 16 HCC patients had no mutation, one was homozygous and six were heterozygous for the H63D mutation; 65 of the 100 subjects with chronic hepatitis were homozygous for the wild-type alleles, six were homozygous and 29 heterozygous for H63D; 69 of the 100 diabetics had no mutation, three were homozygous and 27 heterozygous for the H63D, while one was heterozygous for the S65C mutation; 65 of the healthy control individuals were homozygous for the normal alleles, six were homozygous and 29 heterozygous for H63D; 69 of the 100 diabetics had no mutation, three were homozygous and 27 heterozygous for the H63D, while one was heterozygous for the S65C mutation; 65 of the healthy control individuals were homozygous for the normal alleles, six were homozygous and 31 heterozygous for H63D mutation, and, finally, one subject had compound hetero-

Table 1. Baseline characteristics of the study group

<table>
<thead>
<tr>
<th>Clinical status</th>
<th>No. of cases</th>
<th>M/F</th>
<th>Mean age (years ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemochromatosis</td>
<td>4</td>
<td>2/2</td>
<td>37.0 ± 9.0</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>23</td>
<td>21/2</td>
<td>64.22 ± 10.58</td>
</tr>
<tr>
<td>Chronic liver disease</td>
<td>100</td>
<td>70/30</td>
<td>54.47 ± 12.59</td>
</tr>
<tr>
<td>Diabetes</td>
<td>100</td>
<td>58/44</td>
<td>57.3 ± 13.3</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>100</td>
<td>79/21</td>
<td>40.4 ± 9.5</td>
</tr>
</tbody>
</table>

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Table 2. HFE genotypes in subjects with different clinical conditions

<table>
<thead>
<tr>
<th></th>
<th>HH (4 cases)</th>
<th>HCC (23 cases)</th>
<th>Liver disease (100 cases)</th>
<th>Diabetes (100 cases)</th>
<th>Controls (100 cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C282Y homozygous</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C282Y heterozygous</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H63D homozygous</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>H63D heterozygous</td>
<td>2</td>
<td>6</td>
<td>29</td>
<td>27</td>
<td>31</td>
</tr>
<tr>
<td>S65C homozygous</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S65C heterozygous</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Compound heterozygosity</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Wild/wild</td>
<td>2</td>
<td>16</td>
<td>65</td>
<td>69</td>
<td>65</td>
</tr>
</tbody>
</table>

HH, hereditary haemochromatosis. HCC, hepatocellular carcinoma. *C282Y/H63D.

zygosity for the C282Y and H63D mutations (Table 2). This last subject (a 64-year-old man), subsequently examined by biochemistry and ultrasonography, showed a normal liver function as well as normal glycaemic profile and normal transferrin saturation.

Finally, direct sequencing analysis showed no other mutation at the level of the HFE gene in any of the four haemochromatosis patients.

Discussion

Some data suggest that the world-wide distribution of HFE-related haemochromatosis corresponds to the migration pattern of Celtic peoples and, indeed, this genetic disease is much more frequently observed in individuals of North European origin than in Caucasians from other geographical areas (20). Homozygosity for the HFE pathogenetic mutation inducing the C282Y substitution has been detected in 85–100% of haemochromatosis patients of North European origin (4, 21). Moreover, this mutation appears with high frequency among populations of Celtic descent, reaching the allele frequency of 10% in Irish chromosomes, while data for the HFE genotype distribution in Europeans from Mediterranean countries are still incomplete (4).

In a previous study, Piperno et al. showed that 64% of Italian patients with HH are homozygous for the C282Y mutation, with a decreasing gradient from north to south of the country (15), while Carella et al. found this mutation in 1% of normal chromosomes from a similar population (22).

In this study, we aimed to obtain reliable information about the allele frequency of HFE mutations in the Mediterranean area by examining individuals from Sicily and Calabria, two regions in the south of Italy that are in the centre of the Mediterranean Basin. Moreover, we thought that this kind of genetic analysis might be of further interest because these regions were for centuries under the rule of various different nations, including people from Northern Europe such as the Normans and the Swabians (23).

We found that only one of 654 chromosomes carried the C282Y mutation, corresponding to an allele frequency of 0.15%, which is one of the lowest frequencies reported so far. An equal allele frequency was found for the S65C mutation, which was recently reported to be associated with mild forms of HH. H63D change was detected in 18.6% of the chromosomes examined: it was similarly distributed in the different categories of patients and healthy subjects included in the study, confirming that this polymorphism is widespread in the general population throughout the world (6, 8, 13). The reason(s) for the enormous difference in C282Y distribution between Northern Europe and lands washed by the Mediterranean Sea are difficult to explain, especially when it is considered that North European peoples were present for centuries in Sicily and Calabria. We could speculate that HFE-mutated genotypes were negatively selected in areas where another genetic disorder provoking iron overload – thalassaemia – was highly endemic (thalassa is the ancient Greek name for the Mediterranean Sea). In this context, it seems remarkable that the C282Y mutation was detected neither in four subjects with haemochromatosis, nor in patients with chronic liver disease, HCC or diabetes, while the only case carrying this mutation was a healthy individual with compound heterozygosity for H63D. However, it is noteworthy that non-HFE-related haemochromatosis has recently been reported in Italy, including juvenile haemochromatosis mapped to chromosome 1 (24) and haemochromatosis caused by mutation of the transferrin receptor 2 gene on chromosome 7 (25).

Altogether, these data indicate that in populations from the Mediterranean Basin, HFE polymorphisms have relatively low clinical and diagnostic relevance and confirm that hereditary haemochromatosis is a heterogeneous genetic dis-
order with significant geographic differences, as recently suggested by Pietrangelo et al. (18).

References