Association between HFE mutations and acute myocardial infarction: a study in patients from Northern and Southern Italy

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Abstract

There is interest in the role of iron in age-related diseases such as atherosclerosis. Tissue iron deposition could be harmful, because Fe²⁺ can react with H₂O₂ to form OH⁻ radicals and Fe²⁺ ox can react with O₂ to form reactive oxygen species. Free radicals react with cell membranes and cell organelles and could lead to the development of atherosclerosis by initiating lipid peroxidation. Hereditary hemochromatosis provides an opportunity for studying the effects of iron on cardiovascular disease. Some studies have shown that individuals who carried HFE mutations may be at greater risk of developing coronary heart disease than those without the mutations. In contrast, a large number of studies have reported no association between HFE mutations and coronary heart disease. These studies have possible confounding factors, such as the homogeneity of the population in term of geographical origin among others. We studied the relation between HFE mutations and acute myocardial infarction in two case–control studies involving two sets of subjects representing different age groups from different geographic regions in Italy. The first one was composed of 172 older patients (139 males and 33 females; mean age 67) and 207 healthy controls (91 males and 116 females; mean age: 46) from Emilia-Romagna. The second one was composed of younger 77 patients (75 males and 2 females; mean age 41) and 172 healthy controls (75 males and 97 females, mean age: 38) from Sicily. All patients were genotyped for ApoE alleles, since the ApoE-ε4 allele is considered a risk factor for cardiovascular diseases and can interfere with other genetic and environmental factors by modifying susceptibility to this disease. DNA typing for C282Y and H63D HFE alleles was performed also. There were no significant differences in frequencies of the different HFE alleles between acute myocardial infarction patients and controls in cohorts of both old and young patients. Also taking into account the presence or absence of the ApoE-ε4 allele, no significant differences in H63D allele frequencies were observed. Thus, our study, performed in two samples of genetically homogeneous patients and controls, does not support the suggestion that HFE mutations may be associated with acute myocardial infarction in susceptible individuals.
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Introduction

Hereditary hemochromatosis is an autosomal recessive disorder characterized by iron overload as a result of increased dietary absorption. The principal gene responsible for hereditary hemochromatosis, designated HFE, is located...
on chromosome 6 in the HLA region. This HLA class Ib gene product, HFE, no longer participates in immunity because it has lost its ability to bind peptides due to a definitive closure of the antigen-binding cleft. This protein, instead, has acquired the ability to form complexes with the receptor for iron-binding transferrin [1,2]. The single point mutation 845A, a change from cysteine at position 282 to tyrosine (C282Y), in this gene has been identified as the main genetic basis of hereditary hemochromatosis in patients of Northern European ancestry. Another mutation, 187G, a histidine to aspartate at amino acid 63 (H63D), appears to be associated with milder forms of hereditary hemochromatosis [1–3].

It is well known that HFE heterozygous individuals have a higher iron content [4,5]. In a C282Y mutation high-carrier frequency population such as in Denmark [6], an age-related reduction in the frequency of heterozygosity for C282Y has been reported, suggesting that carrier status is associated with shorter life expectancy. Thus, the heterozygous state would be expected to be associated with specific adverse health consequences. It has been proposed that the heterozygous state may adversely affect the course of other diseases, such as hereditary spherocytosis [7,8], abnormal hemoglobins [9], or chronic hepatitis [10]. Moreover, it has been suggested [11] that male heterozygotes for hereditary hemochromatosis have an increased relative risk for diabetes, colorectal cancer, and hematologic malignancy and females have an increased risk for colonic adenoma and for stomach cancer. Blood lead levels were found to be higher in heterozygotes for hemochromatosis than in controls [12]. Besides, Roberts et al. [13] showed that the incidence of the C282Y mutation is greatly increased in patients with acquired porphyria cutanea tarda and that some homozygotes for this mutation suffer from porphyria cutanea tarda but do not have the classical signs of hemochromatosis.

Most important from a public health point of view is the proposal [14–16], supported by a large Finnish study [17], that heterozygosity for hemochromatosis may be an important risk factor for myocardial infarction and perhaps for severe cardiac bradycardyarrhythmias [18]. However, the data here are contradictory. A study using the extensive data provided by a large epidemiologic follow-up study showed that the risk of coronary artery disease was not related to transferrin saturation. Indeed, the relative risk was slightly decreased at high iron saturations [19]. However, three studies have shown that individuals who carried HFE mutations may be at greater risk of developing coronary heart disease than those without the mutations [20–22]. In contrast, several other studies have reported no association between HFE mutations and coronary heart disease [23–33].

The causes of these discrepancies are not clear. However, association studies are subjected to a number of possible confounding factors, such as the homogeneity of the population in term of geographical origin among others [34]. Thus, we have studied the allele frequencies of HFE mutations (C282Y, H63D) in two sets of ethnically homogeneous patients affected by acute myocardial infarction from Northern and Southern Italy. All patients were genotyped for ApoE alleles. ApoE polymorphisms are able to modulate lipoprotein metabolism at different steps and to influence LDL-cholesterol levels and also other lipoproteins features [35]. ApoE-ε4 allele is considered a risk factor for cardiovascular disease and it has been suggested that ApoE-ε4 status can interfere with other genetic and environmental factors by modifying the susceptibility to this disease [36,37].

**Materials and methods**

In this study two sets of patients and controls (not relatives of patients) were analyzed. The first one was composed of 172 patients (139 males and 33 females; mean age 67, age range: 55–80) affected by acute myocardial infarction, consecutively admitted at the Cardiac Unit of Bologna University Hospital, and 207 healthy controls (91 males and 116 females; mean age 46, age range: 23–65) from Emilia-Romagna. Control subjects from Emilia-Romagna were unrelated hospital staff. The second one was composed of 77 patients (75 males and 2 females; mean age 41, age range: 23–46) affected by acute myocardial infarction, consecutively admitted at the Cardiac Unit of Palermo University Hospital, and 172 healthy controls (75 males and 97 females, mean age 38, age range: 20–55) from Sicily. The Sicilian controls were unrelated medical students and laboratory staff. These age and sex differences observed in both comparisons between patients and controls are not relevant because no significant differences in HFE frequencies have been demonstrated between subjects included in these age ranges according to both sex and age ([38] and our unpublished results). Acute myocardial infarction was diagnosed on the basis of clinical, enzymatic, and electrocardiographic criteria.

DNA typing for C282Y and H63D HFE and ApoE alleles was performed as previously reported [35,39–41]. Allele frequencies were evaluated by gene count and contingency tables (χ² test) were constructed to determine statistical significance of differences in allele frequency for the polymorphisms under study between the different groups. Statistical power of the study was calculated according to Lemeshow et al. [42].

**Results**

Table 1 shows the frequencies of C282Y and H63D alleles in the two sets of patients affected by acute myocardial infarction and controls from North and South Italy. No significant differences in the C282Y and H63D allele frequencies were observed between patients and controls from the same region. No significant difference was also ob-
served when the data from Northern Italy were analyzed according to gender (in female patients, the frequency of 63D allele was 0.09).

As previously discussed, ApoE-e4 allele is considered a risk factor for cardiovascular disease and it has been suggested that ApoE-e4 status can interfere with other genetic and environmental factors by modifying the susceptibility to this disease [35–37]. However, also taking into account the presence or absence of the APOE-e4 allele, no significant differences were observed between carriers of the mutant HFE-H63D allele and those homozygous for the wild-type allele (Table 2).

Discussion

There is substantial interest in the role of iron in atherosclerotic disease. The excessive tissue iron deposition could be harmful, because of the potential of Fe^{2+} to react with H_2O_2 to form OH^- radicals and Fe^{3+} to react with O_2 to form reactive oxygen species. These free radicals react with cell membranes and cell organelles [43] and they are believed to play a role in the development of this age-related chronic inflammatory disease. In fact, it has been reported that these radicals can initiate lipid peroxidation, causing LDL to undergo oxidative modification that targets it for uptake by macrophages and may damage arterial endothelium directly and interfere with normal vasomotor regulation [44–46]. Following the hypothesis that iron overload could be a risk factor for atherosclerosis, hereditary hemochromatosis provides an additional model for studying the effects of iron in these diseases.

Three studies have shown that individuals who carried the HFE mutations might be at greater risk of developing coronary heart disease than those without the mutation [20–22]. Roest et al. [20] followed 12239 Dutch women for 16–18 years and found a risk of mortality from vascular events, either myocardial infarction or cerebrovascular disease, significantly higher in C282Y heterozygotes compared with individuals with the wild-type genotype. However, the association was not significant for deaths from coronary heart disease alone. On the other hand, Tuomainen et al. [21] found that male Finnish carriers of the C282Y mutation had a 2.3-fold risk of first acute myocardial infarction compared with non-carriers. Heterozygous C282Y carrier status seemed to confer a significant increase in risk of coronary heart disease also in a prospective study performed in North Americans [22]. The C282Y mutation was associated with non-significantly increased risk. However, after adjusting for other confounding risk factors the association became stronger. In contrast, a larger number of studies have reported no associations between HFE mutations and atherosclerosis including coronary artery disease and myocardial infarction [23–33,47–49]. Furthermore, the majority of prospective studies have not shown an association of serum ferritin, or other iron measures, with coronary heart disease [50]. However, differences in the study design and/or in the ethnic background, with different gene–gene or gene–environment interactions, might be reasonable explanations for the discrepant results. In fact, different populations represent different gene pools, implying that gene–disease associations are expected to vary between populations [34]. Thus, we have studied the allele frequencies of HFE mutations (C282Y, H63D) in two sets of ethnically homogeneous patients affected by acute myocardial infarction from Northern and Southern Italy.

We studied the relation between HFE mutations and acute myocardial infarction in 172 older patients (mean age 67) and 207 controls from Northern Italy. We were not able to show significant differences in frequencies of the different HFE alleles (C282Y and H63D) between controls and acute myocardial infarction patients. To confirm these negative results, we examined HFE mutations in another set of 77 younger patients (under 45 years) and 172 controls from Sicily. Also in this set of patients, no significant difference in frequencies of the different HFE alleles between controls and patients was observed. Besides, we were unable to find differences in allele distribution among patients carrying or

<p>| Table 2 |
| Frequencies of H63D alleles among patients affected by acute myocardial infarction (ApoE-e4 positive and negative from Northern and Southern Italy (absolute numbers of analyzed genes per group are reported in parentheses)) |</p>
<table>
<thead>
<tr>
<th>Northern patients</th>
<th>Southern patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoE-e4+</td>
<td>0.853 (58)</td>
</tr>
<tr>
<td>ApoE-e4-</td>
<td>0.888 (240)</td>
</tr>
</tbody>
</table>

Note. ApoE typing was available only for 169 Northern patients and for 69 Southern ones. No significant differences (P > 0.05) in the H63D allele frequencies were observed between ApoE-e4 positive and negative patients from the same region.
not carrying the Apo-E4 allele. Thus, our study, performed in two samples of genetically homogeneous patients and controls, does not support the suggestion that HFE mutations may be associated with acute myocardial infarction in susceptible individuals. However, our study shares certain limitations of case–control studies. The subjects were not recruited prospectively; therefore, a survival bias cannot be excluded and a possible effect of HFE heterozygosity on mortality cannot be ruled out. Moreover, our lack of association could be biased as controls could be future or silent diseased cases.

It may be relevant to quote that in another age-related disease, Alzheimer’s disease, iron has been claimed to play a role [51]. So, two studies have suggested that HFE mutations may interact with the mechanisms involved in Alzheimer’s disease such as the disease progression in the preclinical stage leading to an anticipation of clinical diagnosis [52,53]. However, our recent study, performed in a larger sample of patients and controls than the previous ones, does not support the suggestion that the H63D mutation may anticipate sporadic Alzheimer’s disease clinical presentation in susceptible individuals [41].

On the other hand, a recent study performed on a large panel of American individuals, screened for C282Y and H63D mutations, suggests that the clinical penetrance of the homozygous mutation is very low. In fact, in this study, only 1 of the 152 homozygotes had signs and symptoms of hereditary hemochromatosis and, concerning other conditions, there was only a significantly increased prevalence of a history of hepatitis. The authors add that it would be quite extraordinary for common polymorphisms such as HFE mutations to have an adverse phenotypic effect, concluding that one must resist the temptation to conclude that the adverse associations which have been observed are related by cause and effect [38,54]. Accordingly, in two European studies, the observed frequency of C282Y homozygosity in oldest old male English and in oldest old male and female Dutch was not significantly lower than that predicted [33, 55] and in Sicilian and Sardinian populations the HFE mutations are found to be overrepresented in very old people [39,56,57].

Previous and present studies indicate that HFE mutations are poor genetic markers of risk in the general population of patients. A recent study performed in a large number of adult male and female twins from Australia concluded that the proportion of variance in iron parameters ascribable to the HFE gene variations was rather low. Genetic polymorphisms at other loci and non-genetic differences are likely to play a major role in determining the iron status [58]. Therefore, if iron is involved, other factors may easily confound an association between cardiovascular risk and heterozygosity for HFE mutations in the various studies.

Finally, the HFE gene is an HLA gene [2] and in the HLA complex many other genes encode for proteins involved in immune-inflammatory responses [59]. The association between HFE mutations and coronary heart disease reported in patients of Northern Europe ancestry might be attributable to a linkage disequilibrium of the HFE mutations with unknown mutations in other HLA genes specifically present in such populations.

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References

[8] D.M. Mohler, M.S. Whhey, Case report: hemochromatosis heterozygotes may have significant iron overload when they also have hereditary spherocytosis, Am. J. Med Sci (1986) 320–324.


