

## ELECTRONIC LETTER

# Haemochromatosis (*HFE*) gene C282Y mutation and the risk of coronary artery disease and myocardial infarction: a study in 1279 patients undergoing coronary angiography

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Hereditary haemochromatosis is the most common genetic disorder in white people. Its prevalence exceeds the combined incidence of cystic fibrosis, muscular dystrophy, and phenylketonuria.<sup>1</sup> The faulty haemochromatosis gene (*HFE*) (OMIM 235200) was discovered in 1996<sup>2</sup> and is localised on the short arm of chromosome 6. A single mutation, 845A (c845A; GenBank U60319 OMIM 235200.0001) in the *HFE* gene results in the substitution of tyrosine for cysteine at amino acid 282 in the corresponding *HFE* protein and is referred to as the C282Y mutation. About 0.3% of C282Y homozygotes and 10% of heterozygotes are found in white populations.<sup>3-6</sup> A higher prevalence in Ireland, Wales, and Brittany, and in white Australians compared to the south of Europe suggests a Celtic origin for the C282Y mutation.<sup>7</sup> A second mutation in the *HFE* gene (c187G; H63D (GenBank U60319 OMIM 235200.0002)) contributes only in combination with C282Y to iron overload (compound heterozygosity).

Of patients with clinically identified haemochromatosis 80%–90% are homozygous for the C282Y mutation.<sup>8</sup> The disease is characterised by increased iron deposition in the liver, heart, pancreas, and other organs, leading to liver cirrhosis, heart failure owing to cardiomyopathy, and diabetes mellitus. On the other hand, the penetrance of the C282Y mutation is variable; homozygotes without evidence of iron overload have been described.<sup>9</sup> Heterozygotes are in general healthy but have slightly increased concentrations of serum ferritin, serum iron, and increased iron saturation of serum transferrin.<sup>10</sup> In combination with established liver disease, heterozygotes show a worse disease course,<sup>11,12</sup> but there are also conflicting results.<sup>13</sup>

An association between atherosclerotic disease and iron deposition has been suggested for several years. In vitro findings suggest a role for excess iron in atherosclerosis. Iron promotes the oxidation of low density lipoprotein (LDL).<sup>14</sup> The ferritin gene is expressed in advanced atherosclerotic lesions.<sup>15</sup> In an animal model of atherosclerosis an iron

deficient diet reduces the progression of atherosclerosis.<sup>16</sup> Epidemiological studies showed an decreased risk of myocardial infarction (MI) in blood donors<sup>17</sup> and an association of high body iron stores with MI.<sup>18</sup> Heterozygosity for the C282Y mutation was reported to be a risk factor for first MI in men<sup>19</sup> and cerebrovascular death in women.<sup>20</sup> We examined the prevalence and extent of coronary artery disease (CAD) and MI in relation to the C282Y mutation in a large population with angiographically confirmed coronary status.

## METHODS

### Study population

The C282Y mutation in the *HFE* gene was analysed in 1279 white people, born in Germany, consecutive patients who underwent elective coronary angiography at our institution for diagnostic purposes according to guidelines. This population included patients with angina-like chest pain and non-invasive tests suggesting ischaemia, patients with depressed left ventricular ejection fraction of unknown origin, and patients with valvular heart disease. Conventional risk factors alone did not lead to coronary angiography. Patients with previously performed coronary bypass surgery or percutaneous transluminal coronary angioplasty (PTCA) were excluded from the analysis because of their treated coronary status. The protocol of this study was approved by the ethics committee of the University of Jena and patients gave written informed consent.

### *HFE* C282Y genotyping

Genomic DNA was extracted from blood leucocytes using a standard DNA extraction kit (QIAamp, QIAGEN GmbH, Hilden, Germany). A 237 bp fragment of the *HFE* gene containing the mutation site was first amplified by polymerase chain reaction (PCR) with the primers 5'-GTG ACC TCT TCA GTG ACC-3' and 5'-AAT GAG GGG CTG ATC CAG-3'.<sup>21</sup> PCR cycles were three minutes at 95°C, 35 cycles of 30 seconds at 95°C, 45 seconds at 48.4°C, and 45 seconds at 72°C, followed by a final extension step of five minutes at 72°C. The amplified fragment was digested (two hours at 37°C) with the restriction enzyme *Sna*BI (Promega, Madison, WI, USA), products were electrophoresed on 2% agarose gels, and visualised by ethidium bromide staining under UV light. Assays for the C282Y mutation were always performed blindly in relation to other data of the respective person.

## Key points

- The C282Y mutation in the *HFE* gene was investigated in a series of 1279 consecutive patients with determined coronary status.
- The mutation was not associated with an increased risk of coronary artery disease or severity of coronary lesions.
- Furthermore, there was no linkage between the C282Y mutation and previous myocardial infarction or depressed left ventricular ejection fraction.

**Abbreviations:** CAD, coronary artery disease; *HFE*, haemochromatosis; LDL, low density lipoprotein; MI, myocardial infarction; PCR, polymerase chain reaction; PTCA, percutaneous transluminal coronary angioplasty

**Table 1** Frequencies of the C282Y mutation and corresponding allele frequencies in the whole study population and in subgroups

	No	Wild type (%)	C282Y mutation (%)	Mutant allele frequency
Whole population	1279	90.9	9.1	0.047
No CAD	435	92.6	7.4	0.038
Single vessel disease	246	89.4	10.6	0.054
Double vessel disease	223	88.8	11.2	0.058
Triple vessel disease	375	90.9	9.1	0.047
No MI	811	90.6	9.4	0.048
Previous MI	468	91.2	8.8	0.045

CAD, coronary artery disease; MI, myocardial infarction.

### Presence and extent of CAD and MI

A luminal stenosis of a great coronary vessel of 50% or more established the diagnosis of CAD. Accordingly, single to triple vessel disease was classified. In addition, the Gensini score<sup>22,23</sup> and the atherosclerosis score<sup>24</sup> were calculated to describe the extent of CAD. The Gensini score represents the functional significance of coronary artery narrowing; a higher score is accompanied by more severe disease. The atherosclerosis score (range 0–3) represents the general grade of atherosclerosis in all coronary segments. Patients were further classified by a history of MI, diagnosed by history, electrocardiography, and detection of abnormalities of wall motion shown by contrast ventriculography.

### Determination of left ventricular ejection fraction

Left ventricular ejection fraction was calculated with a biplane contrast ventriculography during catheterisation of the left side of the heart.

### Evaluation of conventional atherosclerotic risk factors

Demographic data (age, hypertension, diabetes mellitus, smoking) were obtained at the time of coronary angiography. Smoking habits were given as pack years; hypertension and diabetes were defined as binary variables. Serum LDL cholesterol was measured by conventional methods.

### Statistical analysis

The relation of the C282Y mutation to continuous variables was tested with the Mann-Whitney U test. The relation of dichotomy variables was checked with Pearson's  $\chi^2$  analysis. The  $\chi^2$  test was also used to recognise a deviation in genotype frequencies or a deviation from Hardy-Weinberg equilibrium. The relation of the C282Y mutation to CAD or previous MI was determined by a multivariate logistic regression model. In multivariate models, continuous covariates were categorised (body mass index (BMI), steps of 5 kg/m<sup>2</sup>, age, steps of 10 years, LDL cholesterol, steps of 0.5 mmol/l) or dichotomised (smoking less than or more than 10 pack years). The multivariate analyses were also repeated without categorising covariates. Odds ratios with 95% confidence intervals (95% CIs) were calculated as an estimate of relative risk of CAD or MI. The genotype at codon 282 of the *HFE* gene was dichotomised as wild type and heterozygous or homozygous mutation. All tests of significance were two sided; a value of  $p < 0.05$  was considered to be significant. The statistical analyses were computed with SPSS (SPSS, Chicago, IL, USA) statistical software.

## RESULTS

### C282Y mutation frequency in the whole study population and in the subgroups with established CAD or MI

In the entire study population, 116 (9.1%) heterozygotes and one homozygote for the C282Y mutation were detected. The C282Y allele frequency is comparable to other populations of

middle European ancestry: a German population<sup>3</sup> with 9.6% heterozygotes and an Austrian population with 9.4% heterozygotes.<sup>4</sup> The homozygous patient was a 77 year old man with triple vessel disease and previous inferior MI, who had strong conventional risk factors (smoking of 20 pack years and marked hypercholesterolaemia with LDL cholesterol of 6.42 mmol/l).

In the subgroup with CAD the percentage of the heterozygous C282Y mutation was not significantly different from the group without CAD. Patients with single, double, or triple vessel disease were also comparable for the C282Y mutation. In the subgroup of patients (n=468) with previous MI, the same C282Y mutation frequency as in the control group was found. Table 1 summarises these results. The genotype distributions in the whole population and in the mentioned subgroups were not different from the expected Hardy-Weinberg equilibrium.

### Relation of conventional risk factors and the C282Y mutation to the prevalence of CAD

As expected, patients with established CAD had more conventional risk factors (table 2). In a multivariate analysis, male sex ( $p < 0.0001$ ), age ( $p < 0.0001$ ), diabetes mellitus ( $p < 0.0001$ ), smoking more than 10 pack-years ( $p < 0.0001$ ), and LDL cholesterol ( $p < 0.004$ ) could be shown to be strong risk factors for CAD (fig 1). Carriers of the C282Y mutation had a 1.5-fold (95% CI 0.9 to 2.5) risk for the presence of CAD, there was no significant difference ( $p = 0.10$ ). In addition, in low risk subpopulations (for example, age < 60 years, non-smoker, low LDL cholesterol, patients without diabetes) a significant association between the C282Y mutation and CAD could not be detected (data not shown).

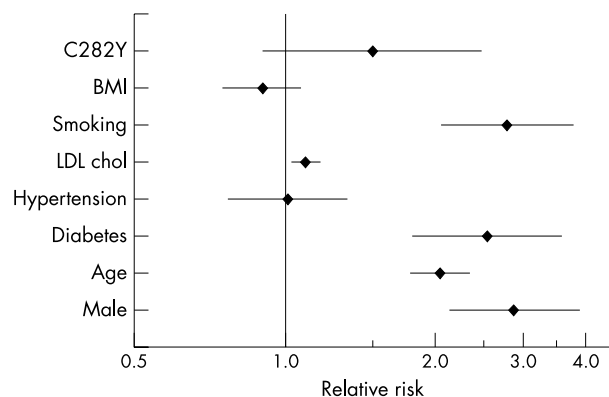
### Association between the C282Y mutation and extent of CAD

Angiographic scores (Gensini score, atherosclerosis score) were calculated in all patients with CAD. In the whole population with CAD, the Gensini score was lower in patients carrying the C282Y mutation compared to wild type patients

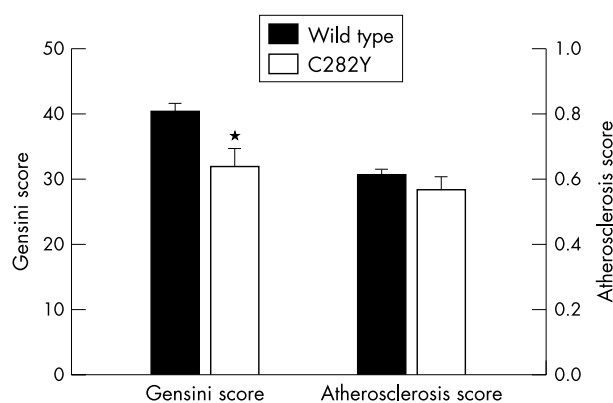
**Table 2** Distribution of conventional risk factors and the C282Y mutation in CAD and controls

	No CAD	CAD	p value
Age (years)	56.6 (9.9)	62.4 (9.1)	<0.001
Men (%)	57.7	78.8	<0.001
BMI (kg/m <sup>2</sup> )	27.2 (3.7)	26.9 (3.5)	NS
Hypertension (%)	56.6	61.3	NS
Diabetes mellitus (%)	14.3	28.4	<0.001
Smoking (pack years)	6.6 (11.6)	15.5 (19.4)	<0.001
LDL cholesterol (mmol/l)	4.1 (1.1)	4.3 (1.2)	0.008
Treatment with statins (%)	7.9	20.9	<0.001
C282Y mutation (%)	7.4	10.1	NS

Continuous variables are given as mean (SD).



**Figure 1** Relative risk of CAD (mean and 95% CI). Relative risk is shown for presence of the C282Y mutation, BMI (per 5 kg/m<sup>2</sup> increase), smoking more than 10 pack years, LDL cholesterol (per 0.5 mmol/l increase), presence of hypertension and diabetes mellitus, age (per decade), and male sex.



**Figure 2** Comparison of mean Gensini and atherosclerotic scores between wild type and the C282Y mutation in all patients with CAD. Values are means (SEM). \* $p < 0.05$  v wild type.

(fig 2). The comparison of atherosclerosis score did not show significant differences. In patients with single, double, or triple vessel disease the scores were comparable in carriers of the C282Y mutation and wild type patients (fig 3).

#### Relation between the C282Y mutation and MI

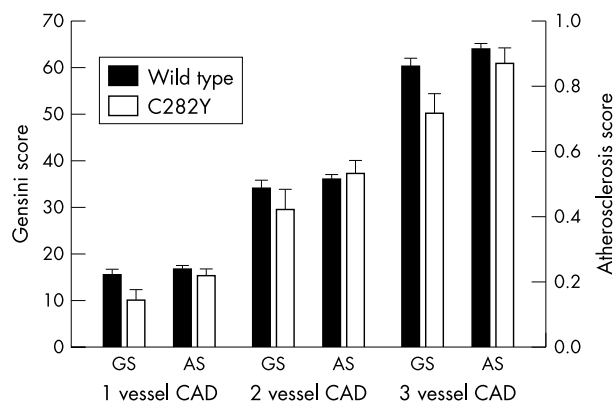
In the subpopulation with established CAD, the percentage of the C282Y mutation was the same in subjects with MI and in control subjects (table 1). In a multivariate analysis including conventional risk factors, an odds ratio of 0.70 (95% CI 0.44 to 1.14) was estimated for carriers of the C282Y mutation. When only men were examined, the results were essentially the same with a relative risk for MI of 0.71 (95% CI 0.41 to 1.22).

#### Influence of the C282Y mutation on left ventricular ejection fraction

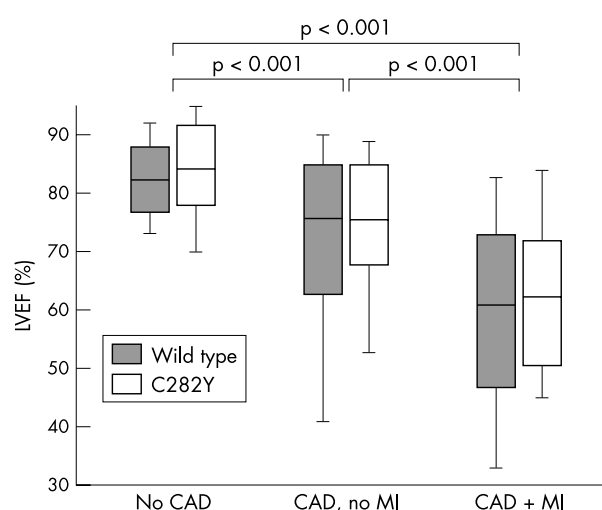
As shown in fig 4, left ventricular ejection fraction was strongly determined by the presence or absence of CAD and MI. Those carrying the 282Y mutation in the *HFE* gene had a similar ejection fraction to those without the mutation.

#### DISCUSSION

The C282Y mutation in the *HFE* gene is the major cause of hereditary haemochromatosis in white people. We evaluated the incidence of the C282Y mutation in the *HFE* gene in 1279 white people whose coronary anatomy was defined by means of coronary angiography. In our study population, one subject was homozygous for the mutation and 9.1% were heterozygous. The frequency of heterozygosity for the C282Y muta-



**Figure 3** Comparison of mean Gensini scores (GS) and atherosclerotic scores (AS) according to the C282Y mutation in patients with single, double, and triple vessel CAD. Values are means (SEM). No significant differences between wild type and the C282Y mutation could be detected in the corresponding groups.



**Figure 4** Left ventricular ejection fraction in patients without CAD (no CAD), with established CAD without MI (CAD, no MI), and patients with CAD and previous MI (CAD+MI). Values are given as mean, 25th/75th centile (bar), and 10th/90th centile (Whisker caps). The differences between groups were significant. Within groups, no differences between wild type and C282Y mutation could be detected.

tion was consistent with the frequency that would be predicted with the Hardy-Weinberg equation.

Heterozygotes have been reported to have slightly increased serum iron concentrations, transferrin saturation, and serum ferritin concentrations.<sup>10</sup> Liver disease in heterozygotes is generally associated with pre-existing disease.<sup>11-12</sup> An influence of excess iron storage on development of atherosclerotic disease has been suggested. Experimental findings indicated an increased lipid peroxidation by iron ions.<sup>14</sup> In an animal model of atherosclerosis an iron deficient diet leads to reduced atherosclerotic lesions.<sup>16</sup> Epidemiological findings suggested an association between the C282Y mutation and the development of CAD or MI. Heterozygous men have an increased risk for first MI.<sup>19</sup> In heterozygous women an increased risk for cardiovascular death was found,<sup>20</sup> but only mortality rates for cerebrovascular death reached significance. In a prospective case-cohort design, an association between the C282Y mutation and development of CAD was reported.<sup>25</sup> In studies with morphological evaluation of atherosclerotic lesions different results were obtained. In a case-control study using ultrasound to determine atherosclerotic plaques in the carotid

and femoral artery, no association between C282Y mutation and atherosclerotic plaque thickness was found.<sup>26</sup> In an angiographically controlled population, ferritin and transferrin concentrations were not associated with CAD.<sup>27</sup> In a study comparing 174 patients with angiographically confirmed CAD and healthy subjects, no relation between the C282Y mutation and CAD could be found.<sup>28</sup> In a population (265 patients) with proven premature CAD a lower frequency of the C282Y mutation compared to healthy controls was found.<sup>29</sup> A postmortem study in 41 cases with iron overload and multiorgan haemochromatosis showed no association with prevalence of CAD.<sup>30</sup>

Coronary angiography is the gold standard for detecting CAD and the extent of coronary atherosclerosis. Our data from a large angiographically controlled population provide no evidence that the C282Y mutation is associated with an increased incidence of CAD or MI. The same findings were made in low risk subgroups (age <60 years, non-smoker, low LDL cholesterol, patients without diabetes). The extent of CAD, represented by angiographic scores and stratification into single, double, or triple vessel disease was not influenced by the C282Y mutation. By contrast with expectations, the Gensini score was even lower in patients with CAD carrying the C282Y mutation. Reduced left ventricular ejection fraction was only determined by the presence of CAD or MI and not by the heterozygous occurrence of the C282Y mutation. These data do not support increased myocardial damage owing to iron overload in heterozygotes, by contrast with the development of cardiomyopathy in several homozygotes with hereditary haemochromatosis.

Our findings in a large cohort of patients are in accordance with the studies already mentioned in discovering morphological lesions. These findings do not invalidate epidemiological studies probing the association between heterozygosity and cardiovascular mortality, as reviewed by Sullivan.<sup>31</sup>

We examined a population undergoing coronary angiography and might therefore be biased towards those with disease related to haemochromatosis. However, two findings argue against a selection bias. (1) The percentage of heterozygotes is identical to other populations of middle European ancestry,<sup>3–6</sup> indicating no over-representation of the C282Y mutation in our population sample. (2) Established risk factors of CAD, such as hypercholesterolaemia, cigarette consumption, age, diabetes mellitus, and male sex could be confirmed in the present study sample. Consequently, unknown risk factors or interactions between these factors and CAD should be recognisable in our population. The only established risk factor in our study population not associated with CAD was arterial hypertension. This is explained by angina-like chest pain in hypertensive patients combined with false positive stress testing owing to left ventricular hypertrophy. This leads to diagnostic coronary angiography and subsequently to an over-representation of hypertensive patients in the group with exclusion of CAD.

In conclusion, in our population of 1279 white people with angiographically confirmed coronary status, there is no evidence of an association between the C282Y mutation in the haemochromatosis gene and prevalence of CAD or previous MI. Moreover, this mutation is not a risk factor for advanced disease or depressed ejection fraction in CAD.

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