The prevalence of haemochromatosis gene mutations in the West of Scotland and their relation to ischaemic heart disease

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Objectives: Excess iron stores have been postulated to enhance the risk of ischaemic heart disease. This study aims to determine whether the two major mutations of the haemochromatosis (HFE) gene (C282Y and H63D) are associated with ischaemic heart disease (IHD) or myocardial infarction (MI).

Design: Cross sectional case-control study.

Setting: The geographical area studied by the MONICA (monitoring trends and determinants in cardiovascular disease) heart attack register for North Glasgow in Scotland, UK.

Patients: 1009 control subjects chosen at random from general practitioner registers were studied. Additionally, 924 subjects who had survived a first MI sustained between 1985 and 1992 were identified from the MONICA register.

Main outcome measures: C282Y and H63D mutations, previous MI, and presence or absence of IHD.

Results: Mutant gene prevalences in the whole control population were as follows: C282Y: homozygote 0.9%, heterozygote 17.7%; H63D: homozygote 2.1%, heterozygote 25.5%; and compound heterozygote: 2.4%. Analysis by \( \chi^2 \) test and logistic regression analysis did not identify any significant difference in genotype prevalence between normal control, IHD control, and MI survivor groups.

Conclusions: The C282Y homozygote and heterozygote prevalences are among the highest reported worldwide. No association between IHD or MI and HFE genotype was identified. However, these results need to be interpreted in the light of the cross sectional case-control nature of the study.

Subjects and Methods
Subjects
A random sample of 1688 subjects aged between 55 and 74 years and living in North Glasgow, Scotland, UK were identified from the patient registers of local general medical practitioners, with the intention of identifying the prevalence of cardiac disease in this population by a two stage, stratified randomisation process. All had to reside within the geographical area previously defined by the World Health Organization’s Scottish MONICA (monitoring trends and determinants in cardiovascular disease) heart attack register for North Glasgow project—that is, within the boundaries of the city of Glasgow north of the River Clyde. It was our intention to recruit a study cohort of 1000 subjects, by oversampling where necessary, and stratified such that there would be a target of 125 men and 125 women within each five year age band. Subjects were excluded if they had a history of severe mental or physical disease that would limit their ability to provide consent or to attend appointments.

These subjects were invited for a clinic visit to provide a detailed medical history and for a physical examination, ECG, and blood sampling. The 12 lead ECG was recorded in a standard fashion at a paper speed of 25 mm/s and subsequently coded according to the Minnesota ECG code for the presence of Q/QS waves (1.1–1.3), left bundle branch block (7.11), ST segment depression (4.1–4.4), and T wave inversion (5.1–5.3). This study achieved a response rate of 59.8%, with 1009 participants attending. Blood samples were obtained.
from 962 subjects. The response rate was higher for men than for women (63.1% v 56.8%; p = 0.008) and was higher in the first 10 years of the age range than in the second (64.7% v 55.4%, p = 0.001). The subjects who had any evidence of current or previous IHD (MI, angina pectoris, coronary revascularisation procedure, or the ECG abnormalities described above) identified from the medical history, physical examination, or ECG were placed into a group denoted “IHD controls”. The remaining subjects, who had no evidence of IHD, were placed into a group denoted “normal controls”.

Nine hundred and twenty-four subjects who had sustained a first validated MI and who attended a follow-up visit were identified from the MONICA register. The MONICA project maintains a database of all coronary events in selected populations. The Glasgow MONICA project identifies all people resident in North Glasgow aged between 25 and 64 years at the time of MI on the basis of hospital discharge coding, death certificate data from the Registrar General for Scotland, and postmortem and general practitioner data. Twenty thousand, two hundred and fifty-eight people were initially identified from the register as having survived a first MI between 1985 and 1992. Five hundred and sixty-five (24.6%) had died, 170 (7.5%) had moved out of the area, 82 (3.6%) were within the study, and 63 (2.8%) were excluded by their general practitioner as being unable to attend because of severe mental or physical disease. The response rate within this eligible group was therefore 66.6%.

All of these remaining subjects had therefore survived MI for a median period of 7.0 years (range 2.5–11.5 years). Stored blood, which had been sampled in 1995–96, was available for 846 of these subjects. This group of subjects was denoted “MI survivors”.

All subjects gave written informed consent to the use of their blood for genetic analysis, and this study conforms to the principles of the Declaration of Helsinki. This study was approved by the local hospital ethics committee.

Laboratory analysis
Venous blood samples were analysed for the C282Y and H63D mutations by standard restriction fragment length polymorphism techniques following amplification by polymerase chain reaction. These mutations are, with very rare exceptions, mutually exclusive on the same chromosome (that is, they are in complete linkage disequilibrium), allowing the subjects to be classified into six genotypes. These genotypes are: wild type (H63H, C282C/ H63H, C282C), H63D homozygote (H63D, C282C/ H63D, C282C), H63D heterozygote (H63D, C282C/ H63H, C282C), compound heterozygote (H63D, C282C/ H63H, C282Y), C282Y homozygote (H63H, C282Y/ H63H, C282Y), and C282Y heterozygote (H63H, C282Y/ H63H, C282C). For logistical reasons, or because of an inadequate sample of blood, genetic analysis was incomplete for 60 (3.3%) of the 1808 subjects from whom blood had been obtained (26 (5.0%) normal controls, 16 (3.6%) IHD controls, and 18 (2.1%) MI survivors).

Statistical analysis

The Hardy-Weinberg equation and the χ² goodness of fit test were used to test for genetic equilibrium. The difference in genotype frequencies between groups (univariate analysis) was compared using the χ² test or Fisher’s exact test, where appropriate, and by using logistic regression analysis (multivariate analysis) to correct for differences in age, sex, and other IHD risk factors. One way analysis of variance was used to compare the means of continuous variables. The 95% confidence interval (CI) of an estimate of prevalence was calculated by the method described by Bland. Significance was taken as being p < 0.05. All analyses were two tailed. Analyses were performed using SPSS statistical software, version 10 (SPSS Inc, Chicago, Illinois, USA).

RESULTS

Table 1 shows the number of subjects in each of the three groups (normal control, IHD control, and MI survivor), together with a breakdown of age, sex, and other IHD risk factors. There was a significant difference, by univariate analysis, between the normal control group and the MI survivor group for all variables (p < 0.01), with the exception of mean serum cholesterol concentration and the proportion of subjects who were diabetic, or were former smokers. Table 1 also shows the odds ratio for each risk factor. There was no difference in age, sex, or other IHD risk factors between the normal control and IHD control group. The overall prevalence of IHD in the entire control group was 46%. This was subdivided as follows: ECG evidence of previous MI (Q/QS waves) 6.6%; left bundle branch block, ST segment depression, or T wave inversion 26.6%; antianginal treatment 9.9%; self reported history of MI 10.7%; and self reported history of angina 16.4% (note that subjects could be included in more than one category).

Table 2 shows the frequencies in the whole control population (normal controls and IHD controls) of the H63D and C282Y homozygotes, heterozygote carriers, compound heterozygotes, and individual allele frequencies. The distributions of these genotypes were all in Hardy-Weinberg equilibrium (p = 0.24, p = 0.74, p = 0.79, for wild type, H63D, and C282Y, respectively).

Table 3 shows the prevalence of each of the six possible genotypes for each subject group. This table also shows the odds ratios and 95% CIs by logistic regression analysis comparing the normal control versus IHD control and normal control versus MI survivor groups, respectively. There was no significant difference in the genotype frequencies (as assessed by univariate analysis) between the groups with the exception of the C282Y heterozygotes, which occurred less frequently in the post-MI group than in the normal control group (p = 0.04). After correction for differences between the two
groups using logistic regression, there were no significant associations. In particular, the lower frequency of C282Y heterozygotes in the MI survivors than in the control group became insignificant (odds ratio 0.70, 95% CI 0.48 to 1.04, p = 0.08). Analysis of men and women separately did not show any significant associations, with the exception of the lower frequency of C282Y heterozygotes in the MI survivors than in the control group, which was confined to women only. This negative association in women between the MI survivor group and C282Y heterozygosity persisted on multivariate adjustment for risk factors or multiple significance testing), which may promote atherogenesis. It may also promote IHD in both sexes, the iron hypothesis may also account for the higher incidence of IHD in men and postmenopausal women. As iron stores increase with age in both sexes, the iron hypothesis may also account for the prevalence between groups (albeit losing significance after adjustment for risk factors or multiple significance testing).

In the absence of randomised controlled studies of iron depletion in normal populations, evidence to support the iron hypothesis has been sought from biochemical studies of iron stores and, with the discovery of the major mutations associated with haemochromatosis in 1996, from studies of the HFE genotype.

Results of studies of iron stores have been conflicting. Biochemical measurement of iron status is prone to interference by multiple factors such as inflammation, recent blood loss, and medication. An early study from Finland showed a positive association between iron stores and IHD, but further large studies and a meta-analysis of all prospective studies have been negative.

Genotype studies are unaffected by the factors discussed above, although these have also shown conflicting results. Cross sectional case–control studies have had similar findngs to our own, namely no association between IHD and HFE genotype. The study by Hetet and colleagues is of particular note, as it showed an association between C282Y heterozygosity and IHD in a subset analysis of women from the same geographical population as that of our study. Analysis of women as a separate subset from our data suggested the opposite. We found a non-significant trend towards a negative association between IHD and C282Y heterozygosity.

Three prospective studies have addressed the association between HFE genotype and IHD. The Utrecht study showed a non-significant increased relative risk (approximately 2) for M1 in postmenopausal women that was associated with C282Y heterozygosity but a significantly increased relative risk for all cardiovascular death. The Finnish and US studies also showed a non-significant increased relative risk of similar magnitude. However, after correction for other risk factors, a significantly increased relative risk was found (between approximately 2 and 3). These three studies did not consider the H63D mutation.

Our study had a cross sectional case–control design and therefore identified only surviving members of the population. In particular, the post-M1 group had all survived between 2–11 years after MI. A survival disadvantage may explain why the prevalences of left ventricular hypertrophy and hypertension were lower in the M1 survival group. If the HFE mutations confer a survival disadvantage to those with IHD or MI, then differences in genotype prevalence apparent in prospective studies may not be detected in a cross sectional case–control study. It is theoretically possible therefore that the one genotype to show a trend towards a differing prevalence between groups (albeit losing significance after adjustment for risk factors or multiple significance testing), namely the lower frequency of C282Y heterozygotes in the M1 survivor group, may confer a survival disadvantage related to iron stores following MI.
Conclusion
The C282Y homozygote and heterozygote prevalence in this West of Scotland population is among the highest reported worldwide. There was no association between IHD or MI and HFE genotype in this large study despite a high prevalence of IHD. However, these results need to be interpreted in the light of the cross sectional case-control nature of our study.

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