Factor V Leiden and other thrombotic risk factors in CHD and myocardial Infarction

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Introduction

Hemostatic imbalance might be an etiological factor in the transition of coronary heart disease (CHD) to myocardial infarction (MI). Several polymorphisms in genes regulating coagulation and hemostasis have been described as risk factors for venous thrombosis. However, their contribution to the development of arterial thrombosis is still unresolved. There are many reports in literature dealing with the role of specific coagulation factors in MI, ischemic stroke or peripheral arterial occlusion (for references see [1]), but for none of them a clear association to CHD or MI was finally accepted.

The aims of the present study were
1. to determine the exact prevalences of mutations and polymorphisms in the genes for FV (FV Leiden mutation Arg506Gln and haplotype FV HR2, His1299Arg), MTHFR (677C>T), FII (20210G>A), FVII (Arg353Gln), FXIII (Val34Leu) and ACE (insertion/deletion polymorphism in intron 16), known to be risk factors or discussed as protective factors (FVII, FXIII) for venous thrombosis, in a group of angiographically confirmed CHD patients,
2. to determine an association between individual genetic markers and/or combinations of them and CHD and
3. to characterize the relationship of genetic marker frequency and the degree of coronary artery stenosis.

Subjects and Methods

Subjects

We have analyzed 132 (102 male, 30 female) angiographically characterized patients with CHD, admitted to the Clinic for Internal Medicine at University Hospital Greifswald, North-Eastern Germany. 89 of them had a history of non-fatal MI. With regard to the number of stenoses 38 of them had a single vessel disease, 39 a double vessel disease and 53 a triple vessel disease. In two cases no result of angiography was available. As a control group we investigated 155 (72 male, 83 female) participants of a population-based cross-sectional epidemiological study of the same region (Study of Health in Pomerania SHIP [2]), having no sign of CHD (i.e. persons with history of deep vein thrombosis, MI or stroke were excluded).
Molecular genetic methods for detection of sequence variants

For the DNA analysis of the variants of FV, FII, MTHFR and ACE blood samples were soaked onto filter paper cards and used for PCR as described previously [3].

The detection of the molecular markers has been performed by standard methods by PCR and restriction analysis (FV Leiden: [4]; FII: [5]; MTHFR: [6]). For the analysis of the newly described FV haplotype HR2 we detected the mutation 4070 A(R1)/G(R2) (His1299Arg) in exon 13 of FV as described by Lunghi et al. [7]. The analysis of the I/D polymorphism in intron 16 of the ACE gene was performed according to the protocols described by Rigat et al. [8] and for control of mistyping the D/D genotype by Odawara et al. [9]. For the other mutations/polymorphisms genomic DNA was extracted from blood samples by standard methods. The Arg353Gln polymorphism in exon 8 of FVII [10] was analyzed by PCR amplification and digestion with Msp I. Primer were designed from the FVII sequence [11]. The FXIII Val34Leu polymorphism was analyzed by PCR amplification and heteroduplex analysis [12].

Statistics

For statistical analysis allele frequencies were calculated by counting genes from the observed genotypes. Differences in allele frequencies and categorical variables like sex, history of hypertension or diabetes between groups were tested for heterogeneity by \( \chi^2 \) test. T-Test for two independent samples was used to compare means of age, body mass index (BMI) and plasma levels of triglycerides, total cholesterol and fibrinogen between groups. Adjusting for classical cardiovascular risk factors was performed by bivariate logistic regression. All analyses were performed using SPSS version 10.07 for Windows (SPSS GmbH Software München, Germany).

Results

Table 1 shows the clinical characteristics of patients and controls and the distribution of classical cardiovascular risk factors in these groups. As expected, some of these factors were more common in patients than in controls. As expected significantly more males than females had an CHD. In all CHD patients there were more persons with a history of diabetes mellitus (p<0.0011) than in controls. However, in this specific group of patients we found significantly lower plasma levels of total cholesterol (p=0.005) and a lower BMI (p=0.021). In patients with MI, sex distribution (p<0.0001), BMI (p=0.016), total cholesterol (p=0.006), fibrinogen levels (p=0.001) and diabetes frequency (p=0.0005) were significantly different to controls. Patients with single-vessel disease were significantly younger than our controls (p=0.004), had lower plasma levels of total cholesterol (p=0.007). In tendency there were less persons with hypertension (p=0.0558), and again more men present in that group (p=0.0027) than in the control group. No data were available for current smoking status or use of hormones in patients.