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Regular Article

Platelet glycoprotein GP VI 13254C allele is an independent risk factor of premature myocardial infarction

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ABSTRACT

Aim: The purpose of this study was to asses the impact of haemostatic and platelet receptor gene polymorphisms as an inherited risk factor for premature onset of myocardial infarction (MI).
Methods: Polymorphisms of platelet receptors - GP Ia (807C>T, rs1126643), GP VI (13254T>C, rs1613662), GP IIIa (HPA-1, rs5918), PAR -1 (IVS -14A>T; rs168753), $P2Y_{12}$ (34C>T, rs6785930 and H1/H2 haplotype, rs2046934), and genetic variations of the gene coding for cyclooxygenase-1 (COX-1) (-842A>G, rs10306114 and 50C>T, rs3842787) were investigated. Mutations in the genes coding for coagulation factor V (Q506R (Leiden) mutation, rs6025) and factor II (prothrombin G20210A, rs1799963) were also determined. The prevalence of gene polymorphisms was investigated in 105 consecutive patients with premature MI. This was compared with the same gene polymorphism prevalence in a group of 132 patients in which coronary artery disease had been excluded. Genotyping was done using PCR, followed by melting curve analysis with specific fluorescent hybridization probes.
Results: A significant association between GP VI 13254C allele carriers and premature MI was found ($p = 0.025$). No other differences in prevalence of the investigated polymorphisms between the compared patient populations reached statistical significance. In a logistic regression, which took other cardiovascular risk factors into account, the significance of the GP VI 13254C allele and vascular risk was suggested (OR 1.888, 95% C.I. 1.029 to 3.464, $p = 0.040$). In a binary logistic regression the positive relationship between the GP VI genotype and female gender was observed (OR 3.676; 95% C.I. 1.159 to 11.628; $p = 0.027$). The frequencies of GP VI and GP Ia gene polymorphisms were independent of one another ($p = 0.836$).
Conclusion: The presence of the GP VI 13254C allele is an independent predictor of premature MI.

Introduction

Arterial thrombosis caused by rupture or erosion of an atherosclerotic plaque, leading to platelet adhesion, thrombin generation and subsequent thrombus formation in coronary or cerebral arteries causing myocardial infarction (MI) and stroke, respectively, is a major clinical problem. Epidemiological studies indicate that coronary artery disease and cerebrovascular disease result from complex interactions between genetic susceptibility factors, long-term environmental influences (smoking, obesity), and established, concomitant disorders (diabetes, hypertension, dyslipidemia) [1].

Death from coronary artery disease at an early age is a strong predictor for the presence of a genetic disorder [2]. The impact of haemostatic and platelet receptor gene polymorphisms, as a risk factor for coronary artery thrombosis, remains controversial [3]. However, the significantly higher prevalence of polymorphisms in patients with premature myocardial infarctions suggests the strong likelihood of a genetic component.

Methods

Genetic polymorphisms

The frequency of ten haemostatic, platelet receptor, and enzymes gene polymorphisms were studied. Polymorphisms were chosen with respect to their potential association with enhanced platelet reactivity and thrombin formation respectively; reactivity to adenosine diphosphate (polymorphisms of the **$P2Y_{12}$ receptor** [4,5]), thromboxane A_2 synthesis (polymorphisms of **cyclooxygenase** [6]), reactivity to thrombin (polymorphism of the protease-activated (**PAR**) **-1 receptor** [7]), interaction with collagen (polymorphisms of the **glycoprotein** (**GP**) **VI** [8] and of the **GP Ia** [9]), the final common pathway in platelet activation

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(polymorphism of **GP IIb/IIIa** [10]), and thrombin synthesis (**factor V** [11] and **factor II** mutations [11]).

Subjects

The study population was comprised of a cohort of all consecutive patients who were hospitalized because of premature MI during the three-year period from July 2005 to July 2008 in the Vinohrady Cardiocentre, Prague, Czech Republic [12]. The frequency of genetic polymorphisms were investigated in 105 patients: men aged ≤50 years and women aged ≤55 years. This was compared with the same gene polymorphism prevalence in a group of 132 patients in whom coronary artery disease had been excluded. Patients (which served as the comparison group) were chosen from 1028 patients randomized in the PRAGUE-8 trial [13]. Selected patients underwent elective coronary angiography and had normal coronary angiograms (Table 1). A diagnosis of acute MI had to meet the new criteria of the American College of Cardiology and the European Society of Cardiology definition [14]. Patients with acute MI underwent urgent (STE-MI) or early (≤24 h of hospitalization) (NSTE-MI) coronary angiography. All subjects provided signed informed consent with regard to research genetic testing.

Laboratory analysis

Polymorphisms of platelet receptors - GP Ia (807C>T, rs1126643), GP VI (13254T>C, rs1613662), GP IIIa (HPA-1, rs5918), PAR -1 (IVS -14A>T, rs168753), $P2Y_{12}$ (34C>T, rs6785930 and H1/H2 haplotype, rs2046934), and genetic variations of the gene coding for COX-1 (-842A>G, rs10306114 and 50C>T, rs3842787) were investigated. Mutations in the genes coding for coagulation factor V (Q506R (Leiden) mutation, rs6025) and factor II (prothrombin G20210A, rs1799963) were also determined.

Genomic DNA was isolated from whole blood using a Magna Pure LC nucleic acid extraction system (Roche diagnostics, Mannheim, Germany). Genotyping was done using PCR, followed by melting curve analysis with specific fluorescent hybridization probes. The sequence-specific primers and the fluorophore-labeled probes used in this analysis were designed and obtained from TIB MOLBIOL (Berlin, Germany). Reactions were performed using a LightCycler® 480 (Roche diagnostics). Genotyping results for individual polymorphisms were confirmed independently. As a result, polymorphisms determined using the conventional technique of polymerase chain reaction based on restriction fragment length polymorphism assays were compared to genotypes determined with the LightCycler®.

Ethical review board approval was obtained and the study was conducted according to the ethical principles in the Declaration of Helsinki and Good Clinical Practice guidelines.

Statistical Analysis

Statistical analysis was performed in R software [15]. First, chi-square test of independence in 2 by 2 contingency tables (rows – status having or not having MI risk factor, columns – status having or not having gene polymorphism) was calculated. This test is equivalent to the test of odds ratio being equal to one [16]. These tests were followed by two-sample Student t-test with Welch approximation of degrees of freedom to compare the mean ages, means of INR and mean platelet counts between patients with premature onset of MI and without coronary artery disease. The interactions of particular gene polymorphisms in MI group were tested via chi-square test. Binary logistic regression model was used to estimate odds ratios of having MI with gene polymorphisms as independent variables. Binary logistic regression model was also used to estimate odds ratios of having cardiovascular risk factors (hypertension, hyperlipidemia, diabetes), long-term environmental influences (smoking, obesity) or being female in MI group with gene polymorphisms as independent variables.

The power of the chi-square test (for the particular alternative hypothesis about GP VI 13254C and sample sizes; patients with premature MI n = 105 and patients without CAD n = 132; (Table 2)) is equal to 0.506 [16].

Results

Established cardiovascular risk factors (hypertension, hyperlipidemia, diabetes) were less frequent in patients with premature MI (Table 1). Overall, 73.7% of patients (66% of men and 81% of women) with premature MIs were current cigarette smokers compared to 15.15% of patients without coronary artery disease (risk ratio 6.08, $p < 0.0001$). Half of the patients who suffered a premature MI had a positive family history for cardiovascular disease.

In the two-group comparison of the prevalence of ten genetic polymorphisms, premature MI was associated with a higher prevalence

Table 1
Baseline characteristics.

	Patients with premature MI n = 105	Patients without CAD n = 137	p-value (chi-square test)
Age (y); mean ± SD	47.78 ± 6.13	63.66 ± 9.47	<0.0001*
Male (%)	78.22	34.09	<0.0001
STE-MI (%)	56.00	X	X
NSTE-MI (%)	44.00	X	X
BMI>30 (%)	31.96	31.06	0.4425
Hypertension (%)	45.54	77.27	<0.0001
Current cigarette smoke (%)	73.74	15.15	<0.0001
Diabetes mellitus (%)	7.92	14.39	0.0656
Hyperlipidemia (%)	27.72	52.27	0.0001
Family history positive for CAD (%)	56.60	NA	X
Previous MI (%)	14.90	0	X
Previous PCI (%)	14.90	0	X
Previous CABG (%)	2.00	0	X
Previous stoke (%)	2.00	0	X
No. of diseased vessel		0	X
1-vessel disease (%)	50.51	X	X
2-vessel disease (%)	23.23	X	X
3-vessel disease (%)	20.20	X	X
INR mean ± SD	1.069 ± 0.099	1.059 ± 0.078	0.503*
Platelet count (x 10^9); mean ± SD	251.34 ± 61.99	250.78 ± 59.87	0.946*

NA – data not available; MI – myocardial infarction; CAD – coronary artery disease; PCI – percutaneous coronary intervention; CABG – coronary artery bypass graft; SD – standard deviation; * – p-values from two-sample Student t-test with Welch approximation of degrees of freedom.