RELATION OF THE LEU40ARG VARIANT OF GLYCOPROTEIN IIIA TO PERSONAL AND FAMILY HISTORY OF MYOCARDIAL INFARCTION

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GP1Ib/IIa fibrinogen receptor is a key element of the thrombotic pathway. In this study, we investigated the possible relation of PIA1/A2 polymorphism (1565T>C; Leu33Pro) and a rare 1586T>G (Leu40Arg) variation of GP1IIa gene to personal and family history of myocardial infarction (MI) among 601 patients with angiographically confirmed coronary heart disease. Four hundred and fifteen patients had MI and 94 of individuals reported family history of premature MI. The Arg40 (1586G) variant (n = 4) was present exclusively in MI-patients and significantly correlated with a family history of premature MI (P = 0.013), whereas the Pro33 (1565C; PIA2) allele (n = 204) was similarly prevalent among different groups of patients. These data indicate the importance of the Arg40 variant but do not support a significant role of Pro33 allele in susceptibility to MI.

Key words: myocardial infarction, platelets, GP1IIa, PIA polymorphism, Leu40Arg variation.

INTRODUCTION

Glycoprotein GPIIIa is an integral part of the GPIIb/IIIa receptor, a key element of the thrombotic pathway intimately involved in pathogenesis of myocardial infarction (MI). This platelet-specific receptor upon activation binds fibrinogen, which leads to platelet cross-linking, aggregation and thrombus formation. Alleles-modulating function of GPIIb/IIIa might result in prothrombotic platelet phenotypes and contribute to the inheritance of MI. Two of such potentially pathogenic variations are located in exon 3 of the GPIIIa gene. One is a common 1565T>C exchange leading to Leu33Pro replacement in mature GPIIIa (Newman *et al.*, 1989), known as PIA1/A2 polymorphism. The Pro33 (PIA2) allele confers to the receptor altered conformation (Honda *et al.*, 1995; Kunicki *et al.*, 1995), decreased threshold for activation, increased outside-in signalling and increased fibrinogen binding (Feng *et al.*, 1999; Goodall *et al.*, 1999; Michelson *et al.*, 2000; Vijayan *et al.*, 2000). Another radical variation is a rare 1586T>G transversion that causes substitution of leucine to arginine at amino acid 40 (Unkelbach *et al.*, 1994), which was also recently detected in Latvia (Licis *et al.*, 2006). The effect of Arg40 allele on platelet function is not known. The purpose of this pilot study was to examine the possible relation of the Leu33Pro and Leu40Arg variants to the personal and family history of MI among 601 patients with coronary heart disease (CHD).

METHODS

Study design. We performed a cross-sectional study on 601 patients who underwent scheduled coronary angiography at the Latvian Centre of Cardiology. These patients were chosen from a larger database; general inclusion criteria for which were: (i) age ≤ 70 years, (ii) clinical symptoms typical for or suggestive of coronary heart disease, (iii) no history of previous revascularisation (percutaneous transluminal coronary angioplasty and/or coronary artery bypass grafting). Additional criteria applied specifically for this analysis were (i) availability of information on personal history of myocardial infarction and family history of premature MI (defined as MI in first degree relatives before or at age of 55 years in men and 65 years in women) and (ii) presence of angiographically confirmed CHD (defined as coronary artery stenosis $\geq 50\%$). The following conventional CHD-risk factors were recorded for all patients during hospitalisation for angiography: age, gender, smoking status, diabetes, arterial hypertension, body mass index (BMI), and total cholesterol. The study was approved by the Central Medical Ethics Committee of Latvia and the patients gave written consent to participation.

Determination of genotypes and haplotypes. Genomic DNA was isolated from blood by standard phenol extraction. Oligonucleotides TATGCTCCAATGTACGGG (GPIIIaFw) and ACCTCCACCTTGTGCTCT were used to

PCR-amplify a 377 bp fragment encompassing both variations. Genotyping was then performed by MALDI-TOF MS assay (Kim *et al.*, 2003) employing the ddNTP extension protocol as provided by the manufacturer (Bruker Daltonics). 5'-biotinilated primers TCTTACAGGLCCTGCCTC and CCTTCAGCAGATTCLCCTTC were used to detect PIA and 1586T>G alleles, respectively.

Minor alleles of both substitutions create recognition sites for restriction endonuclease MspI. To identify allelic associations (haplotypes), we applied restriction digestion of PCR products followed by gel electrophoresis in a 2% agarose.

Statistical analysis. SPSS software (version 12.0) was used for statistical calculations. Univariate analysis (UVA) was performed with Pearson's chi-square test or Fisher's exact test as appropriate and odds ratio (OR) was calculated. Spearman's correlation was applied to analyse allele-dosage effect (additive model for PIA polymorphism). Adjustment for conventional CHD-risk factors (multivariate analysis, MVA) was performed with logistic regression using forced-entry method, in which the Exp (β) value characterised the adjusted OR of a genetic factor. A two-sided *P*-value of less than 0.05 was regarded as statistically significant result.

RESULTS

Characteristics of the study population. Data on the study population are summarised in Table 1. Four hundred and fifteen (69.1%) patients had experienced myocardial infarction, 94 (15.6%) individuals reported a positive family history of premature MI, and 64 (10.6%) had both personal and family history of MI.

The distribution of alleles in our study was similar to other European populations (Unkelbach *et al.*, 1994; Kastrati *et al.*, 2000; Nadasi *et al.*, 2005). The four individuals carrying the Arg40 variant were PIA1/A2 heterozygotes and, as revealed by restriction fragment length analysis, the allele was in complete linkage disequilibrium (LD) with PIA2 allele. Interestingly, although the 1565T>C and 1586T>G variations are separated by only 21 nucleotides, the LD pattern varies in Europe and haplotypes are population specific. Thus, in Hungarians the Arg40 variant is mostly associated with the Leu33 allele of the PIA polymorphism (Nadasi *et al.*, 2005), while in Latvian (this study) and in German (Unkelbach *et al.*, 1994) populations it is linked to Pro33 allele. Such a combination of two closely located radical amino acid replacements raises the probability of a pathogenic influence.

Prevalence of Arg40 and PIA2 variants in groups of study population. Regarding the relationship between genotypes and personal history of myocardial infarction, no significant association was detected, but it is remarkable that all carriers of Arg40 allele had previous MI (Table 2).

Table 1

CHARACTERISTICS OF THE STUDY POPULATION (n = 601)

Characterization of CHD and related factors		
	Personal history of MI, n (%)	415 (69.1)
	Family history of premature MI, n (%)	94 (15.6)
	Both personal and family history of MI, n (%)	64 (10.6)
Cardiovascular risk factors		
	Age mean, years (SD)	55.9 (8.2)
	Male, n (%)	478 (79.5)
	Current or former smoker, n (%)	426 (70.9)
	Hypertension, n (%)	383 (63.7)
	Diabetes mellitus, n (%)	66 (11.0)
	BMI mean, kg/m ² (SD)	28.5 (4.4)
	Total cholesterol, mmol/l (SD)	5.6 (1.3)
Alleles and genotypes, n (%)		
T1565C	T (PIA1; Leu33 allele)	998 (83.0)
	C (PIA2; Pro33 allele)	204 (17.0)
	T/T	411 (68.4)
	T/C	176 (29.3)
	C/C	14 (2.3)
T1586G	T (Leu40 allele)	1998 (99.7)
	G (Arg40 allele)	4 (0.3)
	T/T	597 (99.3)
	T/G	4 (0.7)

MI, myocardial infarction; SD, standard deviation; BMI, body mass index.

Comparing the prevalence of genetic variants between individuals with and without family history of MI, Arg40 carriers were more frequent among patients who had first degree relatives with premature MI (3.19 % versus 0.20 %; *P* = 0.013; OR = 16.681, 95%CI 1.716–162.136), whereas no relation was detected for PIA polymorphism. Multivariate analysis was not performed since family history of a disease is not affected by individual's personal disease-risk factors.

The Arg40 variant had a highly significant association (4.69% versus 0.19%; *P* = 0.004; OR = 26.361, 95%CI 2.700–257.363) with patients who had both a personal and family history of myocardial infarction and this association retained statistical significance after adjustment for confounding factors (*P* < 0.01; Exp (β)=25.833, 95%CI 2.449–272.509). The PIA2 allele showed only a weak, statistically non-significant linear trend (additive model, *P* = 0.178 and *P* = 0.193 in UVA and MVA, respectively).

DISCUSSION

A family history of premature coronary heart disease had been recognised as a major risk factor of CHD, which is, at least partly, genetic in nature and largely independent of other personal cardiovascular-risk factors (Marenberg *et al.*, 1994). The data presented here provide a novel observation that a 1586T>G exchange causing a radical amino acid substitution in mature glycoprotein IIIa, the Leu40Arg replacement, is a possible inheritance factor for myocardial infarction.

Table 2

PREVALENCE OF GENOTYPES IN GROUPS OF PATIENTS ACCORDING TO HISTORY OF MI

Characteristic		PIA genotype, n (%)			P; UVA (MVA)			1586T>G genotype, n (%)		P; UVA (MVA)
		A1A1	A1A2	A2A2	additive	dominant	recessive	TT	TG	
Personal history of MI	Yes	284 (68.43)	121 (29.16)	10 (2.41)	0.987 (0.976)	1.000 (0.878)	1.000 (0.718)	411 (99.04)	4 (0.96)	0.317 (0.999)
	No	127 (68.28)	55 (29.57)	4 (2.15)				186 (100)	0 (0)	
Family history of MI	Yes	63 (67.02)	28 (29.79)	3 (3.19)	0.725	0.809	0.468	91 (96.81)	3 (3.19)	0.013
	No	348 (68.64)	148 (29.19)	11 (2.17)				506 (99.80)	1 (0.20)	
Both personal and family history of MI	Yes	39 (60.94)	23 (35.94)	2 (3.12)	0.178 (0.193)	0.200 (0.218)	0.653 (0.500)	61 (95.31)	3 (4.69)	0.004 (0.007)
	No	372 (69.27)	153 (28.49)	12 (2.24)				536 (99.81)	1 (0.19)	

MI, myocardial infarction. UVA and MVA are univariate and multivariate analyses, respectively. In MVA results are adjusted for age, gender, smoking, hypertension, diabetes, body mass index, and total cholesterol.

tion. This agrees with a recent report that the Arg40 variant is characteristic of individuals with other thrombotic conditions (Nadasi *et al.*, 2005). However, more data are needed to reach a definite conclusion on the pathogenic effect of the Arg40 allele, because analysis of low-frequency variants is prone to false positive results. Also, as it is not clear what influence the Leu40Arg substitution has on the action of platelets, further functional studies are needed. At present one can only speculate that the radical nature of this replacement may affect conformation of GPIIIa, as occurs in the case of the PIA1/A2 (Leu33Pro) polymorphism.

The PIA2 (Pro33) allele has not only been shown to alter structure of GPIIIa but was also consistently demonstrated to increase responsiveness of platelets. Yet, epidemiological studies regarding its impact on coronary thrombosis have produced controversial results. The first report that PIA2 increases risk of MI (Weiss *et al.*, 1996) was confirmed by some subsequent analyses, while other studies failed to replicate the initial finding (Mikkelsen *et al.*, 2005). In our patient population, a direct association of the PIA2 allele with myocardial infarction was clearly absent, and only a weak linear trend for a correlation with the combined criterion, both personal and family history of MI, was observed. These findings do not support the view that the Pro33 variant is a significant risk factor for myocardial infarction.

A possible limitation of the present analysis is that it did not involve healthy controls and, therefore, the results regarding personal history of MI have to be considered only in context of confirmed CHD (this limitation does not apply to results on the family history of premature MI). For example, as a component of the vitronectin GPV/GPIIIa receptor in endothelial and vascular smooth muscle cells, the PIA1/A2 polymorphism might affect susceptibility to atherosclerosis (Mikkelsen *et al.*, 2001), masking its impact on myocardial infarction.

In conclusion, our findings show that (i) the Arg40 variant is a possible inheritance factor for myocardial infarction, which merits analysis in a substantially larger population of patients; (ii) the PIA1/A2 polymorphism does not have an importance in susceptibility to MI.

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GLIKOPROTEĪNA IIIA LEU40ARG VARIANTA SAISTĪBA AR MIOKARDA INFARKTA PERSONĪGO UN ĢIMENES VĒSTURI

GPIIb/IIIa fibrinogēna receptors ir centrāls tromboģenēzes elements. Balstoties uz 601 pacientu ar angiogrāfiski apstiprinātu koronāro sirds slimību, pētījumā analizēta GPIIIa gēna PIA1/A2 polimorfisma (1565T>C; Leu33Pro) un retas 1586T>G (Leu40Arg) variācijas asociācija ar miokarda infarktu (MI) un priekšlaicīga MI ģimenes anamnēzi. 415 no pacientiem bija raksturīgs miokarda infarkts un 94 — MI ģimenes vēsture. Arg40 (1586G) variants (n = 4) tika konstatēts vienīgi MI pacientos un būtiski korelēja ar pāragra MI ģimenes anamnēzi ($P = 0.013$). Pro33 (1565C) alēle (n = 204), savukārt, dažādās pacientu grupās bija izplatīta līdzīgi. Šie rezultāti liecina par Arg40 varianta nozīmi, bet neapstiprina Pro33 alēles lomu MI predispozīcijā.