Effects of Platelet Membrane Glycoprotein Polymorphisms on the Risk of Myocardial Infarction in Young Males

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Abstract

Background: Platelet adhesion and aggregation are mediated by specific platelet membrane glycoproteins GPIa/IIa, GPIbα, and GPIIb/IIIa, and are essential steps in thrombus formation and development of acute myocardial infarction.

Objective: To evaluate the risks exerted by each of the following polymorphisms in young males with AMI: HPA-1a/b in GPIIIa; 807C/T in GPIa; and HPA-2a/b, VNTR and Kozak C/T in GPIbα .

Methods: We conducted a case-control study of 100 young males with first AMI before the age of 53 and 119 healthy controls of similar age. All subjects were tested for the above polymorphisms.

Results: The allele frequencies of each of the platelet polymorphisms were not significantly different between the young men with AMI and the controls. Smoking alone was associated with a 9.97-fold risk, and the presence of at least one metabolic risk factor resulted in a 2.57-fold risk of AMI.

Conclusion: The platelet glycoproteins polymorphisms studied are not an independent risk factor for AMI.

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Acute myocardial infarction frequently results from a rupture of an atherosclerotic plaque that is followed by thrombus formation [1]. Platelet adhesion and aggregation have been found to have a crucial role in thrombus formation and development of AMI [2]. At sites of vascular injury when subendothelium is exposed, thrombus formation is initiated by binding of glycoprotein Ib-IX-V to von Willebrand factor [3]. Stable adhesion, activation and aggregation of platelets are then mediated via GPIa/IIa binding to exposed collagen and GPIIb/IIIa binding to vWF and fibrinogen [4]. The three-platelet membrane GP receptors – GPIIb/IIIa, GPIa/IIa and GPIb-IX-V – have highly interactive and additive adhesive effects, ultimately resulting in stable thrombus formation [5].

GPIa/IIa is one of several collagen receptors present on the platelet membrane. Some polymorphisms were identified in the GPIa gene. Two of them – C807T and G873A – are silent and linked, and have been shown to be associated with the receptor density on platelet membrane, resulting in corresponding platelet adhesion to collagen type I and III [6,7]. The 807T/873A homozygosity of the GPIa gene polymorphism was found to be an independent risk factor for AMI in some studies, although not in others [8,9].

GPIbα is the largest polypeptide in the GPIb-IX-V complex and contains the binding region for vWF. It has several polymorphic sites. The first is amino acid dimorphism Thr145Met, known as human platelet antigen-2 [10]. The HPA-2a/b polymorphism is adjacent to the vWF-binding site and might therefore influence the receptor-vWF interaction. The second polymorphism is the variable number of tandem repeats, with four alleles named A, B, C and D representing 4, 3, 2 and 1 repeats of 39 base pairs respectively [11]. HPA-2 polymorphism is in linkage disequilibrium with the VNTR polymorphism: HPA-2a is associated with the D or C variants, whereas HPA-2b is linked with the B and A alleles [11]. The third polymorphism is a single dinucleotide polymorphism C/T at position –5 from the initiator ATG codon (Kozak sequence). The less common C allele is associated with increased expression of the GPIb receptor on the cell membrane [12]. Several studies found an association between GPIbα polymorphisms and arterial thrombosis. The VNTR A-B/Met145 allele was shown to be a risk factor for coronary artery disease or AMI, but others could not confirm such an association [8]. The Kozak polymorphism was found to be associated with acute stroke rather than with AMI [8].

The GPIIb/IIIa is the most abundant receptor in the platelet membrane and is known as the fibrinogen receptor, although it binds other ligands, including vWF. The most frequent alloantigen, which is associated with immune thrombocytopenia, is expressed on GPIIIa and is known as HPA-1 (PI^A). HPA-1a/b polymorphism was identified as a single nucleotide change (T/C) causing an amino acid substitution (Leu33Pro) [13]. Numerous studies examined the role of HPA-1 polymorphism in a wide variety of coronary artery diseases with controversial results. Some showed that HPA-1b is a risk factor for myocardial infarction or restenosis, whereas other reports could not support these findings [8,9].

In a normal population the allele frequency of HPA-1b is 10–19%, of GP1a-C807T it is 36% for the T allele, of GP1b Kozak it is 15% for the C allele, of VNTR it is 10% for the B allele, and of HPA-2 it is 14% for the b allele [8].

AMI = acute myocardial infarction
vWF = von Willebrand factor
GP = glycoprotein

HPA = human platelet antigen
VNTR = variable number of tandem repeats

In the present case-control study we evaluated the risks exerted by each of the following platelet membrane glycoprotein polymorphisms in young males with AMI: HPA-1a/b in GPIIIa, 807 C/T in GPIa, HPA-2a/b, VNTR and Kozak C/T in GPIbα.

Methods

Cases and controls

The study group comprised male patients under the age of 53 who were consecutively admitted to the Coronary Care Unit from March 1994 to March 1997 with an established diagnosis of first AMI, as defined by the Cardiovascular Health Study [14]. Of 115 eligible patients 100 were included in the study. The control group consisted of 119 healthy male subjects of similar age who were enrolled during routine annual examinations of the Israel Defense Forces, which included an exercise stress test. None of the controls had a history or evidence of coronary artery disease as determined by medical history, rest electrocardiogram and ergometry. The Human Subject Ethics Committee of the hospital approved the study and written consent was obtained from all subjects.

Demographic characteristics

Demographic data were obtained for each subject from the medical records of Sheba Medical Center and the Israel Defense Forces. The information included current age (for controls), age at the time of the AMI, ethnic background, smoking history, blood pressure, total serum cholesterol, diabetes status, and history of coronary events.

Determination of platelet GP polymorphisms

Genomic DNA was isolated from 5 ml whole blood by a standard method [15]. The 807 C/T in GPIa gene was detected by polymerase chain reaction using forward primer 5'TATGGTGGGGACCTCACGAA CAC3' modified (underlined nucleotide) for *XmnI* recognition site, and reverse primer 5'GATTTAACTTTCCCAGCGCCTTC3'. The C allele was digested to 210 and 22 bp fragments by *XmnI* restriction enzyme.

The HPA-1 (Pl^A) polymorphism, a substitution of Leu33Pro caused by C/T change in GPIIIa gene, was detected by PCR amplification of 482 bp fragment as described by Simsek et al. [16] followed by *MspI* digestion.

The HPA-2 polymorphism, a substitution of Thr145Met in GPIbα, was detected by PCR amplification of 587 bp fragment using forward primer as described by Ishida et al. [11], and the reverse primer 5'TATGGGCTTTGGTGGGGAACTTGACC3' followed by *BsaHI* digestion.

Identification of the VNTR polymorphism was carried out by PCR amplification using forward primer 5'CCACTACTGAACCAACCC CAAGC3' and reverse primer 5'GCTTGTGGCAGACACCAGGATGG3'.

The Kozak C/T polymorphism in the 5' of the GPIbα gene was detected by PCR using forward primer 5'TCCACTC AAGGCTCCCTTGC3' and reverse primer 5'GGCGAGTGTAA GGCAT CAGG3' followed by digestion with *AvaII*.

PCR = polymerase chain reaction

Statistical analyses

Differences in baseline characteristics between patients and healthy controls were assessed by the chi-square test for categorical variables or Fisher's exact test according to the size of the cells examined, and *t*-test for continuous parameters. Univariate odds ratio and 95% confidence interval were estimated for each platelet receptor's polymorphism. To estimate the effect of various risk factors on the occurrence of myocardial infarction, an unconditional multivariate logistic regression model was designed that included age, ethnic origin, smoking status and presence of metabolic risk factors such as hypertension, hypercholesterolemia or diabetes, as controlling variables.

Results

Study population characteristics

Demographic information and the prevalence of the corresponding risk factor among cases and controls are shown in Table 1. More than half the AMI patients (57%) were of European-American origin compared to 30% of the controls.

Among the AMI patients 57% were smokers and 55% had at least one atherogenic metabolic risk factor (17% hypertension, 14% diabetes and 41% hypercholesterolemia). The corresponding figures among the controls were 28.6% for smokers and 29.4% for any metabolic risk factors (9.2% hypertension, 0% diabetes and 24.3% hypercholesterolemia). These differences were statistically significant except for hypertension [Table 1].

Allele frequencies of platelet receptor polymorphisms

Table 2 summarizes the allele frequencies for the corresponding polymorphism of platelet glycoproteins among cases and controls.

Table 1. Demographic and clinical data of AMI patients and controls

	AMI patients (n = 100)		Controls (n = 119)		*P*
	No.	%	No.	%	
Age					
Mean ± SD	42.5 ± 4.2		40.6 ± 4.1		0.03
(range)	(29–52)		(29–52)		
Origin					
Asia-Africa	34	34.0	66	55.5	
Europe-America	57	57.0	36	30.3	0.001
Israel and mixed	9	9.0	17	14.2	
Smoking					
Never	39	39.0	85	71.4	
Ever	57	57.0	34	28.6	< 0.001
Unknown	4	4.0	0		
Hypertension*					
Yes	17	17.0	11	9.2	0.13
No	83	83.0	108	90.8	
Diabetes mellitus					
Yes	14	14.0	0	0	< 0.001
No	86	86.0	119	100	
Hypercholesterolemia**					
Yes	41	41.0	29	24.3	
No	59	59.0	90	75.6	0.009

* Systolic blood pressure =140 mmHg on admission

** Total serum cholesterol level > 200 mg/dl on admission.

Table 2. Allele frequency of platelet membrane glycoprotein polymorphisms among AMI patients and controls

Gene	Polymorphism	AMI (n=100) %	Controls (n=119) %	OR	95% CI
GPIIIa	HPA-1b	11	14.3	0.74	0.42–1.31
GPIa	807T	42	37.8	1.48	0.75–2.93
GPIb	HPA-2b	13	9.7	1.40	0.77–2.53
GPIb	VNTR-A+B	8	5.5	1.20	0.92–1.57
GPIb	Kozak-C	13	11.3	1.17	0.66–2.07

The alleles 807T, HPA-2b, VNTR-A+B and Kozak-C were more common among the AMI patients (42%, 13%, 8% and 13% respectively) compared to the controls (37.8%, 9.7%, 5.5% and 11.3% respectively). However, these differences did not reach statistical significance [Table 2]. In contrast, the HPA-1b was less common among AMI patients (11%) than among the controls (14%).

To overcome the differences between the cases and controls with regard to ethnic origin, age, smoking and hypercholesterolemia, these variables were included in a multivariate stepwise logistic regression model. Since the prevalence of VNTR-A+B and Kozak-C alleles was very similar between the two groups, only 807T and HPA-2b were analyzed by the multivariate logistic model [Table 3]. Following adjustment for the above variables, the differences in frequency of either 807T or HPA-2b alleles were not statistically significant (OR = 1.26, 95% CI 0.59–2.7 for 807T; OR = 1.19, 95% CI 0.57–2.51 for HPA-2b). As shown in Table 3, smoking or the presence of any metabolic risk factor was associated with increased risk of AMI (OR = 3.91, 95% CI 2.22–6.88, and OR = 2.93, 95% CI 1.68–5.12, respectively).

The interaction between the metabolic risk factors, smoking and the platelet polymorphisms was further analyzed following inclusion of these variables in the logistic model [Table 4]. The presence of any platelet polymorphisms in the absence of smoking or metabolic risk factors was not associated with statistically significant risk of AMI (OR = 1.76, 95% CI 0.69–4.52). The presence of any metabolic factor increased the risk of AMI 2.6-fold, whereas smoking alone increased the risk almost 10-fold. When smoking and metabolic risk factors were included in the logistic model (regardless of platelet polymorphisms), the presence of these two risk factors conferred an OR of 11.4 (95% CI 1.67–29.86) adjusted for age and ethnic origin. However, when any platelet polymorphisms, any metabolic factors and smoking were analyzed simultaneously, the risk of AMI increased over 14-fold (OR = 14.43, 95% CI 4.54–45.87).

OR = odds ratio
CI = confidence interval

Table 3. Effect of smoking, diabetes mellitus, hypercholesterolemia, and platelet polymorphisms HPA-2b and 807T on the risk of AMI

Variable	OR	95% CI
Smoking	3.91	2.22–6.88
Diabetes mellitus	40.06	2.36–681.2
Hypercholesterolemia	2.16	1.21–3.84
GPIb HPA-2b	1.19*	0.57–2.51
GPIa 807T	1.26*	0.59–2.70

* Following adjustment for age, origin, smoking and hypercholesterolemia

Table 4. Effect of concurrent presence of platelet membrane glycoprotein polymorphisms, smoking and metabolic factors on the risk of AMI

Any platelet polymorphisms	Any metabolic risk factors *	Smoking	OR	95% CI
–	–	–	1.0	
+	–	–	1.76	0.69–4.52
–	+	–	2.57	1.33–4.95
–	–	+	9.97	2.88–34.6
+	+	+	14.43	4.54–45.87

+ = presence, – = absence
* Hypertension, diabetes mellitus or hypercholesterolemia

Discussion

In the present study of young males with AMI, the platelet GP polymorphisms (HPA-1a/b in GPIIIa, 807 C/T in GPIa, and HPA-2a/b, VNTR and Kozak C/T in GPIbα) were not found to be independent risk factors for AMI. Combination of any of the above polymorphisms with both smoking and metabolic risk factors slightly increased the risk of AMI as compared to the risk conferred by metabolic risk factors and smoking alone (from 11 to 14-fold).

In accordance with our results, others also found that HPA-1b, HPA-2b or 807T alleles are not independent risk factors for AMI [8]. A review of the role of platelet membrane polymorphisms in myocardial infarction [8] shows that with regard to HPA-1, 12 studies were negative whereas only 7 were positive. Moreover, a recent meta-analysis of 34 studies on HPA-1 and coronary artery disease concludes that the risk for AMI was low [17]. With regard to GPIb polymorphisms, similar to our results, the majority of studies failed to find a correlation with AMI [8]. With respect to the correlation between AMI and GPIa 807T, the number of positive and negative studies is equal, leaving the issue inconclusive [8]. These conflicting results, obtained from various studies, may stem from the differences in populations or the specific outcome studied. In addition, in most of the studies the role of platelet GP receptor polymorphisms in AMI was analyzed with regard to the risk conferred by simple gene-disease associations. Due to population diversity and the multigenic etiology of AMI, gene-environmental interactions may be more valuable in defining the risk for AMI.

It was recently proposed that the platelet receptors GPIb-IX-V and GPIIb/IIIa may have a role in thrombogenesis rather than in atherogenesis [18], whereas GPIa/IIa was found to be a predictor of the extent of atherogenesis [19]. This is based on the fact that GPIIb/IIIa knockout mice were shown to develop atherogenesis [20], and Glanzmann thrombasthenia patients lacking the GPIIb/IIIa complex were shown to have carotid atherogenesis [21]. Moreover, in a coronary artery disease trial, antiplatelet therapy with inhibitors of GPIIb/IIIa (abciximab) demonstrated beneficial effects [22]. Finally, a recent prospective study that analyzed the effect of HPA-1b allele on the outcome in a cohort of coronary artery bypass

surgery patients with established atherosclerosis found that this allele was significantly more represented among patients who developed AMI or died [23].

In conclusion, the present study of young males with AMI did not find the platelet glycoprotein polymorphisms (HPA-1a/b in GPIIIa, 807 C/T in GPIa, and HPA-2a/b, VNTR and Kozak C/T in GPIbα to be independent risk factors for AMI.

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