The Role of Human Platelet Antigen 1 Polymorphism in Development of Coronary Artery Stenosis in Iranian Population

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Abstract

Background- Aggregation is the final step in activation of platelets and is mediated by presentation of GPIIb/IIIa receptors on the platelet membrane that binds to fibrinogen and von Willebrand's factor. There are common mutations in GPIII structure that can change the behavior of the molecule and may change the pattern of interaction between platelets and injured endothelium, thus they can have prognostic impact in coronary artery disease (CAD) and acute coronary syndrome. In some large trials, persons homozygous for the PlA2 allele had a greater chance of coronary stenosis and myocardial infarction (MI) than heterozygotes or non-carriers, but other studies did not confirm this association. This is the first study of PlA polymorphism in Iran and is aimed to find a possible association of this mutation and CAD in the Iranian population.

Method- In this case-control study, we chose 200 patients who underwent diagnostic coronary angiography between 2005 and 2006 in Hamedan, Iran. In these patients HPla genotype determination was done using PCR method.

Results- We found no significant association of coronary artery stenosis and PlA2A2 or PlA1A2 genotypes in our patients, p value>0.05. However, there was a significant association between possession of PlA2 allele and occurrence of CAD in patients more than 50 years of age, p value 0.045.

Conclusion- Variations in PlA phenotype do not seem to have an association with ischemic heart disease, but the PlA2 allele may have a role in the development of atherosclerosis and MI in persons more than 50 years of age ***(Iranian Heart Journal 2009; 10 (3):22-26).***

Key words: coronary artery disease ■ human platelet antigens ■ polymorphism

GPIIb-IIIa is the most abundant platelet receptor, with 40–80,000 GPIIb-IIIa complexes per platelet.[1] GPIIb/IIIa is an integrin receptor[2] composed of two subunits, IIb (CD41) and IIIa (CD61). Each subunit is produced by separate genes that lie on the long arm of chromosome 17 at q21-22.

Fibrinogen is the main receptor of GPIIb/IIIa due to its high plasma concentration but other adhesive and non-adhesive ligands bind to it, including vWF, fibronectin, vitronectin, red cell ICAM-4[3], prothrombin, and thrombospondin.

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GPIIb/IIIa has a key role in platelet aggregation and thrombus formation. The resultant thrombus formation can lead to the development of acute coronary syndromes and the sudden death of patients with coronary heart disease. GPIIIa (b3-integrin) is also present in the endothelium and vascular smooth muscle cells (VSMCs).[4,5] Its function is related to VSMC responses to endothelial injuries caused by, e.g., hemodynamic shear stress, smoking, diabetes, and hypertension.[6,7]
Platelet PlA polymorphism of the GPIIIa gene is caused by a single point mutation in exon 2 of the GPIIIa gene, which leads to substitution of leucine (PlA1) for proline (PlA2). The functional importance of this mutation as a risk factor for MI was first suggested by Weiss and colleagues.[8] Other studies on associations between the PlA2 allele and acute coronary events have, however, been controversial.[9-11] More controversy was added by the recent findings that possession of the PlA2 allele may cause either less or more fibrinogen binding than patients homozygous for PlA1 and also by the finding that the response to thrombin differs between the genotypes.[12]
In Iran, there has not been such a study to date. Therefore, the purpose of this study is to evaluate the effect of PlA polymorphism on the occurrence of coronary artery stenosis and possibly acute ischemic events in the Iranian population.

Methods

This is an observational case-control study and the population of the study was selected from patients who underwent coronary angiography for suspected ischemic heart disease in Ekbatan Hospital, Hamedan, Iran between 2005 and 2006.
In the case group, we had 100 patients with documented coronary artery disease which was defined as >50% involvement of at least one epicardial coronary artery. In the control group, there were 100 patients who had no evidence of coronary artery obstruction on angiography. Of the patients with coronary artery disease, 33% had involvement of one coronary artery, 38% of them had two vessel-disease and 29% had three vessel-disease.
Data about conventional risk factors was gathered by history taking or from the patients' medical records.
In this study, HPLA genotype was determined by PCR (polymerase chain reaction) method. A sample containing 7cc of venous blood was taken from a peripheral vein and was sent to the laboratory for DNA extraction. First the sample was mixed with 10cc EDTA and 25cc of LYSE I and the mixture centrifuged for 7-12 minutes. The sediment was again mixed with phosphate buffers and centrifuged. The resultant final sediment was mixed with LYSE II, sodium disulfate and propanol to reveal DNA molecules. These DNA molecules were then separated and kept in a refrigerator for one week. After this time, samples of DNA were used for amplification. Finally agarose gel electrophoresis and addition of ethidium-bromide was done to separate bands of DNA for final analysis under ultraviolet light.

Statistical analysis

The data analysis for HPLA genotype prevalence and frequency of PLA2 allele was based on ANCOVA, in which the possible confounding effects of age, body mass index, diabetes, hypertension, and smoking (if data were available) were taken into account by including them into the model as covariates. The computation was carried out with SPSS 13 Statistical Software (2004 version). The odds ratios and their 95% confidence intervals were calculated with Confidence Interval Analysis (CIA) software using Pearson chi-square on a personal computer.

Results

In the case group, 72% of patients were male and 28% were female, and in the control group 44% were male and 56% female. The mean age of the patients was 55.6 years. 21%

of the patients and 46% of the controls were less than 50 years old while 79% of patients and 46% of controls were more than 50 years old(p-value <0.05)

The prevalence of A1A1 genotype was 73% in the CAD group and 82% in the control group, with no significant difference between the two groups (p-value >0.05). Likewise the prevalence of A1A2 and A2A2 genotypes was not significantly different (A1A2 prevalence 16% in the normal group and 27% in the CAD group; A2A2 prevalence: 0% in CAD group and 2% in controls, p-value >0.05, Table I).

Table I. Prevalence of different genotypes of HPLA1 in case and control groups.

	A1A1 allele		A1A2 allele		A2A2 allele	
	case	control	case	control	case	control
number	73	82	27	16	0	2
percent	73	82	27	16	0	2

After analysis of A2 allele prevalence in the two groups, we found that the overall prevalence of A2 allele was not different significantly between the two groups, but when data was analyzed according to age it became clear that the A2 allele was more prevalent in CAD patients over 50 years of age than in their age-matched controls, but below 50 years of age there was no difference between the case group and their controls in the prevalence of A2 allele (Table II).

Table II. Prevalence of A2 allele in patients above 50 years of age (p value: 0.045).

	Patients with A2 allele		Patients without A2 allele	
	number	percent	number	percent
case	8	38.1	13	61.9
control	8	17.4	38	82.6

Discussion

Here we present the results of the first PCR study on the association of PlA polymorphism with coronary atherosclerosis, coronary narrowing, and MI in Iran.

From the analyzed data mentioned above, it seems that the overall frequency of PLA polymorphism in CAD patients is not different from the normal population, but in special subgroups such as persons more than 50 years of age, possession of PLA2 allele could be a risk factor for coronary stenosis and possibly occurrence of myocardial infarction.

Weiss et al.[8] were the first to report an association between the PlA polymorphism and MI. They found that the PlA2 allele was positively associated with MI and that this association was even stronger in their group of patients <60 years old. This finding has been supported by other studies,[10,11,14] but conflicting results have also been reported[9,13] in similar series comprising patients with MI as well as in a large, prospective series of 14,000 men.[15]

In their results, Ridker et al.[15] could confirm no connection between PlA polymorphism and cardiovascular events.

In an interesting study, Mikkelsson et al.[16] found a significant relation between possession of the PlA2 allele and slower progression of coronary artery stenosis. However, rate of coronary thrombosis was higher among patients with PlA2 allele. These results were based on autopsy series of 300 middle-aged Finnish men who died of sudden cardiovascular death or violent events. These results thus support the concept of the functional importance of PlA polymorphism. The higher prevalence of the PlA2 allele among men with MI associated with coronary thrombosis is likely due to the thinner fibrous caps of their atheromatous plaques and/or more reactive platelets. On the other hand, in PlA1 homozygotes, intimal hyperplasia may be more extensive, resulting in progressive coronary stenosis with stable plaques and "silent" occlusion of the vessel lumen.

In a large study in Denmark in 2003, Bojesen[17] reported a 3-4 time higher risk of cardiovascular disease in men who were homozygous for PlA2 allele (A2A2)

compared to heterozygotes or non-carriers (A1A2 or A1A1).

Conflicting results were found in other studies; in a relatively large study by Sungha et al.[18] in South Korea in 2004, there was no association between GPIIb/IIIa and GPIa polymorphism with occurrence of coronary artery disease or MI. Similar results have been observed in other studies carried out in Japan, Thailand and Germany.[19-21]

In our country, Iran, there has not been such a study to date, so we did not have access to a local database for comparison. Furthermore, the study is under-strength due to the low volume of population studied. On the other hand, the effect of confounding factors such as age, sex and conventional risk factors could be important because there was no randomization during sampling, although this problem was partially overcome by various analytic methods. Despite these pitfalls, the results are compatible with what has been observed in some studies carried out in other countries; for example, the importance of PlA2 allele possession in the occurrence of coronary events has been noted in some large trials. Controversies about the role of GP IIb/IIIa polymorphism may be partially due to different ethnicities included in the studies. For example, in nearly all studies performed in the Far East we see the same results (no relationship between IIb/IIIa polymorphism and ischemic heart disease). On the other hand the results of studies carried out in Scandinavian countries show opposite results. So the genetic differences among human races or possibly the effect of various environmental factors in different geographical areas may explain these conflicting results. Other factors such as sex and age could be important, as we noted, it seems that the effect of PlA2 allele possession is more important in the occurrence of coronary artery disease or cardiac events among men with 50 years of age or more. This may necessitate further studies in special high risk subgroups such as diabetics or older ages.

In conclusion we found no relationship between HPlA genotype and development of atherosclerotic narrowing of coronary arteries in Iranian patients but analytic results suggest an association between PIA2 allele possession and CAD in patients more than 50 years old. Our results to some extent support the concept of functional importance of HPLA polymorphism on occurrence and progression of atherosclerosis. Although interesting, these results need to be confirmed by larger clinical trials in the field of common mutations of platelet receptors in Iranian population.

Conflict of Interest

No conflicts of interest have been claimed by the authors.

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