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THE PREVALENCE OF PAI-1 4G/5G GENE VARIANT IN SERBIAN POPULATION

Abstract

Introduction: Plasminogen activator inhibitor 1 (PAI-1) has a major role in inhibition of fibrinolysis and normal haemostasis.

The presence of the PAI-1 4G/4G genotype leads to increased expression of PAI-1. High blood level of PAI-1 is associated with many diseases such as thrombosis, cerebral insult, myocardial infarction, pregnancy loss, preeclampsia, insulin resistance, type 2 diabetes, breast cancer and asthma.

In this study, the prevalence of PAI-1 4G/5G gene variant was determined in healthy subjects from Serbian population.

Methods: The study was carried out in a group of 210 healthy subjects (105 women and 105 men). The presence of PAI-1 4G/5G gene variant was detected by PCR-RFLP analysis.

Results: The prevalence of PAI-1 4G/4G genotype was 34.76% and it was increased compared to PAI-1 5G/5G genotype (19.05%). The most frequent was PAI-1 4G/5G genotype (46.19%). Allelic frequency for 4G allele was higher (0.58) compared to 5G allele (0.42).

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Conclusions: The prevalence of PAI-1 4G/5G gene variant in Serbian population is similar to the neighboring populations. Results of this study represent the first data for Serbian population. This study could be useful for further research where the role of PAI-1 4G/5G gene variant will be assessed in the pathogenesis of many diseases.

Keywords: PAI-1 4G/5G, plasminogen activator inhibitor 1

Introduction

Haemostasis represents one of the most important homeostasis mechanisms. It has major role in providing liquid state of blood and its normal flow through the circulation, while in the case of blood vessel damage it can ensure blood clot formation and preventing hemorrhage. In maintenance of this very complex balance several factors are included: the endothelium of blood vessels, platelets, coagulation factors, coagulation inhibitors and fibrinolytic system (1).

The fibrinolytic system has a role to prevent the pathological extension of the blood clots removing fibrin from the circulation. The central enzyme of this system is the plasmin generated by activation of plasminogen. Plasmin activity is regulated by a complex network of activators and inhibitors of fibrinolysis. The main inhibitors of fibrinolysis are: plasminogen activator inhibitor (PAI-1 and PAI-2) and α2-antiplasmin (2).

Plasminogen activator inhibitor type 1 (PAI-1) is a 55kD glycoprotein synthesized by endothelial cells, hepatocytes and megakaryocytes (3). PAI-1 acts as an inhibitor of endogenous fibrinolytic activity due to its ability to inhibit the activity of tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) (4-6). The gene for PAI-1 is located on chromosome 7 (7q21.3-q22.1) and contains 8 introns and 9 exons (3). At position -675bp in the promoter region of PAI-1 gene an insertion /deletion of a single guanosine is described, marked as 4G/5G gene variant. Allelic variant of 5 guanosines (5G) contains overlapping binding sites for activator and repressor of transcription, which leads to normal expression levels of PAI-1. In contrast, the 4G allelic variant, related only with a transcription activator, leads to increased level of PAI-1 in blood (3).

Increased expression of PAI-1 leads to reduced fibrinolysis, which may represent a risk factor for a number of cardiovascular diseases: myocardial infarction (7, 8), stroke (9), and deep vein thrombosis (3). Studies have shown that PAI-1 4G/5G gene variant may be one of the risk factors for spontaneous miscarriage (10, 11) and preeclampsia (12). Furthermore, increased expression of PAI-1 is associated with insulin resistance (13) and type 2 diabetes (14). Also, the important role of this genetic variant has been demonstrated in the pathogenesis of breast cancer (15) and asthma (16).

The prevalence of PAI-1 4G/5G gene variant varies depending on ethnicity (17-22). The frequency of this gene variant was determined for a number of populations, but there is no data observed for Serbian population.

The aim of this study was to determine the frequency of PAI-1 4G/5G gene variant in healthy Serbian population.

Materials and methods

Our study included 210 healthy subjects (105 women and 105 men; aged 39±11.25 years) with no history of thrombotic event. For the isolation of DNA peripheral blood lymphocytes and buccal mucosa cells were used. Blood samples from subjects were taken on 3.8% sodium citrate as anticoagulant, and buccal mucosa samples were taken with sterile swab. Genomic DNA was isolated using the QIAamp DNA Blood MiniKit (QIAGEN, Germany) according to manufacturer's standard protocol.

The PAI-1 4G/5G gene variant was detected by PCR-RFLP. Polymerase chain reaction was carried out in a 25μL reaction volume containing: 1x buffer A (Kapa Biosystems, Boston, USA), 2.5 mM $MgCl_2$; 200μM dNTP; 10pmol of primer Pa and Pb primer; 1U Kapa Taq polymerase (Kapa Biosystems, Boston, USA) and 200 ng of DNA.The thermal cycle profile and primers used in PCR are given in Table 1.

PCR products were digested by Bse*II* restriction enzyme (Biolabs, NewEngland) and analyzed on 10% polyacrylamide gel electrophoresis. Normal (77 and 21 bp) and mutant (98 bp) allele were separated based on the size of the restriction fragments. The DNA was visualized by silver staining (24).

Statistical analysis

The determination of Hardy-Weinberg equilibrium (Hardy-Weinberg equilibrium) was performed by using the online software, Hardy-Weinberg equilibrium calculator (http://www.oege.org/software/hardy-weinberg.html).

Results

Our study included 210 healthy subjects from the territory of Serbia. Genotyping of PAI-1 4G/5G was performed by PCR-RFLP method (Fig. 1). Our results showed that PAI-1 5G/5G genotype was present in 40 (19.05%) subjects, 97 (46.19%) were heterozygous carriers of PAI-1 4G/5G gene variant, while 73 (34.76%) subjects were carriers of homozygous PAI-1 4G allele. Allelic frequency of the 4G allele was 0.58 and frequency of the 5G allele was 0.42. The study group was in Hardy-Weinberg equilibrium (χ^2=0.59).

Discussion

In this study we determined the prevalence of PAI-1 4G/5G gene variant in healthy Serbian population. Our results showed that the frequency of 4G allele is 0.58, which is in concordance with the results obtained for the neighboring populations (17-19). In a study of Alfirevic et al. the same frequency of 4G allele was found in the Croatian population (17) (Table 2). Similar results were obtained by Spiroski et al. for the Macedonian population in a study involving 82 healthy subjects (40 women and 42 men; aged 40.7±11.3 years) (18). Nossikoff et al. examined the prevalence of PAI-1 4G/5G gene variant in Bulgarian patients with myocardial infarction (54 patients) and controls (85 healthy subjects).They showed that in a healthy Bulgarian population the frequency of 4G allele was slightly decreased (0.42) (19). In the Greek study, including patients suffered from premature myocardial infarction and healthy subjects, it has been shown that frequency of 4G allele is 0.52 in a healthy Greek population (20). On the other hand, in the population of Spain (21) and Italy (22) the 4G allele frequency was slightly lower (0.49 and 0.47, respectively).

All studies, including ours, have shown a high frequency of 4G allele in different populations (17, 18, 20). High frequency of 4G allele in the healthy population may indicate a potential protective role of this gene variant in certain disorders.

Some studies have shown that PAI-1 can reduce the diseases risk due to its protective action (5, 7).The presence of 4G allele may lead to increased levels of PAI-1 which can cause a reduction in proteolysis and plaque stabilization during the inflammatory process in the brain tissue. Local mechanisms within brain tissue may be involved in the protective effect of PAI-1 and thereby contribute to reduced risk of stroke (5). In the Dutch study, which included people aged 65 to 84 years, it has been shown that the presence of PAI-1 4G/4G genotype reduces the risk of stroke, transient ischemic attack, and death caused by the presence of cardiovascular disease (5). The inhibitory effect of PAI-1 4G/4G genotype on cell migration may lead to a reduced risk of coronary artery disease (7).

Our study shows that the prevalence of PAI-1 4G/5G gene variant in healthy Serbian population is similar to the neighboring populations. These results provide important information for future studies which will investigate potential role of PAI-1 4G/5G gene variant in pathogenesis of various disorders.

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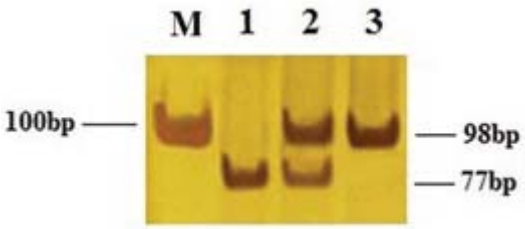
Table 1. The thermal cycle profile and primers used in PCR

Primer Pa (23)	5' CACAGAGAGAGTTCTGGCCACGT 3'
Primer Pb (23)	5' CCAACAGAGGACTCTTGGTCT 3'
Thermal cycle profile	94 °C/5 min (94 °C/30 sec, 61 °C/30 sec, 72 °C/30 sec) 33 ciklusa 72 °C/10 min

Table 2. Genotype and allelic frequency ofPAI-1 4G/5G gene variant in different populations

Genotype	Serbia	Italy	Macedonia	Croatia	Bulgaria	Greece	Spain
4G/4G (%)	34,76	24,36	24,4	32	18	20,3	21
4G/5G (%)	46,19	50,26	62,2	52	49	63,3	52
5G/5G (%)	19,05	25,38	13,4	16	33	16,4	27
Allelic frequency	Serbia	Italy	Macedonia	Croatia	Bulgaria	Greece	Spain
4G allel	0,58	0,49	0,55	0,58	0,42	0,52	0,47
5G allel	0,42	0,51	0,45	0,42	0,58	0,48	0,53

Figure 1. Polyacrylamide gel electrophoresis of the PAI-1 4G/5G gene variant



M- 100bp Ladder

1- PAI-1 5G/5G genotype

2- PAI-1 4G/5G genotype

3- PAI-1 4G/4G genotype

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