Effect of plasminogen activator inhibitor-1 4G/5G polymorphism in Turkish deep vein thromboembolic patients with and without prothrombin 20210 G-A

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ABSTRACT

Prothrombin 20210 G-A mutation is a common factor that predispose to thrombosis. The effect of the mutations in PAI-1 gene on the risk of thrombosis is controversial. We aimed to evaluate the role of these two polymorphisms in Turkish patients with deep vein thrombosis. Although there was no statistically significant difference in patient and control group for the distribution of PAI-1 4G/5G polymorphism in the present study, the risk of thrombosis increased from 3.4 fold to 8.4 in patients carrying PT20210 A and PAI-1 4G.

Key Words: Prothrombin 20210 G-A, PAI-1 4G/5G, Deep vein thrombosis.

ÖZET

Plazminojen aktivatör inhibitör-1 4G/5G polimorfizminin, protrombin 20210 G-A taşıyan ve taşımayan derin ven tromboembolili Türk hastalarda etkisi

Tromboza yol açan edinsel mutasyonlar içinde protrombin 20210 G-A en önemlilerindendir. PAI-1 polimorfizminin etkisi ise tartışmalıdır. Biz bu çalışmada, her iki mutasyonun derin ven trombozlu Türk hastalarında etkisini araştırdık. Kontrol grubu ile hasta grubu arasında PAI-1 4G/5G polimorfizm farkı saptanmazken PT20210A ve PAI-1 4G'yi birlikte taşımanın tromboz riskini 3.8'den 8.4 kata arttırdığını gösterdik.

Anahtar Kelimeler: Protrombin 20210 G-A, PAI-1 4G/5G, Derin ven trombozu.

INTRODUCTION

Several inherited factors that predispose to thrombosis have been identified[1]. The most common cause of thrombophilia is the G-A substitution at the nucleotide 1691 of Factor V gene leading a single amino acid alteration in one of the three cleavage sites, ie Arg instead of Gln at position 506. This common mutation causes activated protein C resistance[2]. Other common mutation is prothrombin 20210 G-A alteration which causes a "gain of function" in the coagulation system with an increase of prothrombin levels associated with an increased potential to form thrombin[3]. The prevalence of FV1691A and PT20210A varies among populations; it was reported between 7-10% and 2.6 in healthy Turkish population[4,5]. Although most of the case reports and series suggest that FV1691A and PT20210A are risk factors, data is controversial for the occurrence of deep vein thrombosis (DVT). Reason could be other genetic and/or environmental factors which may influence and determine the prothrombotic role of the FV1691 G-A and PT20210 G-A.

A decreased fibrinolytic activity due to increased levels of plasminogen activator inhibitor-1 (PAI-1) has been shown in patients suffering deep vein thrombosis[6]. Elevated plasma PAI-1 levels are associated with the 4G allele of a 4G/5G insertion/deletion polymorphism located in the promoter region 675 bp upstream from the transcription start sequence of the PAI-1 gene[7,8]. The thrombotic risk of carrying PAI-1 4G allele was found to be controversial in previous studies[7-15]. In older women it was suggested as an important contribution of PAI-1 in cerebrovascular pathology. PAI-1 4G/4G homozygotes had a markedly reduced risk of cerebrovascular mortality compared with PAI-1 5G/5G homozygotes[11]. However, it was not the case in pediatric stroke patients[13]. The discrepancy between two studies would lie on the explanation that PAI-1 may protect against destabilization of the atherosclerotic plaque in elderly patients which is not present in pediatric cases.

Although PT20210A mutation is one of the most common inherited risk factor of venous thrombosis, the prothrombotic potential of PT20210A is significantly milder than that verified for the factor V Leiden. Moreover, the absence of association with recurrent DVT, the identification of asymptomatic homozygous carriers and the great clinical variability between carriers of this polymorphism have led some authors to suggest that this polymorphism could not be considered as a genetic risk factor for DVT[16-19]. These data led to the assumption that additional prothrombotic risk and/or factors may be necessary for the clinical manifestation of thrombosis.

As there are no existing data in the Turkish population, we aimed to evaluate the role of these two polymorphisms in Turkish patients with DVT.

MATERIALS and METHODS

The case-control study included 90 patients with DVT. Eighty-nine healthy unrelated individuals from Ankara without family histories of thrombosis, stroke, or Behçet's disease were selected as a control group. DNA was extracted by conventional methods and polymerase chain reaction of the PAI-1 4G/5G polymorphism was performed according to previously described method by using primers 5'CACAGAGAGAGTCTGGCCACGT-3' and 5'-CCAACAGAGGACTCTTGGTCT-3'. Amplification was performed for 35 cycles with annealing temperature of 60°C (Ericomp, USA). Amplified 98/99-bp product was digested with Bse LI (Fermentas, Vilnius, Lithuania) at 55°C and subjected to 6% polyacrylamide gel electrophoresis[20]. FV1691 G-A and PT20210 G-A mutations were analyzed according to previously described techniques[2,21].

RESULTS

Genotype distributions of PAI-1 4G/5G and PT20210 G-A are given in Table 1 and Table 2. There was no statistically significant difference in patient and control group for the distribution of PAI-1 4G/5G polymorp-

Table 1. Distribution of PAI-1 4G/5G polymorphism in Turkish population

	Controls		Patients		
	n: 89	%	n: 90	%	
PAI-1[a]					
5G/5G	23	25.8	15	16.6	
5G/4G	48	53.9	46	51.1	
4G/4G	18	20.2	29	32.2	
PT[b]					
GG/86	86	92.9	78	65.11	1
GA/3	3	3.3	12	13.3	4.4 (CI)

[a] Allele frequency 4G controls: 0.50, [b] Allele frequency 4G patients s: 0.57 (p= 0.61)

Table 2. Distribution of PAI-1 4G/5G and PT 20210 G-A polymorphisms

PAI-1		Control	Patients	
-675 4G/5G	PT 20210 G-A	n: 89	n: 90	OR
5G/5G	G/G	22	13	1
4G/5G	G/G	47	41	1.47
4G/4G	G/G	17	24	2.4
5G/5G	G/A	1	2	3.4
4G/5G	G/A	1	5	8.46
4G/4G	G/A	1	5	8.46

hism (p= 0.61). There was a significant difference for the PT20210A mutation between both groups (p= 0.05). It is interesting that 4G/4G homozygotes have a 2.4 fold risk for DVT. Moreover, the risk of thrombosis increases from 3.4 fold to 8.46 in patients carrying PT20210A and PAI-1 4G (OR 2.5 to 0.5 CI) which is significantly important.

DISCUSSION

PAI-1 4G allele carriers have potentially decreased fibrinolytic activity constituting an additional prothrombotic factor[6-9]. Previous data indicated that carrying the PAI-1 4G allele either in heterozygous or homozygous state did not have an independent effect on the thrombotic risk but carrying 4G allele in homozygous state increased the risk in the presence of FV1691A[10]. Further another polymorphism -A844G at the PAI-1 promoter was also shown to have the same effect with FVL[22].

Identification of asymptomatic carriers and great clinical variability between carriers of genetic factors factor V Leiden and PT20210A is important for a more accurate risk assessment for venous thrombosis. Although there was no statistically significant difference in patient and control group for the distribution of PAI-1 4G/5G polymorphism in the present study, the risk of thrombosis increases from 3.4 fold to 8.4 in patients carrying PT20210A and PAI-1 4G. Our data indicated that, PAI-1 4G/5G polymorphism should be screened in carriers of FV1691A and PT20210G whether they have symptoms of thrombosis or not.

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