Protein Z G79A polymorphism in Turkish pediatric cerebral infarct patients

Türk çocukluk çağı serebral iskemi hastalarında protein Z G79A polimorfizmi

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Abstract

Objective: Protein Z (PZ) plays an enhancer role in coagulation as an anticoagulant. In this study G79A polymorphism was investigated in Turkish pediatric stroke patients.

Material and Methods: Ninety-one pediatric stroke patients with cerebral ischemia and 70 control subjects were analyzed for PZ G79A and also factor V Leiden (FVL) and prothrombin (PT) mutations.

Results: PZ 79 'A' allele in homozygous state was found in five patients (5.5%), while it was found in only one control subject (1.4%), and it appeared to be a risk factor for pediatric ischemia [OR=3.94 (0.44-35.1)]. When patients and controls who had FVL and PT carriers were excluded, AA genotype carried a risk [OR=3.88 (0.41-36.5)]. In addition, plasma PZ levels were measured in 21 stroke patients and 52 controls. Plasma PZ levels were not different between stroke patients (501,0 $ngml^{-1}$ ± 158,3 $ngml^{-1}$) and controls (447,3 $ngml^{-1}$ ± 166,0 $ngml^{-1}$). However, the plasma levels of PZ were decreased in patients with AA genotype. This is the first study in which G79A polymorphism was investigated in Turkish pediatric stroke patients

Conclusion: Our data showed that carrying 79 AA genotype could be a genetic risk factor for cerebral infarct in pediatric patients.(*Turk J Hematol 2008; 25: 133-5)*

Key words: Protein Z, PROZ, G79A polymorphism, pediatric stroke.

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Özet

Amaç: Protein Z (PZ)'nin koagülasyonu artırıcı yönde etkisinin yanısıra antikoagülant olarak da rol oynadığı gösterilmiştir. Bu çalışmada G79A polimorfizminin Türk çocukluk çağı iskemi hastalarındaki oynadığı rol incelenmek istenmiştir.

Gereç ve Yöntemler: İskemik infarktüs geçiren 91 çocuk inme hastası ve 70 kontrol G79A polimorfizmi ve Faktör V G1691A (FVL), Protrombin G20210A (PT G20210A) mutasyonları açısından incelenmiştir.

Bulgular: 'A' alleli beş hastada (%5,5) homozigot olarak bulunurken yalnızca bir kontrolde (%1,4) homozigotluğu saptanmış ve çocukluk çağı inme için risk faktörü olarak görünmektedir [OR=3,94 (0,44-35,1)]. FVL ve PT mutasyonlarını taşıyan hasta ve kontroller çıkartıldığında AA genotipi yine risk getirmektedir [OR=3,88 (0,41-36,5)]. Ayrıca 21 inme hastası

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ve 52 kontrolde plazma protein Z düzeylerine bakılmıştır. Hasta (501,0 $ngml^{-1}$ ± 158,3 $ngml^{-1}$) ve kontrollerde (447,3 $ngml^{-1}$ ± 166,0 $ngml^{-1}$) plazma protein Z düzeyleri farklı bulunmamıştır. Ancak AA genotipi taşıyan hastalarda plazma protein Z düzeyi düşük bulunmuştur. G79A polimorfizminin Türk çocukluk çağı iskemi hastalarındaki oynadığı rolü inceleyen ilk çalışmadır.

Sonuç: Sonuçlarımız, 79 AA genotipini taşımanın çocukluk çağı iskemik infarktüs hastalarında genetik bir risk faktörü olabileceğini göstermiştir. *(Turk J Hematol 2008; 25: 133-5)*

Anahtar kelimeler: Protein Z, PROZ, G79A polimorfizmi, inme

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Introduction

Protein Z (PZ) is a vitamin K-dependent glycoprotein with structural similarities with many serine proteases like coagulation factors VII, IX, X and protein C [1]. It is shown that thrombin can bind to the phospholipid surfaces in the presence of PZ but not in its absence [2]. It also acts as a cofactor of PZ-dependent protease inhibitor (PZI). PZI play a role in coagulation as an inhibitor of activated factor Xa. PZ enhances this inhibition and suppresses thrombus formation [3-5]. PZ is encoded by a 14 kb gene (PROZ) localized at 13q34 and consisted of eight exons and one alternative exon [6]. Several nucleotide substitutions have been reported in the PZ gene [7]. Although the associations among these substitutions, especially the G79A polymorphism, and PZ plasma levels were investigated in stroke [8-10], no study has been conducted in pediatric stroke. Thus, the aim of this study was to determine the role of G79A polymorphism in intron F of the PZ gene in Turkish children with ischemic infarct.

Materials and Methods

Ninety-one pediatric stroke patients with cerebral ischemia (42 female, 49 male) and 70 control subjects (35 female, 35 male) with no familial history of a vascular disease or thrombosis were included. Cerebral infarcts were detected by magnetic resonance imaging. None of the patients was under anticoagulant therapy at the time of the blood analysis. The G79A polymorphism of intron F of the PZ gene was analyzed according to a previously reported method [8] from the DNA bank of the Pediatric Molecular Genetics Department of Ankara University. 320 bp of the intron F of the PZ gene was amplified by polymerase chain reaction (PCR) using primers as forward 5'-TAACACCATAGACAGAGTCCGAT ATTCGC-3' and reverse 5'-ATGAACTCGGCATTAGAACATGGTTGGAA-3'. The G79A polymorphism, in intron F, was analyzed by KspAI (Fermentas, Lithuania) restriction endonuclease enzyme digestion. When A allele was present, PCR product was yielded in two fragments (221 bp and 99 bp) while G allele was not digested. Also, factor V (FV)1691 G-A and prothrombin (PT)20210 G-A mutations were analyzed with Light Cycler (Roche, Germany) method as reported before [11]. Plasma PZ levels were measured in 21 newly diagnosed stroke patients (presentation age 0.1-16 years, median: 4.9 years) after a 6-month period from the first attack and 52 age-matched controls (presentation age 0.3-18 years, median: 4.1 years) by using Protein Z Asserachrom enzyme-linked immunosorbent assay kits (France). Informed consent was obtained from each individual and/or her/his parents. Statistical analysis was 3 performed using SPSS software (SPSS Inc., Chicago, IL). The groups were compared using chi-square test.

Results and Conclusion

In this study, we investigated the role of the intron F G79A polymorphism of the PZ gene and its effects on expression of the plasma PZ levels in pediatric stroke patients. Firstly, genotype distribution of the G79A polymorphism was tabled between patient and control groups. The frequency of the A allele was higher in patients (0.242) than in controls (0.214); however, the difference was not significant (OR: 1.16; 95%CI: 0,68 to 1,98; p=0,57). Also, there was no significant difference among the stroke patients and controls for the distribution of GG and GA genotypes. Five patients had 'A' allele in homozygous state (5.5%); however, it was present in only one control subject (1.4%) (OR: 3.94; 95%CI: 0.44 to 35.1; p=0.62) (Table 1). Previous studies have stated that homozygosity for the A allele of the G79A polymorphism decreases the risk of cerebral ischemia. There was a negative association between ischemia and the A allele. Those investigators hypothesized that the G79A polymorphism of intron F of the PZ gene has a protective role against ischemia [8,9]. However,

Table 1. Distribution of PZ intron F G79A polymorphism in control and stroke patients

Intron F G79A	n	GG n (%)	GA n (%)	AA n (%)	A allele frequency	p	OR (95%CI)
Controls	70	41 (58.5)	28 (40.0)	1 (1.4)	0.214	0.57	1
Patients	91	52 (57.1)	34 (37.4)	5 (5.5)*	0.242	0.57	1.16 (0.68-1.98)

* p=0.62; OR: 3.94 (0.44-35.1)

Table 2. Distribution of PZ intron F G79A polymorphism after exclusion of FV1691A and PT20210A carriers

Intron F G79A	n	GG n (%)	GA n (%)	AA n (%)	A allele frequency	p	OR (95%CI)
Controls	57	34 (59.6)	22 (38.6)	1 (1.7)	0.210	0.65	1
Patients	65	35 (53.8)	26 (40.0)	4 (6.1)*	0.261	0.65	1.33 (0.73-2.41)

*p=0.57; OR: 3.88 (0.41-36.5)

our results conflict with these conclusions, and the 'A' allele in homozygous state was pointed out as a risk factor for pediatric ischemia. Also, in a recent study, a lower prevalence of the 'A' allele was shown in patients with hemorrhagic stroke when compared with ischemic stroke or controls [12].

The most common cause of thrombophilia is the G-A substitution at the nucleotide 1691 of FV gene which causes activated protein C resistance [13]. Another common mutation, PT20210 G-A alteration, is associated with an increased potential to form PT [14]. It was shown that FV1691 G-A and PT20210 G-A were associated with cerebral infarct risk in children independently [15]. In PZ G79A genotype studies, Lichy et al. [8] (2004) found no correlation between FV Leiden (FVL) and PT mutations with PZ genotypes in juvenile stroke patients. In our pediatric patient group, 17 (18.7%) of the 91 patients were heterozygous for the FV1691 G-A mutation. Ten (10.9%) of the patients carried the PT20210 G-A mutation. The frequency of the G79A polymorphism in stroke patients with FVL and PT mutations was 0.294 and 0.300, respectively, which was not significant when compared to controls. Although 4 out of the 5 patients with homozygous G79A polymorphism carried neither FVL nor PT mutations, 1 of them had G79A polymorphism and also FVL mutation. Because FV1691 G-A and PT20210 G-A mutations were additional risk factors for ischemia, they were excluded to evaluate the direct effect of the G79A polymorphism on pediatric stroke. [16] When patients and controls who had FVL and PT20210A carriers were excluded, carrying 79 AA genotype was determined as a possible risk factor for cerebral infarct (OR: 3.88; 95%CI: 0.41 to 36.5; p=0.57) (Table 2).

Plasma PZ levels were not different between stroke patients (501,0 $ngml^{-1}$ ± 158,3 $ngml^{-1}$) and controls (447,3 $ngml^{-1}$ ± 166,0 $ngml^{-1}$). In patients whose plasma PZ levels were measured according to genotype, AA genotype decreased the plasma levels of PZ, in concordance with the other studies in which the association between low plasma PZ levels and 79 AA genotype was demonstrated [8-10].

In conclusion, this is the first study of the G79A polymorphism in pediatric stroke patients. Our results did not correlate with the previous reports, which reported a protective role of G79A polymorphism in cerebral ischemia. According to our results, having 79 AA genotype and low plasma PZ levels may be risk factors for pediatric ischemia. This could be because of the differences between adult and childhood stroke, which was shown in previous studies [9]. Additionally, there might be an association between this polymorphism and ischemia in our pediatric patient group as a risk factor.

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