Factor V A4070G (His1299Arg) mutation in Turkish pediatric patients with thrombosis

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ABSTRACT

The role of the prothrombotic mutations in pediatric thrombosis are being investigated. Mutations in the factor V gene (FV G1691A and FV A4070G) leading to activated protein C resistance, the main pathological condition in thrombosis and prothrombotic mutations contributing to thrombosis have been identified. The aim of this study is to reveal the role of FV A4070G mutation in pediatric thrombosis. 314 patients with thrombosis at pediatric age including also 111 stroke patients and 127 healthy controls were included to the study. The FV A4070G mutation was evaluated independently and also the combined effects with other prothrombotic mutations were also investigated. In pediatric stroke group FV A4070G was found not to be a risk factor with an OR of 1.04 (CI 95%: 0.56-1.93, p= 0.884). Further studies are needed to study the role of FV A4070G with other risk factors belong to HR2 haplotype.

Key Words: Thrombosis, Stroke, FV G1691A mutation, FV A4070G mutation, Prothrombin G20210A mutation.

ÖZET

Türk pediatrik trombozlu hastalarda Faktör V A4070G (His1299Arg) mutasyonu

Pediatrik trombozda protrombotik mutasyonların rolü araştırılmaktadır. Faktör V geninde, trombozda başlıca patolojik bir durum olan aktive protein C direncine neden olan mutasyonlar (FV G1691A ve FV A4070G) ile tromboza katkıda bulunan bazı protrombotik mutasyonlar belirlenmiştir. Bu çalışmanın amacı pediatrik yaş grubundaki bireylerde FV A4070G mutasyonunun rolünü ortaya koymaktır. Çalışmaya 111'i inme tanılı 314 pediatrik yaştaki trombozlu birey ile 127 sağlıklı kontrol dahil edilmiştir. FV A4070G mutasyonu diğer protrombotik mutasyonlarla ve bunlardan bağımsız olarak değerlendirilmiştir. Pediatrik inme grubunda FV A4070G mutasyonunun bir risk faktörü olmadığı belirlenmiştir (OR: 1.04, CI %95: 0.56-1.93, p= 0.884). FV A4070G mutasyonunun rolünü araştırmak için HR2 haplotipine ait diğer polimorfizmlerin de çalışılması gerekmektedir.

Anahtar Kelimeler: Tromboz, İnme, FV G1691A mutasyonu, FV A4070G mutasyonu, Protrombin G20210A mutasyonu.

INTRODUCTION

A common mutation in the factor V (FV) gene (G1691A) was found to be an inherited risk factor causing thrombophilia resulting in a protein with an altered aminoacid sequence at a critical cleavage site and an altered function, named as Factor V Leiden (FVL). This site is cleaved by activated protein C (APC) in normal coagulation mechanism, but due to a delayed cleavage, FVL mutation leads to thrombus formation and creates a protein that is resistant to APC[1].

A4070G (FV His1299Arg) polymorphism in exon 13 of the FV gene (this allele is part of a haplotype named HR2) has been shown to influence circulating FV levels and contribute to the APC resistance phenotype[1-3]. Bernardi et al have defined the HR2 haplotype with six polymorphisms[4]. This haplotype has been found to be frequent in healthy individuals originating from Italy, India, Somali and Turkey (0.08-0.21). These data reveales that HR2 represents a very ancient set of mutations older than FVL, dating back to a time antecedent the migration of man out of Africa[5,6].

His1299Arg polymorphism is also associated with an imbalance between the two functionally different forms of FV with an increase in the more thrombogenic form[7,8]. Compound heterozygosity of FVL and His1299Arg resulting in a significant resistance to APC has been reported[9]. In HR2 carriers with venous thromboembolism (VTE), FV levels has been found to be decreased compared to non-carriers. The decrease in FV levels can be attributed to the low levels of FV mRNA that has the HR2 haplotype. Similar studies have indicated that the HR2 haplotype is associated with decreased FV activity and a low APC normalized ratio[4,10]. It has been found that there is a significant difference in the frequencies of the R2 allele between the cases with VTE and controls with an odds ratio (OR) of 1.8. And it has also been concluded that there is an increased risk of thrombosis in carriers of the R2 allele. To confirm this findings, it has been suggested that the risk of thrombosis when other risk factors of VTE are excluded, the R2 allele might be significantly important. While HR2 haplotype increases the risk of VTE in carriers with FVL by approximately three fold, no association between VTE and HR2 in non-carriers of FVL has been found[6]. On the contrary, it has also been reported that HR2 is not associated with an increased risk of VTE and with the decreased normalized APC ratios[1]. On the other hand, a significant association between the HR2 haplotype and the occurrence of vein thrombosis has been reported[11].

The frequency of FVL and His1299Arg (A4070G) in Turkish population has been found to be 9.8% and 8.5% respectively[12,13]. Previously, we reported that FV His1299Arg polymorphism is not an independent risk factor in thrombosis but it can be important when associated with FVL in VTE[13]. Those results are in accordance with the previously reported studies[6,9].

A polymorphism in exon 25 of the FV gene has been found to be linked with HR2 haplotype. This substitution, Asp2194Gly (A6755G) in the C2 domain of the FV molecule has been claimed to influence domain folding and glycosylation of FV[14]. Later, these findings have been confirmed with a strong linkage of FV A6755G polymorphism with the R2 allele[15]. The HR2 haplotype has been suggested to have a direct effect on FV synthesis, secretion or stability, because the promoter region of the FV gene has no functional variations linked to the R2 allele. The HR2-linked Asp2194Gly mutation has been considered as a functional determinant in the association of the FV R2-allele with moderately reduced FV levels[16].

Pediatric thrombosis patients who carry the HR2 haplotype were not previously analysed. Here we present our compiled data on R2 haplotype in our Turkish pediatric thrombosis patients.

MATERIALS and METHODS

We studied A4070G polymorphism in exon 13 of the FV gene in a case-control study. Our patient group included pediatric thrombosis patients (n= 314). Of which 111 were pediatric stroke with arterial origin. 14 of the patients were diagnosed as deep vein thrombosis. The rest was mentioned in the 'results' section. The study group also included 76 patients with thrombosis with unknown origin. The control group (n= 127) included individuals without a family history of thrombosis and stroke. A written consent was obtained from each individual and/or from one of his/her parents. The mean age of the pediatric thrombosis group was 8.5 while the age range of the control group was 18-60. DNA was extracted by conventional phenol-chloroform method. Amplification of exon 13 of the FV gene was performed by polymerase chain reaction (PCR). 703 bp fragment was amplified to analyse A4070G (His1299Arg) polymorphism of the FV gene, with the use of the primers [forward primer 5'-CAAGTCCTTCCCCACAGATATA-3' and reverse primer 5' GGTTACTTCAAGGACAAAATACCTGTAAAGCT-3'], with an initial denaturation at 95°C 5', denaturation at 95°C 1', annealing at 57°C 1', extension at 72°C 1' and final extension at 72°C 7' for 35 cycles. PCR products were restricted with Rsa I (New England Biolabs, USA). The results were evaluated in 2% agarose gel electrophoresis by ethidium bromide staining. A normal/non-carrier individual carried only a 492 bp fragment, a heterozygous individual carried a 492 bp with a 211 bp fragment and a homozygous individual carried only a 211 bp fragment[1]. FVL was analysed according to previously reported techniques[12,17].

Statistical Analyses

Differences between groups were analysed with Chi-square and Mann-Whitney U test. Unmatched odds ratio and 95% confidence intervals as an estimate of the relative risk of the allele frequency were calculated in the entire study population. The 95% confidence intervals were calculated from a conditional logistic-regression algorithm by the maximum likelihood method.

RESULTS

A list of the pediatric thrombosis cases with underlying diseases and pediatric stroke cases studied were shown below.

Deep vein thrombosis	14
Behcet's disease	7
Budd chiari syndrome	8
Intracardiac thrombosis	25
Malignancies	11
Retinal artery thrombosis	3
Portal vein thrombosis	12
Sinus vein thrombosis	4
Venous occlusive disease during bone marrow transplantation	4
Disseminated intravascular coagulation (DIC)	6
Renal vein thrombosis	1
Postsplenectomy thrombosis	9
Venous catheter thrombosis	19
Protein S deficiency	1
Protein C deficiency	3
Stroke	111

The distribution of FVL in both pediatric thrombosis and pediatric stroke group was shown in Table 1. Among 314 patients, 41 (13.05%) were found to have FV A4070G in heterozygous state. Of the 111 pediatric stroke patients 19 (17.1%) were heterozygous and 2 (1.8%) were homozygotes. The majority of the pediatric thrombosis group had stroke. However polymorphism was not a risk factor in these cases. The two homozygous individuals were diagnosed as stroke and both of them had prothrombin 20210A mutation. In pediatric stroke group FV A4070G was found not to be a risk factor with an OR of 1.04 (CI 95%: 0.56-1.93, p= 0.884) (Table 1).

Table 1. The distribution of A4070G polymorphism and FV G1691A mutation in Turkish pediatric thrombosis patients. A total of 441 people were tested for A4070G and FV G1691A mutations by using the set of primers designed by Luddington et al[1]. The distribution of A4070G polymorphism was shown

		4070 A-G		1691 G-A		1691A/4070G	
	n	n	%	n	%	n	%
Controls	127	20 (3)[1]	15.7	9	7.08	1	0.78
Patients[2]	203	22	10.8	35 (5)	17.2	1	0.49
Stroke	111	19 (2)	17.1	24 (1)	21.6	1	0.9
DVT	14	1	7.1	2	14.2	-	-
ICT	25	3	12	5	20	1	4

[1] Figures between parenthesis show the homozygotes.
[2] The patient group also includes the DVT and ICT group.

We also analysed our data for the combination of His1299Arg polymorphism and FVL. This was presented in Table 2. The allele frequencies of FVL and HR2 haplotype were assessed in the pediatric thrombosis, pediatric stroke and control groups. The frequency of FVL A allele was 0.035 to 0.098 in our control and pediatric patient groups, respectively. The frequencies of R2 allele in the control, pediatric thrombosis and pediatric stroke groups were found to be 0.090, 0.054 and 0.094, respectively (Table 3).

DISCUSSION

Although several reports on HR2 haplotype has been reported, all focused on vein thrombosis and none of them stated the haplotype as a possible health problem in populations where FV1691 A and HR2 haplotype are prevalent.

Frequency of mutations FV G1691A and FV A4070G in Turkish population is high (9.8% and 8.5% respectively)[12,18,19]. So, we studied extensively this haplotype in our population. The high heterozygous freuquency of FVL is an interesting finding as stroke is an arterial type of thrombosis and the previously reported studies all focused on vein thrombosis and there is no compiled or exact data on arterial thrombosis.

We have previously reported 26 FV 1691A carriers among 129 adult thromboembolic patients, six out of which had FV4070G mutation in combination; with a 6.7 fold risk compared to controls. It is interesting that of these six patients, two patients carried prothrombin 20210A mutation and one patient had protein C deficiency at the same ti-

Table 2 . The risk assessment of factor V G1691A and A4070G mutations in control and patient groups. The OR for pediatric thrombosis and pediatric stroke patients was calculated according to the mutation analysis results. The OR for pediatric thrombosis patients is 0.93 implying that this polymorphism is not a risk factor

		Control (n= 127)	%	Patients (n= 314)	%	OR	CI (95%)
G/G	R1R1	99	77.9	216	68.8	0.88	0.64-1.2
G/G	R1R2	16	12.6	37	11.78	0.93	0.5-1.74
G/G	R2R2	3	2.36	2	0.63	0.27	0.04-1.63
G/A	R1R1	8	6.3	51	16.2	2.57	1.18-5.5
G/A	R1R2	1	0.78	2	0.63	0.8	0.07-9
A/A	R1R1	-	-	6	1.91	-	-

Table 3. The frequencies of R2 allele in the control, pediatric thrombosis and pediatric stroke groups. The p values show that the difference between control, pediatric thrombosis and pediatric stroke patients is not significant

	n	4070G allele frequency	p
Controls	23/254	0.090	-
Pediatric thrombosis	22/406	0.054	0.089
Pediatric stroke	21/222	0.094	0.884

me. Two of the PT 20210A carriers had mesenteric artery thrombosis. The other three had clinical presentation of cerebral infarct, vascular greft thrombosis and Budd Chiari Syndrome[18,20]. Protein C deficient patient was a four-year-old female child with the diagnosis of cerebral thrombosis[20]. This data lead us to study the distribution of FV His1299Arg mutation in fortysix Turkish pediatric cerebral infarct patients below the age of 18[21]. Ten of the patients have been found to carry FV His1299Arg mutation (21.7%), one being homozygous. The cerebral infarct risk for FV 1299 has been found to be 2.5 (CI 95% 0.9-7.2) for all groups[21]. When all underlying possible conditions were excluded, the incidence of FV4070 mutation increased to 33.3%. The risk has also increased to 3.9 (CI 95% 1.2-12.3) indicating a possible role of R2 haplotype in the pathogenesis of the stroke in pediatric patients[21].

Our previous studies in Turkish population revealed FV A4070G heterozygous frequency as 8.5%; if combined with FV 1691A, they conferred a 3-to-6 fold increase in the relative risk of VTE compared with FV1691A alone[18]. Such a high prevalence and the relative risk of this polymorphism in Turkish population led us to determine the frequency of the polymorphism in a large series of Turkish pediatric thrombosis patients.

Our present data showed once more that FV A4070G polymorphism is not an independent genetic risk factor but it can be significant together with FVL mutation[18].

Recently, Bertina et al reported the influence of Asp2194Gly substitution which is in linkage disequilibrium with R2 haplotype, on FV levels, domain folding and glycosylation of Asn2181 using recombinant FV molecules[16]. The authors concluded that Asp2194Gly mutation apart from other R2 haplotype polymorphisms, is a functionally important determinant for decreased FV levels in R2-FV gene carriers. In compound heterozygous FVL/R2 carriers, the increased APCR phenotype is caused by the reduced expression of R2-FV[16]. Castoldi et al put forward the suggestion that the carriership of the R2-FV gene affect the ratio of two functionally different forms of FV[7]. These authors also concluded that the R2 haplotype and R2-linked exon 25 A6755G polymorphism could be candidate markers for prospective studies aimed to understand the role of R2-FV gene in several thrombotic disorders [22-24].

We agree with this suggestion that in R2 haplotype carriers the A6755G polymorphism in the exon 25 of the FV gene must be studied in thrombotic events. As a conclusion, to understand the influence of this haplotype in the coagulation pathway, this polymorphism should be studied with other possible genetic risk factors or other polymorphisms that belongs to R2 haplotype.

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