
R506Q (FV Leiden) and R485K Mutations in the Factor V Gene: Incidence in Deep Venous Thrombosis and Hemophilia A Patients

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ABSTRACT

R506Q (FV Leiden) mutation in exon 10 of the factor V (FV) gene is highly prevalent in European populations and it has been suggested that the coinheritance of FV Leiden mutation may be an important modifier of hemophilia A phenotype. One other substitution R485K in the same exon, with no functional consequences in vitro, is significantly higher in Thailand and has been associated with thrombophilia. In order to see if any correlation exists between R506Q and hemophilia phenotype and between R485K and thrombosis in Turkish patients, DGGE analysis of exon 10 of the FV gene is carried out among deep venous thrombosis (DVT) and hemophilia A patients. Our results indicate that the allelic frequency of the R485K polymorphism is similar to the frequency detected in Europe, and apparently, is not associated with an increased risk of thrombosis in the Turkish population. It is also not possible to show a modifier effect of FV Leiden on hemophilia A phenotype among the limited number of patients included in this study.

Key Words: Mutation, Factor V Leiden, R485K, Deep venous thrombosis, Hemophilia A.

ÖZET

Faktör V Geninde R506Q (FV Leiden) ve R485K Mutasyonları: Derin Venöz Tromboz ve Hemofili A Hastalarında İnsidans

Faktör V (FV) geninde ekson 10'da görülen R506Q (FV Leiden) mutasyonu Avrupa topluluklarında sıklıkla görülmektedir ve bu mutasyonun birlikte kalıtımının hemofili A fenotipini modifiye ettiği düşünülmektedir. Aynı eksonda görülen ve fonksiyonel önemi olmayan diğer bir aminoasit değişikliği, R485K, Tayland'da önemli ölçüde sık bulunmakta ve trombofili ile ilişkili olduğu düşünülmektedir. R506Q ile hemofili A fenotipi arasında ve

R485K ile tromboz arasında bir korelasyonun varlığını göstermek için bir grup DVT ve hemofili A hasta grubunda FV geninin 10. eksonu DGGE ile analiz edilmiştir. Elde edilen bulgular Türk toplumunda R485K polimorfizminin alel sıklığının Avrupa topluluklarında görülen sıklıkta olduğunu ve tromboz riskindeki artışla ilişkili olmadığını göstermektedir. FV Leiden mutasyonunun ise hemofili A fenotipini modifiye eden bir etkisinin olup olmadığını bu çalışma kapsamına alınan kısıtlı hasta sayısı üzerinde gösterme olanağı olmamıştır.

Anahtar Kelimeler: Mutasyon, Faktör V Leiden, R485K, Derin venöz tromboz, Hemofili A.

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INTRODUCTION

Venous thrombosis is a serious clinical condition strongly associated with life threatening pulmonary embolism, which affects 1/1000 individuals every year. Its pathogenesis is multifactorial involving the genetic factors such as mutations affecting proteins of the anticoagulation mechanisms namely antithrombin, protein C, and protein S and an inherited resistance to the anticoagulant action of APC called activated protein C resistance (APCR)^[1]. More than 85% of cases APCR is caused by a single point mutation in the FV gene, a G→A transition at nucleotide position 1691, resulting in an amino acid substitution in exon 10 of the FV gene. The mutation, R506Q, replaces arginine with glutamine at activated protein C (APC) cleavage site and renders the mutant FVa resistant to cleavage by APC^[2]. In its heterozygous form it afflicts affected individuals with a life-long hypercoagulable state and a 5-10 fold increased risk of thrombosis and homozygous cases have 50-100 fold increased risk of thrombosis. World distribution of FV Leiden mutation varies among populations and it has a high prevalence, ranging between 5-10% in European countries where the carrier rate reaches up to 20%, but it was not observed in populations from Asia, Africa and Middle East^[3-6]. The Turkish population displays a high allelic frequency of 4.5-4.9% and a carrier rate of about 10%^[7,8]. Haplotype analysis using 4 polymorphisms show that the FV Leiden mutation occurs on a single exon 13 haplotype suggesting that its primary origin is in a founding population in Europe and is carried to other parts of the

world by migration^[9]. Although genetic drift cannot be ruled out for its persistence in the population, FV Leiden mutation might have provided some selective advantage possibly by reducing blood loss in the absence of medical care. It is also suggested that the coinheritance of FV Leiden mutation may be an important modifier of hemophilia A phenotype since certain identical mutations in the Factor VIII gene are associated with variable phenotypes resulting in severe to mild/moderate hemophilia A^[10-13].

A polymorphism 1628G→A (R485K) close to the site of FV Leiden mutation has been described in Gandrille et al and its allele frequency was shown to be 32.4% in Sub-Saharan Africans, 32.7% in West-Indians, 6.9% in Europe and 59% in the Thai population^[14-16]. Interestingly, this polymorphism was suggested to be a risk factor for thrombosis in the Thai population since the allele frequency is elevated to 80% in thrombosis patients^[16].

In this study, the prevalence of FV Leiden mutation and R485K polymorphism were studied among DVT and hemophilia A patients and apparently healthy individuals to see if R485K is a risk factor for thrombosis and if FV Leiden is a modifier of hemophilia A phenotype.

MATERIALS and METHODS

Peripheral blood samples from 66 DVT, 76 hemophilia A patients and 86 apparently healthy individuals (132, 152 and 172 chromosomes, respectively) were collected in K₂EDTA containing tubes and genomic DNA was extracted by a salting out procedure^[17].

The diagnosis of hemophilia A was based on clinical and hematological data. Factor VIII clotting activity was measured by one-stage clotting method (Diagnostica-Staga) and the clinical criteria of Eyster et al were used to determine disease severity^[18].

FV exon 10 in genomic DNA samples were amplified by PCR using primer pair: upstream GC-clamped 20-mer, FVex10A and downstream 20-mer, FVex10B as described by Gandrille et al, 1995^[14]. PCR products were obtained in 25 µL volume containing 2.5 pmole of each primer pair, 1X Mg⁺² free reaction buffer, 2 mM of Mg⁺² solution, 0.2 mM of each of the four dNTPs, 0.5 U of Taq DNA polymerase. The PCR was performed with the thermocycle program of 94°C for 5 minutes; 30 cycles at 94°C for 1 minute, 52°C for 30 seconds, 72°C for 1 minute and final extension at 72°C for 7 minutes.

DGGE was performed as described by Gandrille et al, 1995 by applying the PCR products to electrophoresis on a 10 to 60% denaturing gradient polyacrylamide gel for 3 hours at 160 volts using a vertical-slab unit (Model SE600; Hoefer Amersham Pharmacia Biotech, Piscataway, NJ, USA)^[14]. DGGE band resolution was increased by forming heteroduplexes by denaturing PCR products at 94°C for 10 minutes followed by 30 minutes reannealing of the DNA strands at 48°C.

DNA sequence analysis was carried out manually using a DNA sequencing kit (T7 Sequenase v2.0; Amersham Life Science, Piscataway, NJ, USA) and a sequencing apparatus (Model S2; BRL, Carlsbad, CA, USA). FVex10B primer was used for sequencing.

RESULTS and CONCLUSIONS

All 142 DVT and hemophilia A patients and 86 apparently healthy individuals were tested for the presence of R506Q mutation and R485K polymorphism. The PCR amplification followed by DGGE analysis of exon 10 of the FV gene was the method of choice since it was possible to observe both mutations (Figure 1). Among all individuals tested FV Leiden homozygotes were detected only in two DVT patients. The presence of FV Leiden mutation detected by the DGGE pattern was also confirmed by DNA sequence analysis in a subset of individuals studied.

Table 1 shows the G1691A (FV Leiden) and G1628A (R485K) allele frequencies in healthy individuals, DVT and hemophilia A patients. The allele frequency of R485K poly-

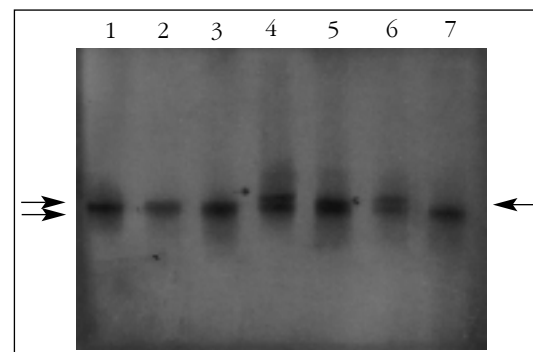


Figure 1. DGGE patterns showing the two different heterozygote patterns of exon 10. Lanes 1-3 and 7 show a normal homozygote subject, lane 4 and 6 a heterozygote subject for the R506Q mutation and lane 5 a heterozygote individual with the R485K polymorphism, respectively. Arrows indicate the three alleles.

Table 1. The allele frequencies of G1691A (FV Leiden) and G1628A (R485K) mutations in the FV gene of healthy individuals and patient groups

	Chromosome no	No of chromosomes with R506Q	R506Q allele frequency (%)	No of chromosomes with R485K	R485K allele frequency (%)
Healthy	172	8	4.7	8	4.7
DVT	132	14	10.6*	9	6.8
Hemophilia A	152	9	5.9	6	3.9

*Significant at 0.05 level (0.01 < p < 0.001).

morphism was found to be 4.7 revealing that the frequency of the R485K polymorphism in Turkey is much lower than the frequency detected in African or Asian populations but is closer to the frequency detected in Europe. The R485K allele frequency was elevated in DVT patients and slightly lowered in hemophilia A patients, compared to the healthy individuals. However, the difference in any of the patient groups was not significant at 0.05 level suggesting that a correlation between this polymorphism and the incidence of thrombosis in Turkish patients does not exist as is shown for the Thai population. The results confirmed the previous allelic frequency of FV Leiden mutation (R506Q) in the Turkish population and its significant role as a risk factor for thrombosis in Turkish patients since χ^2 analysis showed that the allelic frequencies were significantly different at the 0.05 level only in DVT patient group.

The distribution of FV Leiden heterozygotes among individuals mild to severely affected with hemophilia A revealed no indication of a modifier effect of the FV Leiden mutati-

on in hemophilia A phenotype (Table 2). A better test for the hypothesis that the coinheritance of FV Leiden mutation may be an important modifier of hemophilia A phenotype could be to see if patients with identical mutations have differences in their hemophilia A phenotype and FV Leiden genotype. In our group of hemophilia A patients two mutations, namely Arg336stop and Arg2163His, recurred in two and three unrelated patients, respectively. Both patients with Arg336stop mutation had a severe clinical phenotype and they both did not carry the FV Leiden mutation. However, out of the three patients with Arg2163His mutation, two had moderate and one had a severe clinical phenotype. Only one patient with moderate phenotype was a FV Leiden carrier (Table 3). Obviously, the data is not sufficient to conclude whether the variable phenotypic expression of Arg2163His mutation was due to a modifier effect of the FV Leiden mutation.

In conclusion, the frequency of R485K polymorphism is found to be 4.7 in the Turkish population and it is not a risk factor for

Table 2. The distribution of FV Leiden heterozygotes among hemophilia A patients

Hemophilia A phenotype	Number of patients	Number of patients heterozygous	
			for FV Leiden
Severe	41		6
Moderate	26		3
Mild	9		-
Total	76		9

Table 3. Identical hemophilia a mutations in unrelated individuals and their phenotypic effect with relation on the FV Leiden phenotype

Family no	Base change	Amino acid change	Phenotype	R506Q (FV Leiden)
8HA	C1063T	Arg336stop	Severe	No
99HA	C1063T	Arg336stop	Severe	No
55HA	G6545A	Arg2163His	Moderate	Yes
104HA	G6545A	Arg2163His	Severe	No
119HA	G6545A	Arg2163His	Moderate	No

thrombophilia. The observed frequency of the R485K polymorphism supports previous findings in the FVIII and FIX genes that the frequency of polymorphisms in these genes is similar to the populations in Europe^{19,20}. For a comprehensive analysis of the modifier effect of the FV Leiden mutation more hemophilia A patients with recurrent mutations but variable phenotypes need to be studied.

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