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NO SIGNIFICANT ASSOCIATION BETWEEN THE PROMOTER REGION POLYMORPHISMS OF FACTOR VII GENE AND RISK OF VENOUS THROMBOSIS IN CANCER PATIENTS

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Factor VII (FVII) plays an important role in blood coagulation. The role of common polymorphisms influencing the FVII plasma levels in thromboembolic disorders has been evaluated but there is no published data related to the effect of *FVII* gene polymorphisms on the venous thrombosis risk in cancer. *Aim*: To investigate the association of three common functional polymorphisms in the promoter region of *FVII* gene: a decanucleotide insertion at position -323 (-323ins10-bp), a G to T substitution at position -401 (-401GT), and a G to A substitution at position -402 (-401GT) with venous thrombosis in cancer patients. *Materials and Methods*: The study included 60 cancer patients with venous thromboembolism (VTE) (group 1) and 130 cancer patients without VTE (group 2). Genotyping of -323ins10-bp, -401GT, and -402GA polymorphisms in the promoter region of *FVII* gene was performed by the method of single-strand conformation polymorphism analysis and sequencing. Factor V Leiden (FVL) was also determined in all cases. *Results*: The frequency of FVL was significantly greater in cancer patients with VTE compared with group 2 patients ($p < 0.0001$). For each polymorphism of *FVII* gene, the distributions of genotypes and allele frequencies were not significantly different between two groups of patients ($p > 0.05$). The results did not change significantly after the exclusion of patients carrying the FVL ($p > 0.05$). *Conclusions*: The screening for the -323ins10-bp, -401GT, and -402GA polymorphisms of *FVII* gene did not contribute to a meaningful diagnostic investigation in cancer patients with venous thrombosis.

***Key Words*: cancer, factor VII, gene, polymorphism, promoter region, thrombosis.**

Several hereditary risk factors for venous thromboembolism (VTE) have been identified [1]. The polymorphic variants of genes encoding coagulation factors have been investigated as risk factors for venous thrombosis [2]. Among the inherited clotting abnormalities, factor V Leiden (FVL) is the most common cause for venous thrombosis. It is known that factor VII (FVII) plays an important role in the extrinsic pathway of blood coagulation. Its coagulant activity is identified as a potential risk factor for venous or arterial thrombosis. Several studies have provided the evidence for associations between some common polymorphisms of *FVII* gene and FVII blood levels [3–8]. However, other authors have not supported this observation [9].

Three common polymorphisms of the promoter region in *FVII* gene locus; a decanucleotide insertion at position -323 (-323ins10-bp), a G to T substitution at position -401 (-401GT), and a G to A substitution at position -402 (-402GA) have been described and reported to be associated with FVII blood levels. The -323ins10-bp has been extensively studies in relation to FVII plasma level (3–6, 9). It is proposed that -323ins10bp polymorphism may influence the rate of transcription of *FVII* gene. The -323ins10bp is functionally relevant; the rare insertion allele of 10-bp reduces the promoter activity, as compared with the common allele [10]. This insertion allele is related to low blood levels of FVII. According to van't Hooft *et al.* [4] study, -401GT polymorphism is strong linkage disequilibrium with the -323ins10-bp polymorphism.

The -401 T allele was associated with significantly lower plasma levels of FVII than the common -401 G allele, but the rare -402 A allele was associated with significantly higher FVII levels than the common -402 G allele.

On the other hand, there has been controversy whether the genetic variations in blood FVII levels influence the development of VTE [8, 11, 12]. High blood levels of FVII may be related to hypercoagulable state and certain mutations in *FVII* gene locus contribute to the variability in plasma FVII activity. Several studies have failed to find any association of *FVII* gene polymorphisms and venous thrombosis, but not arterial thrombosis [7, 8, 11–13]. A few reports have suggested that those alleles associated with low levels of FVII could play a protective role against myocardial infarction [5, 6]. Other studies, however, failed to detect such as influence [14, 15].

Cancer patients have an increased risk of VTE. Recently we have demonstrated the significant association between FVL and the risk of VTE in patients with cancer [16, 17]. Whether the polymorphism of *FVII* gene is related to an increased risk of VTE in cancer is still an open question. There are only a few published studies about the effect of *FVII* gene polymorphisms on the risk of idiopathic venous thrombosis [7, 8, 11–13]. The published studies have not included in cases with secondary VTE due to acquired risk factors including malignancy. Koster *et al.* [11] reported the first study

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Abbreviations used: CI – confidence intervals; DVT – deep venous thrombosis; FVII – factor VII; FVL – factor V Leiden; OR – odd ratio; SSCP – single-strand conformation polymorphism; VTE – venous thromboembolism.

that examined the risk of venous thrombosis in relation to Msp1 polymorphism of *FVII* gene. The polymorphism was not associated with the risk of deep venous thrombosis (DVT). Besides, no significant association of FVII plasma level with DVT was found.

The thrombotic role of several polymorphisms of genes encoding haemostatic factors has been investigated in cancer patients [16, 17]. Although FVL is an important risk factor for venous thrombosis in cancer, the findings are controversial. If the FVII plays a role as a risk factor for VTE, it can be important to investigate the association between its functional polymorphism and venous thrombosis in cancer patients. In light of the postulation, we have evaluated the possible correlation of promoter region polymorphisms of *FVII* gene with risk of VTE in cancer patients.

MATERIALS AND METHODS

The study population has been described previously [16, 17]. Briefly, two groups of cancer patients were enrolled in the study. Group I consisted of 60 cancer patients with VTE (one patient had thrombosis of the axillary and subclavian vein; two had pulmonary embolism without DVT; other patients had DVT of the lower extremity). Tumor location in this group was as follows: breast (n = 16), lung (n = 17), larynx (n = 2), brain (n = 2), digestive system (n = 11), genitourinary system (n = 3), extremities sarcoma (n = 4), other (n = 5). Group 2 was composed of 130 cancer patients with tumors of breast (n = 76), lung (n = 24), digestive tract (n = 6), larynx (n = 3), brain (n = 5), sarcoma (n = 5), genitourinary system (n = 3), others (n = 8), who had no history of thromboembolic disease during the cancer therapy or follow-up period. In general, tumor stages according to TNM classification were similar in both groups. For example, the distribution of tumor stage for breast cancer in groups 1 and 2 was as follows: early stage (stage I/II) in 8 patients and advanced stage in 8 patients in group 1; early stage in 39 and advanced stage in 37 patients in group 2. All patients with breast cancer in two groups had invasive tumors. The lung cancer patients in both groups had advanced stage of disease. Five of 11 patients with digestive system cancer in group 1 had metastasis. Three of 6 patients with digestive system cancer in group 2 had stage 4 cancer. Before the collection of peripheral blood, all patients gave informed consent to participate in this study. Ethical committee approval was obtained for molecular studies on thrombosis.

DNA was isolated from peripheral blood lymphocytes by the standard phenol-chloroform method. Genomic DNA was amplified by polymerase chain reaction (PCR). Genotyping was performed by single-strand conformation polymorphism (SSCP) analysis and sequencing of all identified patterns. The primer design was based on the sequence of the promoter region of *FVII* gene. The 323ins10-bp, -401GT, 402GA polymorphisms in the promoter region of *FVII* gene were amplified [3, 4, 10, 18]. The PCR reaction started with 5 min at 95 °C and was continued by 34 cycles of 94 °C/1 min, 60 °C/1 min, 72 °C/1 min, and final extension of 10 min at 72 °C (Biometra, Germany). The PCR samples were run on 2% agarose gel.

SSCP was carried out using the following primers: F 5'-GGC CTG GTC TGG AGG CTC TCT TC-3'; R 5'-CGC TGG CAA CAA AAC CGT CCG CTC-3' [15, 18]. The amplified DNA fragment was 214 bp for SSCP. The PCR products were denatured at 99 °C for 7 min and then the resulting single-stranded DNA was loaded on 8% polyacrylamide gel. Electrophoresis was performed with a sequencing apparatus at 130 V of constant power at 4 °C for 10 to 12 h depending on the fragment size. After electrophoresis, gel was silver stained and was visualized under ultraviolet light.

A 315-bp DNA fragment was amplified for DNA sequencing using the following specific primers: F 5'-GTA AGA TGT GGA CCG CTG GA-3' and R 5'-ACA AAA CCG TCC GCT CTG-3'. PCR was carried out after choosing the different band profiles of SSCP analysis.

Prior to sequencing, the samples were purified by using a PCR purification kit (Agencourt, Ampure, Beckman Coulter, USA) and then the DNA sequence analysis was performed using an automatic sequencer [18] (Beckman Coulter CEQ 8000, Beckman Coulter, Fullerton, California, USA).

In addition to these polymorphisms, FVL was determined in all cases [17]. The frequencies of the alleles and genotypes associated with each of three polymorphisms of *FVII* gene and FVL between two groups were compared by chi-square or two-sided Fisher exact test, as appropriate. Haplotype analysis for *FVII* gene was also carried out and the distribution of the haplotypes between two groups was compared. Odds ratios (OR) were calculated as estimate of relative risk, together with 95% confidence intervals (95% CI). All the statistical analyses were also performed after cases with FVL were excluded from the study. All observed genotype and allele frequencies were tested for compliance with Hardy-Weinberg equilibrium. Statistical significance was determined as $p < 0.05$. The statistical analysis was made using SPSS software (SPSS Inc., Chicago, IL).

RESULTS

In all groups, the genotype distributions and allele frequencies were in Hardy-Weinberg equilibrium. The genotypes and allelic frequencies of the -323ins 10-bp, -401GT, -402GA polymorphisms among the patients with and without VTE were summarized in Table 1. The frequencies of these polymorphisms were not significantly different in the two groups ($p > 0.05$). Interestingly, two mutations in the promoter region of *FVII* gene (-401GT and -323ins10-bp) occurred simultaneously.

As shown in Table 2, there was no significant difference in the distribution of six haplotypes between group 1 and 2 patients.

For FVL, we observed the mutation in 25% (15 of 60) and 1.54% (2 of 130) of patients in group 1 and group 2, respectively. This difference between the two groups was found to be statistically significant ($p < 0.0001$).

Table 1. Distribution of three common polymorphisms, -323 ins 10-bp, -401 G/T, and -402 G/A in the promoter region of *FVII* gene in cancer patients with (group 1) and without venous thrombosis (group 2)

FVII Polymorphisms	Group 1 (n = 60)	Group 2 (n = 130)	*p* value
-323 ins10-bp			
w/w	33 (55%)	80 (61.5%)	
ins/w	25 (41.6%)	45 (34.6%)	0.9
ins/ins	2 (3.3%)	5 (3.8%)	
w allele	0.76	0.79	
ins allele	0.24	0.21	
-401 G/T			
GG	33 (55%)	80 (61.5%)	
GT	25 (41.6%)	45 (34.6%)	0.9
TT	2 (3.3%)	5 (3.8%)	
G allele	0.76	0.79	
T allele	0.24	0.21	
-402 G/A			
GG	48 (80%)	89 (68.5%)	
GA	11 (18.3%)	34 (26.2%)	0.7
AA	1 (1.6%)	7 (5.4%)	
G allele	0.89	0.815	
A allele	0.11	0.185.	

In Tables 1, 3: Ins — insertion; w — wild type.

Table 2. Distribution of haplotype of FVII in patients with (group 1) and without thrombosis (group 2)

Haplotype	Group 1 (n = 60)	Group 2 (n = 130)	OR (CI)	*p* value
I	25 (41.6%)	46 (35.4%)	1	
II	1 (1.6%)	7 (5.4%)	0.3 (0.03–2.3)	0.6
III	7 (11.6%)	27 (20.8%)	0.5 (0.2–1.3)	0.2
IV	21 (35%)	38 (29.2%)	1 (0.5–2)	0.9
V	4 (6.6%)	7 (5.4%)	1 (0.3–3.9)	0.8
VI	2 (3.3%)	5 (3.8%)	0.7 (0.1–4.1)	0.9

I: -402GG/-401GG/-323w/w; II: -402AA/-401GG/-323w/w;
III: -402GA/-401GG/-323w/w; IV: -402GG/-401GT/-323ins/w;
V: -402GA/-401GT/-323ins/w; VI: -402GG/-401TT/-323ins/ins
Notes: OR – odds ratio; CI – 95% confidence interval.

We next calculated the prevalence of the -323ins10-bp, -401GT, -402GA polymorphisms in two groups patients after excluding FVL mutation positive patients. No difference was also detected in the distribution of -323ins10-bp,-401GT, -402GA genotypes in cancer patients with VTE versus those without VTE ($p > 0.05$). Their allelic frequencies between two groups were also found to be statistically insignificant (Table 3). In addition, the haplotype frequencies showed a similar distribution among cancer patients with and without VTE ($p > 0.05$) (data not shown).

Table 3. Prevalence of -323 ins 10-bp, -401 G/T, and -402 G/A polymorphisms of *FVII* gene in cancer patients with (group 1) and without venous thrombosis (group 2) after exclusion of patients carriers of FVL

FVII Polymorphisms	Group 1 (n = 45)	Group 2 (n = 128)	*p* value
-323 ins10-bp			
w/w	21 (46.6%)	79 (61.7%)	
ins/w	22 (48.8%)	44 (34.4%)	0.8
ins/ins	2 (4.4%)	5 (3.9%)	
w allele	0.71	0.79	
ins allele	0.29	0.21	
-401 G/T			
GG	21 (46.6%)	79 (61.7%)	
GT	22 (48.8%)	44 (34.4%)	0.8
TT	2 (4.4%)	5 (3.9%)	
G allele	0.71	0.79	
T allele	0.29	0.21	
-402 G/A			
GG	37 (82.2%)	87 (67.9%)	
GA	7 (15.5%)	34 (26.5%)	0.8
AA	1 (2.2%)	7 (5.5%)	
G allele	0.9	0.813	
A allele	0.1	0.187	

DISCUSSION

Thrombosis is one of the most common complications in patients with malignant disease [19, 20]. The pathogenesis of haemostatic disorders in cancer reflects the interaction of different mechanisms including cancer-related factors such as venous stasis, the effects of treatment, especially chemotherapy and patient-specific factors such as thrombophilic status of cancer patients, acquired or congenital disorders of hemostasis. Several molecules of the coagulation and fibrinolytic systems are activated in cancer. Cancer cells can also produce TF and cancer procoagulants which activated the coagulation system [20].

FVII plays a key role in the extrinsic pathway of blood coagulation. High plasma FVII levels can be associated with venous or arterial thrombosis. The polymorphisms in the *FVII* gene may contribute to the variations in plasma levels of FVII. Therefore, it may be postulated that these polymorphisms precipitate venous thrombosis. Previous studies demonstrated that the rare alleles of the polymorphisms at positions -401 and -402 were related to marked changes in the rate of *FVII* gene transcription [4]. The -323ins10-bp polymorphism was directly related to the decrease in transcription [10].

The common polymorphisms in the promoter region of *FVII* gene may influence FVII blood levels because they may modulate its transcription [4, 9, 10]. Corral *et al.* [21] showed that carriers of the -323ins allele had an increased risk for intracranial hemorrhage and they found statistically significant differences in the prevalence of *FVII* -323ins10-bp polymorphism between patients and controls.

On the other hand, some studies have investigated the role of common polymorphisms influencing the FVII plasma levels in thrombotic disorders with conflicting results. There have been a few studies which evaluate the relationship between idiopathic VTE and some common polymorphisms of *FVII* gene [7, 8, 11–13]. The studies failed to show an association between the risk of VTE and the polymorphisms known to modulate blood FVII levels. Corral and colleagues [12] demonstrated no significant association between -323ins10-bp polymorphism and the risk of DVT. More recently, Folsom *et al.* [8] have shown that *FVII* 402GA polymorphism is not associated with VTE occurrence. It should be emphasized that the published studies have not included in cases with secondary VTE due to acquired risk factors including malignancy.

To our knowledge, the present study is the first to address the potential association between venous thrombosis and the common promoter region polymorphisms of *FVII* gene in cancer patients. Our studies showed that the promoter region polymorphisms were not strong determinants of venous thrombosis in cancer patients.

According to some previous studies, the -401GT polymorphism is the linkage disequilibrium with -323ins10-bp polymorphism [3, 4, 10]. In agreement with the reports, 401GT polymorphism showed complete allelic association with -323ins10-bp polymorphism in our series.

FVL is a well-established risk factor in the development of DVT [1, 2]. It is the most common genetic defect causing thrombosis among Caucasians. In general, the risk of venous thrombosis is 5–10-fold higher in cases carrying heterozygous and 50–80-fold higher in cases with homozygous for FVL. However, the role of hereditary thrombophilia in cancer patients with VTE is still unclear [16, 17, 19]. Some authors have been evaluated the role of FVL mutation on the thrombosis risk in malignancy [17]. Previously, we demonstrated a significant association between FVL and venous thrombosis risk in cancer patients [16, 17]. In this study, the frequency FVL in cancer patients with VTE was significantly higher than in those without VTE. The high prevalence of FVL in cancer patients with thrombosis can be associated with the high frequency among healthy Turkish population [22]. After exclusion of patient carriers of FVL, the relationship between the *FVII* gene polymorphisms and VTE was evaluated in our series. When the statistical analysis was also performed, the same insignificant results were observed. Accordingly it can be suggested that the screening for *FVII* polymorphisms does not contribute to a meaningful diagnostic investigation of thrombophilia in cancer patients with VTE.

Although the present study is the first to evaluate the association between venous thrombosis risk and the promoter region polymorphisms of *FVII* gene among cancer patients, there are a few limitations. Firstly, we have not determined FVII plasma levels in our cases. Other limitation is its small size. Another limitation of our study is that patients with different types of cancer were enrolled. Therefore the interpretation of the results can be complicated.

In conclusion, our study has suggested that three promoter polymorphisms of *FVII* gene; 323ins10-bp, -401GT, and -402GA are not contributing variants to VTE occurrence in cancer patients. FVL is the significant risk factor for the development of VTE but there is no additive effect of these polymorphisms of *FVII* gene in cancer. However, further larger studies including different ethnic population are required to better clarify the association of *FVII* polymorphisms with the thrombosis risk.

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