Factor V Gene (1691A and 4070G) and Prothrombin Gene 20210A Mutations in Patients with Behçet's Disease

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Key Words

Behçet's disease · Venous thrombosis · Factor V gene 1691A mutation · Factor V gene 4070G mutation · Prothrombin gene 20210A mutation

Abstract

Objectives: Behçet's disease (BD) is a chronic inflammatory disorder of still unknown etiology, characterized by endothelial cell injury/dysfunction and thrombosis and/or aneurysm of large blood vessels. Thrombophilia may play a role in the pathogenesis of thrombosis in BD. The common inherited gene defects, factor V (FV) 1691A (Leiden) and prothrombin (PT) 20210A, are known risk factors for thrombosis. The FV 4070G polymorphism was shown to influence circulating FV levels and to contribute to the activated protein C resistance phenotype. The aim of the study was to evaluate the role of FV 1691A, FV 4070G and PT 20210A gene mutations in Turkish BD patients with and without venous thrombosis. **Methods:** Seventy-one patients with BD (27 with venous thrombosis) and 91 healthy subjects were included in the study. FV 1691A, FV 4070G, and PT 20210A mutations were determined by a method based on PCR-RFLP. **Results:** The frequency of FV 1691A heterozygous muta-

tion in BD patients with venous thrombosis (25.9%) was significantly higher than that in healthy subjects (8.8%; OR = 3.63; 95% CI 1.18–11.2). Although the frequency of this mutation in patients with venous thrombosis was higher than that in the patients without venous thrombosis (11.4%), the difference did not reach a statistically significant level (OR = 2.73; 95% CI 0.77–9.70). In BD patients with thrombosis, the frequencies of FV 4070G and PT 20210A were not significantly different compared to the BD patients without venous thrombosis and healthy subjects. **Conclusions:** Our results suggest that the FV 1691A, FV 4070G, and PT 20210A mutations are unlikely to play an important role in the pathogenesis of thrombosis in patients with BD.

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Introduction

Behçet's disease (BD) is a systemic vasculitis of unknown etiology, characterized by recurrent oral and genital ulcers and uveitis [1]. Cutaneous, articular, neurologic, intestinal, pulmonary, urogenital, and cardiovascular manifestations have also been observed [2]. The prevalence of vascular involvement such as venous or arterial

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thrombosis and arterial aneurysms in BD is estimated to be about 25%. Venous thrombosis is more common than arterial thrombosis (88 vs. 12%). Deep and superficial venous thrombosis of the lower extremities predominates [3].

The pathogenesis of thrombosis in BD is not yet completely understood. The vascular endothelium has procoagulant, anticoagulant, and fibrinolytic properties. Vascular endothelial cells play a critical role in the defense against thrombosis. The pathological hallmark of BD is a nonspecific vasculitis of veins, arteries, and capillaries in all affected organs. Endothelial dysfunction due to vascular inflammation is considered to be an important factor of thrombosis. Endothelial products reflecting endothelial activation and/or injury are increased in patients with BD [4–6]. However, endothelial injury itself cannot clearly account for the hypercoagulable status of BD, because other vasculitis syndromes do not increase the risk of thrombosis. Moreover, endothelial activation markers do not differ between BD patients with and without thrombosis. Several studies have proposed a potential mechanism to explain hypercoagulability in BD. However, no consistent primary abnormality of the coagulation, anticoagulant, or fibrinolytic systems has yet been confirmed in BD [4]. Other factors including genetic defects may be the underlying cause a prothrombotic state in BD.

Thrombophilia may play a role in the pathogenesis of thrombosis in BD. Activated protein C (APC) resistance is now recognized as one of the most common inherited risk factors for venous thrombosis. Most cases of APC resistance are caused by a point mutation at nucleotide 1691 in the factor V (FV) gene (arginine 506 to glutamine), referred to as the FV Leiden mutation. The mutated FVa is less sensitive to APC-mediated inactivation than normal FV [4]. Subjects heterozygous for the FV Leiden mutation are exposed to a 5- to 10-fold increased risk of thrombosis, while in homozygous subjects the thrombotic risk is increased 50- to 100-fold. It is also suggested that the Leiden mutation increases the risk of deep venous thrombosis in BD [7].

A guanine to adenine base substitution at position 20210 of the 3'-untranslated region of the prothrombin (PT) gene raises PT levels. This mutation is associated with a threefold increased risk of venous thrombosis, and has been reported to be the second most common inherited hypercoagulable state. The prevalence of heterozygous 20210 carriers varies from 1 to 5% in the healthy white population [4]. The frequency of FV 1691A and PT 20210A mutations was found to be 10.4 and 2.7%, respectively, in the Turkish population [8, 9].

Recently, a genetic component in the FV gene that contributes to APC resistance both in the presence and absence of FV Leiden was reported. This highly conserved FV gene haplotype, called the R2 polymorphism, is characterized by an A→G mutation at position 4070 in exon 13 that predicts a His 1299 Arg substitution [10]. The presence of the FV 4070 A→G mutation has been reported in different populations including the Turkish. Akar et al. [9] reported the frequency of the FV 4070G mutation to be 8.5% in a healthy Turkish population. Previous reports on the effect of the 4070G mutation on the occurrence of deep vein thrombosis is controversial [11, 12]. Akar et al. [12] revealed that the FV 1691A mutation is a risk factor for thromboembolism but FV 4070G is not.

The aim of this study was to evaluate the role of FV Leiden, FV 4070G and PT 20210A gene mutations for the development of venous thrombosis in Turkish BD patients.

Patients and Methods

Seventy-one patients, 43 males and 28 females, fulfilling the International Study Group criteria for the diagnosis of BD [13] were included in the study. Patients were recruited from a hospital-based sample. The mean age was 35.7 ± 9.5 (range 17–55) years, and mean disease duration was 80 ± 60 (range 6–264) months in patients with BD. Ninety-one healthy subjects of the same ethnic origin (56 males and 35 females) without any familial history of thromboembolism were also included in the study as a control group. The mean age of the control group was 33.8 ± 8.5 years. The study was approved by the Committee on Medical Ethics of the Ankara University Faculty of Medicine, and written informed consent was obtained from all subjects after an explanation of the purpose of the study.

The detailed clinical characteristics were recorded for each patient. Patients who had recurrent oral and genital ulcers, skin lesions and positive pathergy testing were diagnosed as having mucocutaneous BD. BD with vascular involvement described patients with venous and arterial thromboses and/or aneurysm. Isolated superficial thrombophlebitis without accompanying deep vein thrombosis was not considered as having vascular involvement, and such patients were included in the mucocutaneous group. Patients with mucocutaneous BD who had a disease duration of <5 years were excluded from the study as the risk of developing vascular involvement is high in the first 5 years following the diagnosis. The diagnosis of vascular involvement was made on clinical grounds by color Doppler ultrasonographic and/or angiography using computed tomographic or magnetic resonance techniques where appropriate. Patients with parenchymal lesions were defined as having neurological involvement.

DNA was extracted by conventional methods and polymerase chain reaction (PCR) of the FV gene was performed according to previously described methods using for FV 1691 G→A primers 5'-TCAGGCAGGAACAACACC-3' and 5'-GTTACTTCAAGGAA-CAAAATACCTGTAAAGCT-3', and FV 4070 A→G primers 5'-CAA-

Table 1. The demographic and clinical characteristics of patient groups

	All patients	Patients with venous thrombosis	Patients without venous thrombosis
Number	71	27	44
Age, years	35.7 ± 9.5	36.3 ± 8.2	35.4 ± 10.2
Sex (male/female)	43/28	23/4*	20/24
Oral ulcers	71 (100%)	27 (100%)	44 (100%)
Genital ulcers	63 (88.7%)	22 (81.5%)	41 (93.2%)
Papulopustular eruption	47 (66.2%)	19 (70.4%)	28 (63.6%)
Erythema nodosum	41 (57.7%)	21 (77.8%)**	20 (45.5%)
Positive pathergy test	34 (47.9%)	12 (63.2%)	22 (57.9%)
Ocular involvement	28 (39.4%)	11 (40.7%)	17 (38.6%)
Arthritis	9 (12.7%)	6 (22.2%)	3 (6.8%)
Neurologic involvement	3 (4.2%)	2 (7.4%)	1 (2.3%)
Superficial thrombophlebitis	26 (36.6%)	22 (81.5%)	4 (9.1%)
Deep vein thrombosis	27 (38.0%)	27 (100%)	
Inferior caval vein thrombosis	4 (5.6%)	4 (14.8%)	
Superior caval vein thrombosis	3 (4.2%)	3 (11.1%)	
Arterial involvement	13 (18.3%)	12 (44.4%)	1 (2.3%)

Categorical data are expressed as number of subjects with percentages in parentheses.

* $p = 0.001$; ** $p < 0.05$ vs. patients without venous thrombosis.

GTCCTTCCCCACAGATATA-3' and 5'-AGATCTGCAAAGAG-GGGCAT-3'. Amplification was performed for 35 cycles with an annealing temperature of 57°C (Ericomp, USA). Amplified DNA was digested with *HindIII* enzyme (Promega, Madison, Wisc., USA) for FV 1691 G→A, and *RsaI* enzyme (Promega) for FV 4070 A→G at 37°C and subjected to 2% agarose gel electrophoresis [11, 14]. The detection of the 20210A allele in the PT gene was performed according to a previously described PCR method using the primers 5'-TCTAGAAACAGTTGCCTGGC-3' (nt 19889–19908) and a mutagenic primer 5'-ATAGCACTGGGAGCATTGAAGC-3' (nt 20333–20212). The *HindIII* enzyme (Promega) was used to determine the mutation [15].

Comparison of means and percentages was made with the unpaired Student's *t* test and χ^2 test with Yates correction or Fisher's exact test, as appropriate. The associations between genotype frequency and BD or venous thrombosis are expressed as odds ratio (OR) with 95% confidence intervals (CI).

Results

The demographic and clinical characteristics of all BD patients and the patients with or without venous thrombosis are shown in table 1. Twenty-seven patients had venous thrombosis and 44 patients had no thrombotic events. The distribution of mutations and vascular involvement characteristics in 27 BD patients with venous thrombosis are shown in table 2. All patients with venous

thrombosis had deep vein thrombosis of the lower extremities, and 4 had inferior vena cava and 3 superior vena cava thrombosis. Isolated caval vein thrombosis was not seen. Superficial thrombophlebitis was seen in 26 patients with BD, 22 of them also had deep vein thrombosis. Aneurysm formation, or thrombotic occlusion in pulmonary, brachial, iliac and femoral arteries were present in 13 patients. Arterial involvement was associated with venous thrombosis in 12 patients.

In patients with venous thrombosis, the frequency of male sex was significantly higher compared to the patients without venous thrombosis (85.2 vs. 45.5%, respectively; $p = 0.001$). The frequency of erythema nodosum was significantly higher in patients with venous thrombosis compared to the patients without venous thrombosis (77.8 vs. 45.5%, respectively; $p < 0.05$). Other demographic and clinical characteristics were found to be similar between both groups.

The genotype distribution of FV 1691A, FV 4070G and PT 20210A mutations are given in table 3. All subjects with these mutations were heterozygous carriers. Two BD patients, 1 in the venous thrombosis and 1 in the mucocutaneous group, carried both FV 1691A and PT 20210A mutations. Although the frequency of FV 1691A heterozygotes in patients with BD was higher than that in healthy subjects (16.9 vs. 8.8%), the difference did not

Table 2. Mutation and thrombosis data of our BD patients with venous thrombosis

Patient No.	Age years	Sex	FV 1691A	FV 4070G	PT 20210A	DVT	CVT	A	ST
1	31	M	-	-	-	+	-	+	+
2	36	M	-	-	-	+	-	-	+
3	40	F	-	-	-	+	-	-	+
4	53	M	-	-	-	+	-	-	+
5	38	M	+	-	-	+	-	+	+
6	31	M	-	-	-	+	-	+	+
7	38	M	+	-	-	+	+ ¹	-	+
8	35	F	-	-	-	+	-	-	-
9	41	M	-	-	-	+	+ ²	+	+
10	30	M	-	-	-	+	-	+	+
11	23	M	+	-	+	+	-	-	+
12	21	M	-	-	-	+	+ ¹	+	+
13	28	M	-	-	-	+	-	-	-
14	30	M	-	-	-	+	-	+	+
15	34	F	-	-	+	+	-	+	+
16	36	M	-	-	-	+	-	+	+
17	29	M	-	-	-	+	-	-	+
18	29	F	-	-	-	+	-	-	+
19	47	M	-	+	-	+	+ ¹	+	-
20	43	M	-	-	-	+	-	-	-
21	30	M	-	-	-	+	-	-	+
22	47	M	+	-	-	+	-	-	+
23	36	M	-	-	-	+	+ ²	+	-
24	36	M	+	-	-	+	+ ²	-	+
25	41	M	+	-	-	+	+ ¹	-	+
26	33	M	-	+	-	+	-	-	+
27	55	M	+	-	-	+	-	-	+

DVT = Deep vein thrombosis; ST = superficial thrombophlebitis; CVT = caval venous thrombosis; A = arterial lesion; M = male; F = female.

¹ Inferior vena cava.

² Superior vena cava.

Table 3. Distribution of genetic polymorphisms in patients with Behçet's disease and healthy subjects

	All patients	Patients with venous thrombosis	Patients without venous thrombosis	Healthy subjects
Factor V 1691A				
GG	59 (83.1%)	20 (74.1%)	39 (88.6%)	83 (91.2%)
GA	12 (16.9%)	7 (25.9%)	5 (11.4%)	8 (8.8%)
Factor V 4070G				
AA	66 (93.0%)	25 (92.6%)	41 (93.2%)	83 (91.2%)
AG	5 (7.0%)	2 (7.4%)	3 (6.8%)	8 (8.8%)
Prothrombin 20210A				
GG	64 (90.1%)	25 (92.6%)	39 (88.6%)	88 (96.7%)
GA	7 (9.9%)	2 (7.4%)	5 (11.4%)	3 (3.3%)
Data are presented as the number of subjects with percentages in parentheses.				

Table 4. The relationships between procoagulant mutations and clinical findings in patients with Behçet's disease

	FV 1691A	FV 4070G	PT 20210A
Genital ulcers			
Present	11 (17.5%)	5 (7.9%)	6 (9.5%)
Absent	1 (12.5%)	0 (0.0%)	1 (12.5%)
Papulopustular eruption			
Present	8 (17.0%)	2 (4.3%)	3 (6.4%)
Absent	4 (16.7%)	3 (12.5%)	4 (16.7%)
Erythema nodosum			
Present	9 (22.0%)	4 (9.8%)	4 (9.8%)
Absent	3 (10.0%)	1 (3.3%)	3 (10.0%)
Positive pathergy test			
Present	4 (11.8%)	5 (14.7%)	3 (8.8%)
Absent	4 (17.4%)	0 (0.0%)	2 (8.7%)
Ocular involvement			
Present	5 (17.9%)	2 (7.1%)	1 (3.6%)
Absent	7 (16.3%)	3 (7.0%)	6 (14.0%)
Arthritis			
Present	4 (44.4%)*	0 (0.0%)	0 (0.0%)
Absent	8 (12.9%)	5 (8.1%)	7 (11.3%)
Neurologic involvement			
Present	0 (0.0%)	1 (33.3%)	2 (66.7%)*
Absent	12 (17.6%)	4 (5.9%)	5 (7.4%)
Superficial thrombophlebitis			
Present	7 (26.9%)	1 (3.8%)	3 (11.5%)
Absent	5 (11.1%)	4 (8.9%)	4 (8.9%)
Arterial involvement			
Present	1 (7.7%)	1 (7.7%)	1 (7.7%)
Absent	11 (19.0%)	4 (6.9%)	6 (10.3%)

Data are presented as number of subjects with percentages in parentheses.

* $p < 0.05$.

reach a statistically significant level (OR = 2.11; 95% CI 0.81–5.48). The FV 1691A mutation was detected in 7 of 27 BD patients with venous thrombosis (25.9%), and in 5 of 44 BD patients without thrombosis (11.4%). The frequency of heterozygous carriers of the FV 1691A mutation in BD patients with venous thrombosis was significantly higher compared to healthy subjects (OR = 3.63; 95% CI 1.18–11.2; $p < 0.05$). However, there was no significant difference between the BD patients with and without venous thrombosis (OR = 2.73; 95% CI 0.77–9.70; $p = 0.11$).

The frequency of FV 4070G heterozygotes in BD patients was similar to that in healthy controls (7.0 and

8.8%, respectively; OR = 0.79; 95% CI 0.25–2.52). This mutation frequency in BD patients with venous thrombosis (7.4%) was not found to be significantly different from that of BD patients without venous thrombosis (6.8%) or healthy subjects (OR = 1.09; 95% CI 0.17–7.00 and OR = 0.83; 95% CI 0.16–4.16, respectively).

Although the frequency of PT 20210A heterozygotes in patients with BD was higher than that in healthy controls (9.9 and 3.3%, respectively), the difference did not reach a statistically significant level (OR = 3.21; 95% CI 0.80–12.9). This mutation was detected in 2 of BD patients with thrombosis (7.4%), and in 5 of BD patients without thrombosis (11.4%). No statistically significant difference between the BD patients with venous thrombosis and the BD patients without venous thrombosis (OR = 0.62; 95% CI 0.11–3.47) or healthy subjects (OR = 2.35; 95% CI 0.37–14.8) in prevalence of PT 20210A mutation was obtained.

The relationships between the tested gene mutations and other clinical findings of BD are shown in table 4. There was a significant correlation between the presence of FV 1691A mutation and arthritis (OR = 5.40; 95% CI 1.19–24.4, $p < 0.05$). Although the number of the patients was very small, the PT 20210A mutation frequency was significantly higher in patients with neurologic involvement than in the patients without neurologic involvement (66.7 and 7.4%, respectively; OR = 25.2; 95% CI 1.93–328.4, $p < 0.05$). Except for these, the FV 1691A, FV 4070G and PT 20210A mutations did not show any association with other clinical characteristics, including arterial involvement and superficial thrombophlebitis.

Discussion

Although a thrombotic tendency is one of the major characteristics of BD, the pathogenetic mechanisms leading to venous thrombosis are not yet fully understood. Most thrombotic abnormalities have been attributed to the endothelial injury secondary to vasculitis. However, endothelial injury itself cannot clearly account for the hypercoagulable status of BD, because other vasculitis syndromes do not increase the risk of thrombosis, and the endothelial activation markers do not differ between BD patients with and without thrombosis. Recently, it has been suggested that procoagulant mutations may play a role in the development of thrombotic events in BD patients. Several groups have studied the contribution of FV 1691A (Leiden) and PT 20210A mutations to thrombotic tendency in BD patients [16–23]. However, there

are some controversies in this issue mainly resulting from features of the study populations. To our knowledge, this is the first study to examine the association of the three common procoagulant mutations, FV 1691A, PT 20210A, and FV 4070A, with venous thrombosis in BD patients. The risk of developing vascular involvement in patients with BD is high in the first 5 years following diagnosis [3]. Patients without venous thrombosis who had a disease duration of <5 years were excluded from the present study. This approach provided a more favorable examination of the association between procoagulant mutations and development of venous thrombosis.

The prevalence of the FV 1691A mutation in BD varies from 0 to 38% [16–23]. In our study the frequency of heterozygous carriers of the FV 1691A mutation was 16.9% in all BD patients, 25.9% in BD patients with venous thrombosis, and 8.8% in healthy subjects. Although the frequency of the FV 1691A mutation in BD patients with venous thrombosis was found to be significantly higher than in healthy subjects, the difference between BD patients with and without venous thrombosis did not reach a statistically significant level. Previous reports on the association between the FV 1691A mutation and venous thrombosis in BD patients are controversial, with studies favoring [17, 19] or opposing [16, 18, 22, 23] this association. Verity et al. [21] reported that the FV 1691A mutation was significantly associated with ocular disease in BD patients but not with deep vein thrombosis. The association of the FV 1691A mutation with venous thrombosis in Turkish BD patients has been investigated in three studies, one reported a significant relation with thrombosis [17], whereas the others did not [20, 23]. Gül et al. [17] compared the frequency of the FV 1691A mutation in 32 BD patients with venous thrombosis and 32 patients without venous thrombosis, and found that the presence of FV 1691A heterozygosity in patients with BD was associated with a 5.8-fold increase in the risk of venous thrombosis. However, Toydemir et al. [23] did not find a significant difference in the frequency of the FV 1691A mutation in BD patients with and without venous thrombosis. The study of Öner et al. [20] was concerned only with 5 patients with venous thrombosis, therefore, no conclusion could be drawn from this study.

The PT 20210A mutation was investigated in two large studies, one reported no increase in the frequency of the PT gene mutation [23], and the other reported a significant relation with thrombosis [24]. Gül et al. [24] reported that the heterozygous PT gene 20210A mutation was found in 31.3% of BD patients with venous thrombosis and in 3.1% of BD patients without thrombosis. In addi-

tion, 3 patients with BD who had recurrent venous thrombosis, arterial thrombosis, or intracardiac thrombosis associated with the PT 20210A mutation have been described [25, 26]. We observed no difference in the frequency of the PT gene mutation between patients with BD and control subjects (9.5 and 3.3%, respectively). There was also no significant difference in the frequency of the PT 20210A mutation in BD patients with and without thrombosis (7.1 and 10.7%, respectively).

We evaluated the thrombotic risk of the FV 4070A mutation, which to our knowledge has not been studied before in patients with BD. The frequency of this mutation was 6.0% in all BD patients, 3.3% in BD patients with venous thrombosis, 7.4% in BD patients without thrombosis, and 8.8% in healthy subjects. When these groups were compared with each other, the differences were not found to be statistically significant.

Although vascular disease and thrombosis are the most important features of BD, the pathogenesis of thrombosis is not yet completely understood. Our data suggest that procoagulant mutations do not seem to play an important role in the pathogenesis of thrombosis in patients with BD. The development of thrombosis in BD patients requires additional trigger events such as other defects in the coagulation pathway, anticoagulant factors, and fibrinolysis, along with environmental factors.

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