

# Hereditary Thrombophilia Risk Factors In Patients With Venous Thromboembolism

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## ABSTRACT

Venous thromboembolic disease (VTE) is a serious cause of mortality and morbidity with an incidence of 0.1% per year. The etiology of VTE is multifactorial and associated with acquired and hereditary conditions. The aim of our study was to investigate the presence of rare genetic mutations in VTE patients and to evaluate their distribution according to thrombosis sites.

The study group consisted of 107 patients who underwent genetic testing for thrombophilia due to VTE between 2015 and 2017. Patients with VTE who underwent thrombophilic genetic examination by polymerase chain reaction method were included in the study. Antithrombin III, protein C and protein S deficiency patients diagnosed with biochemical tests were excluded from the study. The demographic characteristics and clinical results of the patients were obtained from the hospital archive. The control group consisted of 112 healthy individuals randomly selected from the community. The patient group and the control group were compared.

The mean age of the patients was 39.6 years. The female/male ratio was 1.7. Advanced age and female gender were statistically significantly more frequent in the patient group (In both,  $p=0.0001$ ). Factor V G1691A and B-Fibrinogen-455 genetic defects were statistically significantly higher in the patient group (Respectively;  $p=0.008$ ,  $p=0.049$ ). The most frequent (54.2%) PAI-4G&5G heterozygous gene defect was found in the patients. Deep venous thrombosis was detected in 54.2%, intraabdominal VTE in 4.7%, pulmonary embolism in 27.1%, and cerebral venous thrombosis in 30.8% of the VTE patients.

Thrombophilic genetic mutations are not uncommon in patients with VTE. Thrombophilic genetic mutations in VTE etiology and in selected patients should be investigated. In patients without known thrombophilia mutations, rare thrombophilic mutations should be examined.

**Key Words:** Venous thromboembolic disease, hereditary thrombophilia, rare thrombophilic mutations

## Introduction

Venous thromboembolic disease (VTE) is the formation of venous occlusion due to pathologic thrombus development in veins. According to the theory described for the first time by Rudolph Virchow in 1856, stasis, venous wall damage and local hypercoagulability in blood are the three main causes in the VTE etiology (1).

The annual incidence of VTE is 0.001% in children and 0.1% in adults (1,2). The incidence increases with age, reaching 1% over the age of 85 years(1,2). Of the VTE patients, two thirds have lower limb deep vein thrombosis (DVT) and one third have pulmonary embolism (PE) (2). The major complications of VTE are post-thrombotic syndrome, major bleeding due to anticoagulation, recurrence and death (1,2).

Even though VTE can be due to inherited or acquired causes, multiple causes are concomitantly

present in many of the patients. Today it is estimated that the susceptibility to thrombosis in more than 60% of the cases is linked to the genetic components (2-4).

Factor V Leiden is the most common and well-studied genetic cause of VTE, followed by the prothrombin G20210A gene mutation and deficiencies in protein S, protein C, and antithrombin (2,4). Some other mutations and polymorphisms have also been proposed as genetically determined procoagulant risk factors for VTE (3-5).

Hereditary defects that cause VTE continue to be studied. In this study, we focused on rare genetic mutations, which are reported to be the cause of VTE. Rare genetic mutations in patients and their relationship with the presence of VTE in various localizations were examined. Thus, the role and importance of rare genetic mutations in VTE etiology was examined.

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## Materials and Methods

**Study Group:** Between 2015 and 2017, patients with VTD who underwent genetic examination for thrombophilia in our hospital were examined for hereditary thrombophilic disorders and compared with the healthy control group. For the study, 15/216 numbered and 15/08/2017 dated Ethics Committee approval was obtained from Bezmialem Vakif University. Our study received written approval from all individuals.

In our hospital, the diagnosis of antithrombin III, protein C and protein S deficiency was usually determined by biochemical tests. Patients with 164 VTD who were biochemically determined to have antithrombin III, protein C and protein S deficiency and were not genetically tested were excluded from the study. Forty-four patients with genetically determined antithrombin III, protein C and protein S deficiency were excluded from the study for homogenization of the study. Patients with arterial thrombus, retinal vein thrombus, thoracic outlet syndrome and catheter-related upper extremity thrombus were excluded.

A total of 107 VTD patients with the following genetic tests were included in the study: Factor V mutation analyses (G1691A-Leiden and H1299R-FVR2), Factor II prothrombin (G20210A), Methylenetetrahydrofolate reductase (MTHFR) mutation analyses (C677T and A1298C), Factor XIII (V34L), Beta-fibrinogen (-455 G-A), Plasminogen activator inhibitor 1 (PAI-1) (4G/5G), Angiotensin converting enzyme (ACE) (I/D), Human platelet antigen-1 (Glycoprotein IIIa-GPIIIa) (HPA-1) (a/b), Apolipoprotein B (ApoB, R3500Q) (ApoB-100), Apolipoprotein E (ApoE) (e2, e3 and e3 alleles).<sup>4</sup> Heterozygous and homozygous mutations in thrombophilic gene mutations were evaluated in all subjects. In this respect, both groups were compared.

The control group consisted of 112 people. The control group was randomly selected healthy individuals. There was no consanguinity between the control group and the individuals in the patient group. Thrombophilic genetic tests performed in the study group were also performed in the control group. The genetic mutations of the patients with VTD were compared with those of the control group. Demographic characteristics of patients in the study group, thrombosis regions and thrombophilic genetic mutations in these areas were examined.

**Study Investigations:** It was found that physical examination, D-dimer measurement, VTD

imaging methods such as doppler ultrasonography (DS), venography, computed tomography (CT), magnetic resonance imaging (MR), ventilation-perfusion scintigraphy and angiography were used in these patients (1,4).

While determining the gene mutations of the individuals undergone genetic test for thrombophilia, venous blood sample was taken into a tube with ethylenediaminetetraacetic acid (EDTA) and DNA isolations were made from the peripheral blood leukocytes in 200 µl of peripheral blood samples for two weeks using High Pure PCR Template Preparation Kit. Then, the gene sequences of thrombophilic mutations were reproduced in vitro from the isolated DNAs with the multiplex PCR method and in vitro amplification was carried out using double primers with the PCR multiplex method on the gene chains to probe defects utilizing a mutation detection kit. After hybridization, the mutations in the thrombophilic genes were examined in the LightCycler device and the presence of homozygous and heterozygous genotype was identified with the Melting Curve program.

**Statistical Analysis:** The data obtained in this study were analyzed with the licensed SPSS 21 package program. Descriptive statistical data, mean and standard deviation of continuous variables were shown. Categorical variables were represented by frequency and percentage. The Chi-square ( $\chi^2$ ) analysis was carried out in analysis of the differences of nominal variables between the groups. In cases where the expected values in the cells of the tables did not have sufficient volume, the Fisher's Exact Test or the Pearson Chi-square analysis using Monte Carlo simulation was performed depending on the suitability of the data. While interpreting the results, a significance level of 0.05 was used; it was indicated that there is a significant difference in the case of  $p < 0,05$ , whereas there was not a significant difference in the case of  $p > 0.05$ .

## Results

The mean age was 31.7 in the control group and the Female/Male ratio was 10.2. The mean age was 39.6 years in the VTD patient group, and the Female/Male ratio was 1.7. The age of the patient group was statistically significantly higher than the control group ( $p = 0.0001$ ). The female ratio was 63.6% in the patient group and 91.1% in the control group; there was a statistically significant difference between the groups ( $p = 0.0001$ ). (Table 1).

**Table 1.** The demographic and clinical characteristics of the patient and control groups

Parameters	Patient Group (n:107) n (%)	Control Group (n:112) n (%)	Test
			p
Age (year), (mean)	2-75 (39.6)	2-59 (31.8)	0.0001¥
Female/Male	1.7	10.2	0.0001♠
Heterozygous Defect , (mean)	0-8 (3.6)	1-8 (2.9)	0.0001¥
Homozygous Defect, (mean)	0-4 (1.2)	0-3 (1)	0.204¥
Common gene defect: ACE I/D, n (%)	88, (82.2%)	83 (74.1%)	0.197♠
Rare Genetic Mutations:	26, (24.3%)	12, (10.7%)	0.008♠
B-Fibrinogen-455, n (%)	62, (57.9%)	49, (43.8%)	0.049♠
Factor V G1691A, n (%)			

¥: The independent t-test

♠: Chi-square analysis

Of the 436 thrombophilic gene defects identified in the control group, 73.6% were heterozygous, 26.4% were homozygous defects. Of the 511 thrombophilic gene defects identified in the patient group, 75.1% were heterozygous and 24.8% were homozygous disorders. The number of heterozygous defects was significantly higher in the patient group and there was a statistically significant difference between the groups ( $p=0.0001$ ) (Table 1).

Proportionally, the most common thrombophilic gene defect was ACE I/D gene defect with 82.2% in the patient group and ACE I/D gene defect with 74.1% in the control group. Statistically, the rate of Factor V G1691A mutation was 24.3% in the patient group, and 10.7% in the control group; there was a statistically significant difference between the groups ( $p=0.008$ ). Again, the rate of patients with B-Fibrinogen-455 gene mutation was 57.9% in the patient group, and 43.8% in the control group; there was a statistically significant difference between the groups ( $p=0.049$ ). According to heterozygous/homozygous differentiation, Factor V G1691A heterozygous disorder was statistically significantly higher in the patient group ( $p=0.008$ ) (Table 2).

In our study, 68% of ACE gene mutations were found in women in the patient group and statistically significant ( $p=0.032$ ). In the control group, 75% of Factor V G1691A gene mutations were found in women and were statistically significant ( $p=0.039$ ).

In the patient group, DVT was found as 54.2%, intraabdominal VTD (IAVTD) as 4.7%, PE as 27.1% and cerebral vein thrombosis (CVT) as 30.8%. In our study, Factor V G1691A (n:20, %76.9) and MTHFRA 1298C (n:39, %62.9) thrombophilic gene defects were statistically significantly higher in the DVT patients

(Respectively;  $p=0.008$ ,  $p=0.034$ ). Factor V G1691A (n:22, 29.7%) thrombophilic gene defects were statistically significantly higher in the CVT patients ( $p=0.049$ ). Statistically, DVT was significantly more common in females ( $p=0.004$ ) while CVT was significantly less common in females ( $p=0.009$ ) (Table 2-3).

## Discussion

Venous thrombosis and associated complications are the most common emergency vascular disease after acute ischemic heart disease and stroke (5). The annual incidence of VTD is 104-183 per 100,000(2,3). The annual incidence of VTD increases with age, reaching 1% over 85 years of age (6,7). The F/M ratio has been reported as 1.2 in the literature, while it was found to be 1.7 in our patient group (5,7). In our study, VTD was more common in women.

The etiological factors of VTD are defined as hereditary and acquired (8,9). According to frequency order, the major hereditary causes are active factor V Leiden mutation, prothrombin G20210A mutation, etc (10,11). The major acquired causes include major surgery, active cancer, immobilization, inpatient treatment, trauma, pregnancy, oral contraceptive use, etc (12-17). In thrombophilic gene tests that we performed for VTD and hereditary causes, Factor V G1691A and B-Fibrinogen-455 gene mutations were statistically significantly higher in the patient group. The ACE gene mutation was statistically significantly higher in females in the patient group.

The most common venous thromboembolic disease is DVT. Without PE, the annual incidence of DVT is 45-117 per 100,000 people and the incidence of PE is 29-78 per 100,000 people

**Table 2.** Thrombophilic mutation types according to the groups

Thrombophilic Gene	Mutation Presence	Groups						Test	
		Patient		Control		Total		Value	p <sup>φ</sup>
		n	%	n	%	n	%		
Factor V G1691A	Normal	81	75,7	100	89,3	181	82,6	7,042	0,008
	Mutational	26	24,3	12	10,7	38	17,4		
Mutation Types	Heterozygous	23	21,5	12	10,7	35	16,0	9,559	0,008
	Homozygous	3	2,8	0	0,0	3	1,4		
Factor V H1299R	Normal	81	75,7	83	74,1	164	74,9	0,074	0,789
	Mutational	26	24,3	29	25,9	55	25,1		
Mutation Types	Heterozygous	24	22,4	28	25,0	52	23,7	0,558	0,757
	Homozygous	2	1,9	1	,9	3	1,4		
Factor II G20210A	Normal	99	92,5	108	96,4	207	94,5	1,611	0,204
	Mutational	8	7,5	4	3,6	12	5,5		
Mutation Types	Heterozygous	7	6,5	4	3,6	11	5,0	2,492	0,288
	Homozygous	1	0,9	0	0,0	1	0,5		
MTHFR C677T	Normal	46	43,0	58	51,8	104	47,5	1,697	0,193
	Mutational	61	57,0	54	48,2	115	52,5		
Mutation Types	Heterozygous	50	46,7	41	36,6	91	41,6	2,328	0,312
	Homozygous	11	10,3	13	11,6	24	11,0		
MTHFR A1298C	Normal	45	42,1	56	50,0	101	46,1	1,39	0,238
	Mutational	62	57,9	56	50,0	118	53,9		
Mutation Types	Heterozygous	47	43,9	45	40,2	92	42,0	1,744	0,418
	Homozygous	15	14,0	11	9,8	26	11,9		
Factor XIII V34L	Normal	74	69,2	90	80,4	164	74,9	3,649	0,056
	Mutational	33	30,8	22	19,6	55	25,1		
Mutation Types	Heterozygous	31	29,0	19	17,0	50	22,8	4,559	0,102
	Homozygous	2	1,9	3	2,7	5	2,3		
B-Fibrinogen-455	Normal	45	42,1	63	56,3	108	49,3	3,861	0,049
	Mutational	62	57,9	49	43,8	111	50,7		
Mutation Types	Heterozygous	51	47,7	43	38,4	94	42,9	5,04	0,08
	Homozygous	11	10,3	6	5,4	17	7,8		
PAI-1 4G&5G	Normal	28	26,2	33	29,5	61	27,9	0,155	0,694
	Mutational	79	73,8	79	70,5	158	72,1		
Mutation Types	Heterozygous	58	54,2	57	50,9	115	52,5	0,328	0,849
	Homozygous	21	19,6	22	19,6	43	19,6		
ACE Gene mutation	Normal	19	17,8	29	25,9	48	21,9	1,668	0,197
	Mutational	88	82,2	83	74,1	171	78,1		
Mutation Types	Heterozygous	46	43,0	41	36,6	87	39,7	2,258	0,323
	Homozygous	42	39,3	42	37,5	84	38,4		
HPA-1	Normal	74	69,2	87	77,7	161	73,5	1,626	0,202
	Mutational	33	30,8	25	22,3	58	26,5		
Mutation Types	Heterozygous	30	28,0	21	18,8	51	23,3	2,677	0,262
	Homozygous	3	2,8	4	3,6	7	3,2		
ApoB R3500 Q	Normal	107	100,0	112	100,0	219	100,0	-	-
	Mutational	0	0,0	0	0,0	0	0,0		
ApoE Genotype	Normal	74	69,2	89	79,5	163	74,4	3,054	0,081
	Mutational	33	30,8	23	20,5	56	25,6		
Mutation Types	Heterozygous	18	16,8	10	8,9	28	12,8	3,697	0,157
	Homozygous	15	14,0	13	11,6	28	12,8		

φ: Chi-square analysis

**Table 3.** The demographic and clinical characteristics of the VTE patient subgroups

Parameters	DVT (n:58), n (%)	IAVT, n (n:5) (%)	PE (n:29), n (%)	CVT (n:33), n (%)
Age, (year) (mean)	17-76, (40.1)	31-63, (53.4)	17-75, (42.3)	16-59, (36)
Gender (F/M) (p <sup>ϕ</sup> )	3.1, (p=0.004)	0.7, (p>0.05)	1.9, (p>0.05)	0.8, (p=0.009)
Most Common Mutation, n (%)	ACE, 50 (86.2%)	PAI-4G&5G, 6 (100%)	PAI-4G&5G, 26 (89.6%)	ACE, 25 (75.8%)
Statistically Significant Mutation, n (%) (p <sup>ϕ</sup> )	FactorVG1691A 20, (76,9%) (p=0.008)	-	-	FactorVG1691A 22, 29.7% (p=0.049)
	MTHFRA1298C 39, (62.9%) (p=0.034)			

VTE= Venous thromboembolism, DVT=Deep vein thrombosis, IAVT=Intraabdominal vein thrombosis  
PE=Pulmonary embolism, CVT=Cerebral vein thrombosis

ϕ: Chi-square analysis

(6,18). In clinic, DVT is seen in 10% of the PE patients (18). PE develops in approximately 20% of untreated DVTs and progresses fatally with a rate of 10-20% (9,19). In our study, DVT was detected in 54.2%, and PE in 27.1% of the VTD patients. DVT was statistically significantly more common in female patients. In our study, the statistically significant defect was Factor V G1691A (Leiden) mutation in patients similar to the literature.

Factor V G1691A mutation is the most common cause of hereditary thrombophilia (8,10). The incidence of this mutation is between 2-14% in the population (20). In our study, the rate of Factor V heterozygous Leiden mutation was statistically significant higher in the VTD patients. Moreover, factor V G1691A mutation was statistically significantly higher in the DVT and CVT patients.

Factor V H1299R mutation was seen in 2.3% of the population; while it was found as 24.3% in our study (20). Prothrombin G20210A mutation was seen at 6% in VTD; while it was found as 7.5% in our study (2,10).

If the enzyme activity decreases as a result of the mutation in the MTHFR enzyme gene, the plasma homocysteine level increases and it is a risk factor for venous thrombosis (2,9,20). Sixty percent of the white race carry the MTHFR allele, of which 1.4-15% are TT (homozygous) (10). In our study, MTHFR C677T TT (homozygous) ratio was 10.3% in VTD patients.

MTHFRA 1298 C mutation has been detected as 4.8% in the VTD patients, and it has been reported to increase the VTD risk (20). In our

study, the rate of MTHFRA 1298 CC homozygous mutation was 14% in VTD patients. In addition, MTHFRA 1298 C mutation was statistically significant in the DVT patients.

In the Caucasian population, Factor XIII V34L polymorphism has been reported to be heterozygous by 32-43% and homozygous by 4-10% (21,22). In our study, the rate of Factor XIII V34L mutation was 30.8% in VTD patients, of which 1.9% were LL homozygous.

β-Fibrinogen-455 G/A (β Hae III) gene polymorphism occurs in 8-10% of the normal population (13,20). In our study, the rate of β-Fibrinogen -455G/A mutation was 57.9% in VTD patients, of which 10.3% were of AA homozygous genotype.

4G/5G genotype of PAI-4G&5G gene polymorphism, which increases the VTD risk, occurs in 48% of healthy individuals (10,20). In our study, the rate of PAI-4G&5G gene mutation was 73.8% in the patients, of which 19.6% were of 4G/4G homozygous genotype.

In the white race, the distribution of ACE gene polymorphism, which increases the VTD risk, is in the form of II genotype by 25%, ID genotype by 50%, and DD genotype by 25%. Angiotensin II forms are more common in the DD genotype (10,20,21). In our study, ACE gene mutation was detected as 82,2% in the VTD patients. Of these, 39.3% had D/D homozygous genotype. ACE gene mutation was statistically significantly higher in the female patients.

In East Asia, the rate of heterozygous HPA-1 (a/b) is 5-22%, and the rate of homozygous HPA-

1 (b/b) is 0-0.5% (10,24). In our study, the rate of heterozygous HPA-1 (a/b) was 28% and the rate of homozygous HPA-1 (b/b) was 2.8% in the VTD patients.

In Europe, of the population, 95% carries at least one apoE-e3 allele, 27% carries at least one apoE-e4 allele, and 16% carries at least one apoE-e2 allele (25). In our study, the of Apo E gene mutation was 30.3% in the VTD patients, of which 14% consisted of homozygous ApoE mutation. However, in our study, Apo E mutation rates were not statistically significant.

VTD frequently recurs (1). The recurrence rate of VTD is 12.9% within one year and 30.4% within ten years (2,3). The risk of recurrence is highest within the first 6-12 months (3,6). As long as the anticoagulant therapy is continued, the recurrence of VTD is prevented by 95%. However, VTD recurs in 20-50% of the patients within the first 10 years following the discontinuation of the treatment (6).

In those with two or more combined thrombophilic defects, VTD occurs at a younger age and the risk of recurrent VTD is higher (8). Life-long anticoagulant therapy is recommended for those with combined thrombophilic anomalies and to the high-risk group of recurrent VTD (8,9). A family screening may be performed to confirm thrombophilic disorder in a patient presenting with VTD (9).

The common venous thromboembolic disease in general population causes significant complications. Therefore, the etiology should be well known and appropriate measures should be taken. Nowadays, many mutations have been found to cause venous thromboembolic disease. The cause of hereditary thrombophilia may not always be antithrombin III, protein C or protein S deficiency. In this case, genetic mutations that are rarely seen in the diagnosis and treatment planning of VTD should be considered.

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