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Factor V Leiden mutation frequency and geographical distribution in Turkish population

Eray Yıldız[1], Funda Müşerref Türkmen[2]

[1]Department of Internal Medicine, Division of Allergy and Clinical Immunology, Meram School Medicine, Necmettin Erbakan University, Konya, Turkey
[2]Department of Internal Medicine, Haydarpasa Numune Education and Research Hospital, Istanbul, Turkey

ABSTRACT

Background and Objective: Thrombophilia is a term used to define the conditions creating a tendency toward thrombosis. Factor V Leiden (FVL) is the most frequently observed genetic risk factor, and its frequency varies among societies and ethnicities. In this study, our aim is to identify the frequency of FVL mutation in patients with thrombosis, the frequency of FVL mutation for each thrombosis disease, whether there is any difference in the geographical distribution of FVL mutation in the Turkish population, correlation with age and gender, and correlation with arterial and venous thrombosis. **Methods:** This is an observational case–control and retrospective study. Cases with the FVL mutation examination with clinical provisional diagnosis of arterial and/or venous thrombosis delivered and with the thrombosis proven by radiological visualization methods and laboratory examinations have been planned to be considered and assessed as cases with thrombosis. **Results:** A total of 67 patients with thrombosis and 22 patients without thrombosis have been included within the study. Twenty-six of the cases with thrombosis were from the Black Sea region, 21 were from Eastern Anatolia, 12 were from Central Anatolia, 5 were from Marmara, and 3 were from Southeastern Anatolia. Eleven of the cases without thrombosis were from the Black Sea region, 1 was from Eastern Anatolia, 5 were from Central Anatolia, 2 were from Marmara, 1 was from Southeastern Anatolia, and 2 were from the Aegean region. The significance was resulted from the identification of thrombosis prevalence rate as significantly high in the Eastern Anatolian region. **Discussion:** FVL mutation frequency is quite common in our country, and there are significant differences particularly in terms of regional distribution. Furthermore, FVL mutation is solely not the risk factor for thrombosis, and other coexisting genetic and acquired risk factors are substantial causes for the development of thrombosis.

Key words: Factor V Leiden mutation, thrombophilia, Turkish population

Address for Correspondence:
Dr. Eray Yıldız, Department of Internal Medicine, Division of Allergy and Clinical Immunology, Meram School Medicine, Necmettin Erbakan University, Konya, Turkey
E-mail: drerayyldz@gmail.com

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INTRODUCTION

Thrombophilia (Thrombo-philia: favoring thrombosis) is a term used to define the conditions creating a tendency toward thrombosis. Its pathogenesis is multi-factorial and includes acquired and genetic factors.[1-3]

Factor V Leiden (FVL) is the most frequently observed genetic risk factor, and its frequency varies among societies and ethnicities.[4-6] The frequency of FVL mutation in the Turkish population has been reported to be between 7.9% and 10.9%.[1,7] The FVL mutation carrier ratio has been determined as 7.9% in a trial conducted by Akar *et al.* with 4276 patients.[1] In a trial conducted with adult Turkish patients with thrombosis, the mutation frequency has been identified as 30.8% and in another trial, the frequency of heterozygote FVL mutation has been identified as 6.3%.[8,9]

Although individuals carrying inherited thrombophilia have a genetic risk and the risk of thrombosis increases accordingly, it is possible for them to not have any thrombotic episode throughout their lives. This situation indicates that genetic causes

solely are not sufficient and certain acquired factors do contribute to the development of thrombosis.[1,10,11]

FVL and PT G20210A gene mutations are the most frequently observed genetic risk factors for thrombosis, which bear a high risk in terms of venous thromboembolism. Numerous trials are available indicating the FVL gene mutation as the risk factor for venous thromboembolism. However, only a limited number of trials have been conducted with respect to their roles in arterial thrombosis, and the outcomes of such trials are controversial.[10,12-18]

Our objective in this trial designed to be an observational case–control and retrospective study is to identify the frequency of FVL mutation in patients with thrombosis, the frequency of FVL mutation for each thrombosis disease, whether there is any difference in the geographical distribution of FVL mutation in the Turkish population, correlation with age and gender, and correlation with the arterial and venous thrombosis.

METHODS

This trial has been designed to be an observational case–control and retrospective study and has been conducted at the Haydarpasa Numune Training and Research Hospital, Ministry of Health, Republic of Turkey. Cases with the FVL mutation examination with clinical provisional diagnosis of arterial and/or venous thrombosis delivered and with the thrombosis proven by radiological visualization methods (Doppler ultrasound, computed tomography, magnetic resonance imaging [MRI], or MRI angiography) and laboratory examinations have been planned to be considered and assessed as cases with thrombosis. Individuals with similar age and gender with thrombosis not identified as a result of radiological visualization methods and laboratory examinations have been taken as the control group.

Individuals between the ages of 15 and 80 with arterial and/or venous thrombosis identified clinically and the thrombosis diagnosis proven by appropriate radiological methods, with FVL mutation examination requested in terms of thrombosis etiology, not received warfarin treatment, and with age, gender, birthplace (the regions have been identified on the map of geographical regions of Turkey based on the birthplace of the patient), and family history information accessed have been included in the trial. Patients not fulfilling these criteria have been excluded from the trial.

The age, gender, birthplace, family history of thrombosis, medications used, thrombosis area (central/peripheral localization, arterial/venous localization) data, and the FVL mutation analysis outcomes of cases included in the trial have been recorded.

Ischemiccerebrovascular illness (CVI) localized within the central nervous system and thrombosis causing venous sinus thrombosis have been recorded as "central" while others have been recorded as "peripheral."

FVL mutation analysis has been studied by the real-time method. Dissolution processes have been performed in erythrocyte lysis, leukocyte extraction, leukocyte lysis, protein denaturation, DNA precipitation in isopropyl and 70% ethanol, and post–alcohol flushing TE (Tris-EDTA) buffer from genomic DNA extraction peripheral blood leukocytes from the blood taken.

In order for FVL to be identified, 5 microliter (100–300 ngr) genomic DNA in 20 microliter volume has been used and exposed to the PCR process in the LightCycler 2.0 device at 95^0C for 30 seconds of denaturation and 10 seconds at 55^0C in 45 cycles and 5 seconds at 72^0C. Finally, a melting curve analysis has been performed from 45^0C to 80^0C by increments of 0.1^0C. Mutant samples yielded the peak point at 55^0C in the melting analysis while the wild type samples reached the peak point at 65^0C.

Statistical analysis

The findings obtained from the trial have been assessed by using Number Cruncher Statistical System (NCSS) 2007&PASS 2008 Statistical Software (Utah, USA) for the statistical analysis. Student's *t*-test has been used for the comparison of the parameters, revealing a normal distribution between the two groups in the comparison of the descriptive statistical methods (mean, standard deviation, frequency) as well as the quantitative data. The Mann–Whitney U test has been used for the comparison of parameters not revealing a normal distribution between the two groups. The chi-squared test and Fisher's exact test have been used for the comparison of qualitative data. Significance has been assessed as $P < 0.05$.

RESULTS

A total of 67 patients with thrombosis and 22 patients without thrombosis have been included in the study. No statistically significant difference has been found between the mean age and age distribution of the cases with thrombosis and the control group ($P = 0.803$, $P = 0.751$, respectively) (Table 1).

When the thrombosis distribution was reviewed in cases with thrombosis, deep vein thrombosis (DVT) was observed most frequently followed by CVI and sinus thrombosis. Thrombosis localization was venous in 41 of

Table 1: Demographical features of cases with thrombosis and control group

	Cases with thrombosis (*n* = 67)	Control group (*n* = 22)	*P*
	Mean ± SD	Mean ± SD	
Age	42.94 ± 14.52	42.09 ± 11.19	0.803
Gender	*n* (%)	*n* (%)	
Female	37 (55.2%)	13 (59.1%)	0.751
Male	30 (44.8%)	9 (40.9%)	

the cases, while 26 has been identified as arterial. Again, 36 of the thrombosis cases were peripherally localized, while 31 were centrally localized. The family histories of four patients were positive. The general characteristics of patients with thrombosis are summarized in Table 2.

FVL mutation has been identified as positive in 18 (26.9%) cases with thrombosis and in 5 (22.7%) of the control group. Heterozygote has been identified as positive in 16 (23.9%) of the cases with FVL mutation with thrombosis while homozygote as positive in 2 (3%) (Table 3). No statistically significant difference was available in the ratios of FVL mutation positivity between patients with thrombosis and the control group ($P = 0.700$).

The positivity of family history in cases with thrombosis was 6% (Table 2). FVL mutation was identified to be positive in 1/4 (25%) of the cases with thrombosis with family history and in 17/63 (27%) of cases without family history. No statistically significant difference was available in the ratios of FVL mutation positivity in terms of the existence of family history ($P = 1.000$).

FVL mutation was identified to be positive in 5/26 (19.2%) of the cases with thrombosis localized as arterial and in 13/41 (31.7%) of cases with thrombosis localized as venous. No statistically significant difference was available in the ratios of FVL mutation positivity in terms of thrombosis localization ($P = 0.262$) (Table 4).

Table 2: General characteristics of cases with thrombosis

		n = 67	%
Thrombosis	Deep veins	21	31.3
	Cerebrovascular	18	26.9
	Venous sinus	13	19.4
	Portal vein	4	6.0
	Fistule	3	4.5
	Renal artery	3	4.5
	Splenic artery	2	3.0
	Superior mesenteric artery	2	3.0
	Left atrium	1	1.5
Thrombosis Localization	Arterial	26	38.8
	Venous	41	61.2
Thrombosis area	Central	31	46.3
	Peripheral	36	53.7
Family history	Yes	4	6.0
	No	63	94.0
Total		67	100

Table 3: FVL mutation distribution

		n	%
FVL Mutation	Negative	49	73.1
	Heterozygote	16	23.9
	Homozygote	2	3.0

Table 4: Thrombosis localization with FVL mutation in cases with thrombosis and assessment of area

		FVL Mutation		
		Negative (*n* = 49) *n* (%)	Positive (*n* = 18) *n* (%)	*P*
Thrombosis Localization	Arterial	21 (80.8%)	5 (19.2%)	0.262
	Venous	28 (68.3%)	13 (31.7%)	

Twenty-six of the cases with thrombosis were from the Black Sea region, 21 were from Eastern Anatolia, 12 were from Central Anatolia, 5 were from Marmara, and 3 were from Southeastern Anatolia. Eleven of the cases without thrombosis were from the Black Sea region, 1 was from Eastern Anatolia, 5 were from Central Anatolia, 2 were from Marmara, 1 was from Southeastern Anatolia, and 2 were from the Aegean region.

A statistically significant difference was available between the distribution of cases with thrombosis and the control group by region ($P = 0.031$). The significance was resulted from the identification of thrombosis prevalence rate as significantly high in the Eastern Anatolian region (Table 5).

DISCUSSION

Thrombophilia can be defined as an increase in the tendency of clotting in blood. It is a substantial cause of morbidity and mortality in clinical practice. Numerous genetic (primary) and acquired (secondary) factors causing thrombophilia have been identified. Genetic and acquired factors could be the cause of thrombophilia alone. However, in the majority of cases, both genetic and acquired causes coexist.[1]

FVL mutation is the most frequently observed genetic risk factor, and its frequency may vary among societies and ethnicities. The prevalence of FVL mutation has been reported to be 2%–14% within the normal population, and the difference in distribution has been attributed to ethnic and geographical changes.[1] The prevalence of FVL mutation is high among the European population (4%–15%). However, the incidence is rare in Asian, American, or African individuals.[4-6] Greece and Greek Cypriot societies experience the mutation with a high frequency in Europe. The mutation frequency in Greece has been reported as 14% and in Greek Cypriot society as 12%.[19] FVL mutation frequency in the Turkish population has been reported to be between 7.9% and 10.9%.[1,7] The trial conducted by Ekim *et al.* has reported the FVL mutation frequency as 19%.[20] Vurkun *et al.* has identified Active Protein C (APC) resistance in 22 (4.7%) individuals in a trial conducted with a total of 467 healthy subjects, where the APC resistance and FVL prevalence have been evaluated in a healthy population in Edirne. FVL mutation has been identified in 20 (4.28%) individuals; 18 of those were heterozygotes and 2 were homozygotes.[21]

Various outcomes have been obtained from trials conducted with patients with thrombosis. Gürgey *et al.* have identified that 44 (30.8%) of 146 patients with thrombosis have FVL mutation in a trial researching the frequency of FVL and PT G20210A conducted with adult Turkish patients with thrombosis. Seven (4.8%) homozygous and 38 (26%) heterozygous mutations have been identified among the cases with FVL mutation. PT G20210A mutation has been identified in 10 (6.8%) of 146 patients with thrombosis. In other six (4.1%) cases, both FVL and PT G20210A mutations have been identified.[8] Again, Küpeli *et al.*, in a trial conducted with a total of 80 venous thromboembolism patients, have identified the frequency of heterozygote FVL mutation as 6.3%.[9]

In our trial, FVL mutation has been identified as positive in 18 (26.9%) cases with thrombosis and in 5 (22.7%) of the control group. FVL mutation has been revealed to be positive in 23 (25.8%) of a total of 89 cases. Our outcomes are similar to the trial performed by Gürgey *et al.* [8] However, these are not in agreement with other studies and have higher values than other studies performed. Approximately, FVL mutation has been identified as positive in one out of four individuals and based on our trial, it can be suggested whether FVL mutation positivity is a gene mutation or a polymorphism. However, trials to be conducted with a more extensive and broader population could be useful in this regard.

The FVL mutation frequency in Turkish Cypriots has been revealed to be 12.2%.[22] Therefore, it could be said that the prevalence of this mutation is high in Turkish Cypriots. However, being an island society could be one of the factors affecting this situation. However, in other Turkish societies such as Azerbaijan and Kyrgyzstan, the

Table 5: Geographic distribution of cases with thrombosis and control group

		Cases with thrombosis	Control group
		n (%)	*n* (%)
Geographic area	Black Sea	26 (38.8%)	11 (50.0%)
	Eastern Anatolia	21 (31.3%)	1 (4.5%)
	Central Anatolia	12 (17.9%)	5 (22.7%)
	Marmara	5 (7.5%)	2 (9.1%)
	Southeastern Anatolia	3 (4.5%)	1 (4.5%)
	Aegean	0	2 (9.1%)
	Total	67	22

prevalence of FVL mutation is quite common.[23,24] This suggests that FVL mutation is a significant risk factor for thrombosis development in the Turkish population.

In spite of the rarity in identification of the mutation in the Far East and Africa, the high prevalence rates in Europe, Middle East, and particularly Anatolia, acting as a bridge between Asia and Europe, suggest that the mutation may have spread from Anatolia.

In the literature, FVL mutation was heterozygote in the majority of the patients. However, homozygote mutation was also existent although in a few.[8,20,21,25] It has been identified in our trial that 18 (26.9%) of 67 cases with thrombosis have FVL mutation. Heterozygote was existent in 16 (23.9%) of these 18 cases and 2 (3%) of them have homozygote mutation.

Although there are numerous trials indicating the coexistence of venous thrombosis with FVL mutation, there are a limited number of trials on the coexistence of FVL mutation and arterial thrombosis events such as cerebrovascular events, cardiac problems, and peripheral artery disease. Moreover, the outcomes of these conditions are inconclusive.[10, 12-18]

In a trial conducted by Rahimi *et al*., it has been identified that FVL mutation increases venous sinus thrombosis by 9.8-fold. In another trial, FVL has been identified as more common than controls in the patients with cerebral venous thrombosis.[13] Biswas *et al*. have identified in their trial that DVT frequency in individuals with FVL mutation has been increased by 13.7-fold.[14] Frederici *et al*. have revealed in their trial that FVL mutation increased the risk of recurrent venous thromboembolism by 59%.[15]

In a trial conducted by Pestana *et al*. in Venezuela with a large patient population that was diagnosed with DVT, acute myocardial infarction (AMI), and stroke, they have revealed that FVL mutation increased the risk for DVT by 4.2-fold. However, they could not observe a correlation between AMI and stroke and the FVL mutation.[10] In a study conducted by Ercan *et al*. in Turkey, the existence of FVL mutation has been identified not to increase the risk of coronary artery disease.[16]

Distinct from the trials arguing that the FVL mutation is not a risk factor for arterial thrombosis, in a trial conducted by Özmen *et al*. where the frequency of FVL, MTHFR, and PT gene mutations in arterial thrombosis has been researched, the frequency of FVL mutation has been found to be significantly higher than the control group in patients with peripheral arterial thrombosis.[17] Again, in the trial conducted by Kim *et al*., it has been identified that FVL, prothrombin, and genetic abnormalities specific to the homocysteine metabolism have increased the risk of myocardial infarction and ischemic stroke especially in young patients and females.[18]

In our trial, FVL mutation has been identified as positive in 7 (33.3%) patients out of 21 with DVT, 5 (38.5%) out of 13 patients with CVE, and 2 (11.1%) out of 18 patients with venous sinus thrombosis in the thrombosis case group. FVL mutation has been identified as negative in 21 (80.8%) out of a total of 26 patients with arterial thrombosis and as positive in 5 (19.2%) patients in the thrombosis case group. This suggests the coexistence of arterial thrombosis with FVL mutation in our trial.

Twenty-six (38%) out of 67 cases diagnosed with thrombosis were from the Black Sea region and comprised the highest rate. In the regional comparison of patients with thrombosis and the control group, the ratio of patients from the Eastern Anatolia region was high ($P = 0.031$). In consideration of the regional distribution of FVL positive cases with thrombosis in our trial, it has been revealed that the highest positivity was in the Central Anatolia region with 50%. In our patient group, FVL mutation has been identified as positive in 7 (26.9%) out of 26 patients from the Black Sea region, 2 (9.5%) out of 21 patients from the Eastern Anatolia region, 6 (50%) out of 12 patients from the Central Anatolia region, 2 (40%) out of 5 patients from the Marmara region, and 1 (33.3%) out of 3 patients from the Southeastern Anatolia region.

Anatolia is located at the center of three different continents hosting different civilizations. Therefore, it is an expected outcome that different frequencies of FVL mutations in different regions of the country exist accordingly. However, a fewer number of case–control subjects in our trial may have an influence on this outcome. No trial indicating regional differences in our country is available in the literature. Therefore, there is a need for the performance of such a trial. In case the regional differences can be supported by major trials, substantial information and data can be obtained in genetic terms.

In conclusion, we could say that FVL mutation frequency is quite common in our country and there are significant differences particularly in terms of regional distribution. However, it would be highly convenient and appropriate for more extensive trials with broader populations to be conducted in terms of regional distribution. Furthermore, we could conclude that FVL mutation is solely not the risk factor for thrombosis and other coexisting genetic and acquired risk factors are substantial causes for the development of thrombosis. The major limitations of

this study are the low number of patients and that it is conducted at a single center.

Conflict of Interest

None declared.

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