

## Are Prothrombotic Mutations a Time-to-Event Risk Factor?

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### ABSTRACT

**Background:** Deep vein thrombosis (DVT) represents a common disorder involving genetic and acquired risk factors. It has been proposed that acquired risk factors are more important with aging than genetic factors, indicating different prevalence of prothrombotic mutations throughout the lifespan.

**Objective:** To determine the role of the most frequent prothrombotic genetic risk factors (Factor V [FV] Leiden and Factor II [FII] G20210A mutations) in first-time DVT etiology in patients of different ages.

**Method:** This retrospective study included 701 patients living in Serbia with diagnosed DVT as a first-time thrombotic event.

**Results:** Risk assessment for mutations as age-related markers showed no statistical difference (FV Leiden mutation—OR, 1.027; 95% confidence interval [CI], .87–1.22;  $P = .76$  and FII G20210A mutation—OR, 0.940, 95% CI, .74–1.19;  $P = .61$ ). Our results show similar mutation prevalence regardless of how old the patients were at the time of the first DVT occurrence.

**Conclusion:** Our results indicate that these 2 mutations cannot be used as prognostic marker for time-to-event first DVT in the Serbian population; however, further studies are required.

**Keywords:** FV Leiden, FII G20210A, thrombophilia, deep venous thrombosis, aging, prognostic marker

Deep vein thrombosis (DVT) represents a common disorder with an overall incidence of 1 to 2 per 1000 per year, in which a blood clot forms inside a blood vessel and obstructs blood flow, resulting in blockage of the vessel. It is a multifactorial condition that involves genetic and acquired risk factors.<sup>1,2</sup>

The most important acquired risk factors for DVT are: age, trauma, surgical procedures, immobilization, pregnancy, oral contraceptives and malignant neoplasms.<sup>3,4</sup> The incidence

of thrombotic events increases with age and rises to nearly 1 in 100 per year in patients older than 75 years.<sup>5-7</sup>

Although the frequency of thrombosis occurrence sharply increases with advancing age and represents a significant health issue, data regarding the underlying mechanisms and risk factors in elderly patients are scarce. That population is underrepresented in a number of research studies; also, a limited number of studies are focused on genetic risk factors in patients with DVT who are of advancing age.<sup>8-10</sup>

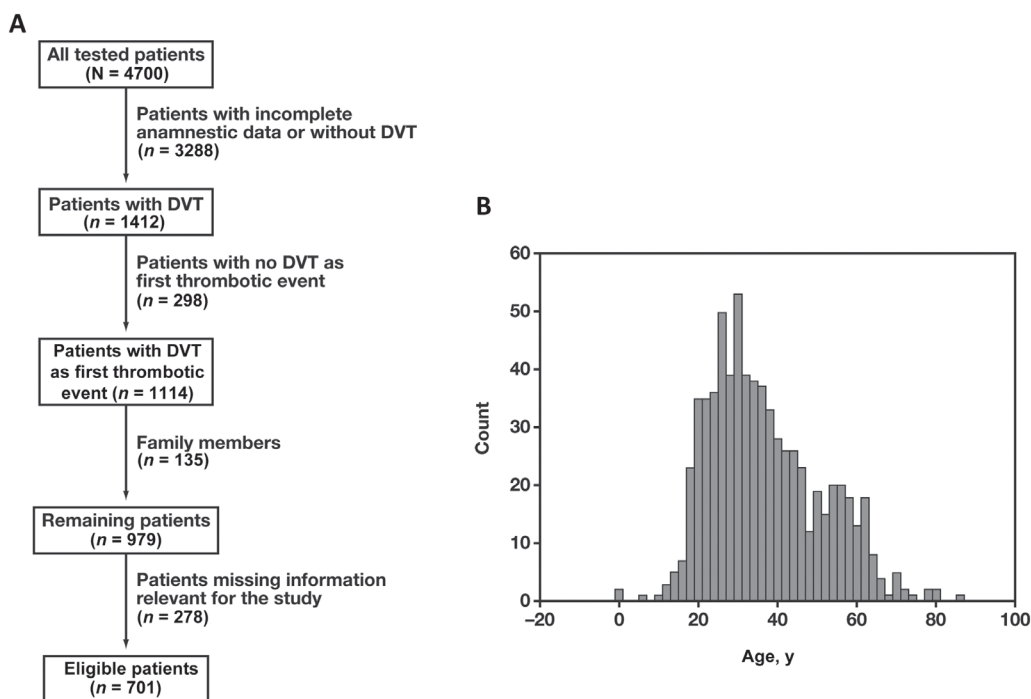
The Factor V (FV) Leiden and Factor II (FII) G20210A mutations are the most frequent prothrombotic genetic risk factors.<sup>1,11</sup> The FV Leiden mutation results in partial resistance of Factor V to the inactivation by its natural inhibitor-protein C.<sup>12</sup> The FII G20210A mutation is associated with increased plasma level of prothrombin.<sup>13</sup> These mutations are correlated with an increase of thrombin generation, leading to thrombosis occurrence. Both mutations are common in healthy white populations (3%–15% and 1%–6%, respectively), with their frequencies increased in patients with DVT, namely, to 15% to 50% for FV Leiden and 6% to 18% for the FII G20210A variant.<sup>13-15</sup> It has been proposed<sup>16,17</sup>

### Abbreviations

DVT, deep vein thrombosis; FV, Factor V; FII, Factor II; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; IQR, interquartile range; OR, odds ratio; CI, confidence interval; wt, noncarriers of mutation; mut, carriers of mutation

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**Figure 1**

Patients in our study. **A**, Selection of the patients for the study. **B**, Distribution of age at onset of first occurrence of deep vein thrombosis (DVT).

that genetic risk factors are relevant for DVT occurrence in young individuals, whereas acquired risk factors are considered more important in elderly patients.

According to this statement, one might assume that the prevalence of these mutations would be less in older patients at first DVT occurrence, compared with younger patients at first DVT occurrence. We investigated the prevalence of the 2 most common prothrombotic genetic risk factors, namely, FV Leiden and FII G20210A mutations, to determine whether they are associated with age at first DVT occurrence.

## Materials and Methods

### Study Design

Patients were selected by searching the Institute of Molecular Genetics and Genetic Engineering, University of Belgrade (IMGGE) database of 4700 patients originating from the geographic area of Serbia (Figure 1). All patients were diagnosed by physicians according to standard

protocols and were referred for routine genetic thrombophilia testing in our institute from 2003 through 2015. Anamnestic data were gathered from the medical histories of the patients. Only patients with objectively diagnosed DVT (via color duplex ultrasonography and/or venography) were considered as possible study subject individuals, and patients who had experienced DVT as their first thrombotic event were selected. We excluded family members of selected patients from the study group (Figure 1). This study was approved by the ethics committee of the IMGGE, Serbia.

### Blood Specimens

Blood specimens from all subjects were taken, with 3.8% sodium citrate as the anticoagulant. Genomic DNA was purified from whole blood using the QIAamp DNA Blood MiniKit (QIAGEN) according to the manufacturer standard protocol. DNA specimens were stored at  $-20^{\circ}\text{C}$  for further usage.

### Polymerase Chain Reaction (PCR)–Restriction Fragment Length Polymorphism (RFLP) Analysis

We tested all subjects for FV Leiden and FII G20210A mutations using PCR-RFLP analysis, as previously described.<sup>18</sup>

The PCR products for FV Leiden mutation detection (generated by primers: 5'TGCCAGTGCTTAACAAGACC3' and 5'TGTTATCACACTGGTGCTAA3') were digested by MnlI (New England BioLabs) restriction enzyme. The PCR products for FII G20210A mutation detection (generated by primers: 5'TCTAGAAACAGTTGCCTGGC3' and 5'ATAGCACTGGGAGCATTGAAGC3') were digested by HindIII (New England BioLabs, Inc) restriction enzyme. Nonmutated and mutated alleles were distinguished by the size of the restriction fragments, using electrophoresis on 10% polyacrylamide gels and visualized by silver staining.

### Statistical Analysis

We performed statistical analysis by using Statistical Package for Social Sciences (SPSS) software, version 20.0, for Windows (SPSS Inc). Data distribution of continuous variables was presented by median and interquartile range (IQR). We performed age distribution between studied groups via Mann-Whitney testing. Risk assessment was presented by odds ratio (OR), 95% confidence interval (CI), and *P* value. *P* values of less than .05 were considered statistically significant. In the assessment of prognostic marker for DVT event, we used multinomial logistic regression, with risk assessment for both examined mutations. Also, risk factors were estimated for their contribution to onset of DVT occurrence using Kaplan Meier analysis and Cox regression.

## Results

The study group baseline data are shown in **Table 1**. The sex ratio was balanced, with a median age of 39 years and a median age at first DVT of 34 years. One quarter of the patients carried FV Leiden mutation (23.1% heterozygous and 1.9% homozygous carriers), whereas 11% carried FII G20210A mutation (10.6% heterozygous and 0.4% homozygous mutation). Concerning the low frequency of individuals who carried the homozygous mutation, we analyzed mutation carriers regardless of homozygous/heterozygous type (hereinafter, carriers).

The median age of first DVT episode was 33 years for FV Leiden (range, 15–78 years), 34 years for FII G20210A (range, 13–79 years), and 31 years (range, 20–80 years) for carriers of both mutations (IQR: 20, 23, and 23, respectively). Median age of first DVT episode for individuals not carrying a mutation (hereinafter, noncarriers) was 34 years

**Table 1. Baseline Characteristics of the Study Group<sup>a</sup>**

Variable	Units of Measurement
Median age, y (IQR)	39 (21)
Median age of first DVT occurrence, y (IQR)	34 (20)
Sex, %	
Male	48.9
Female	51.1
FV Leiden mutation carrier, no. (%)	175 (25.0)
FII G20210A mutation carrier, no. (%)	77 (11.0)

IQR, interquartile range; DVT, deep venous thrombosis; FV, Factor V; FII, Factor II.  
<sup>a</sup>*n* = 701.

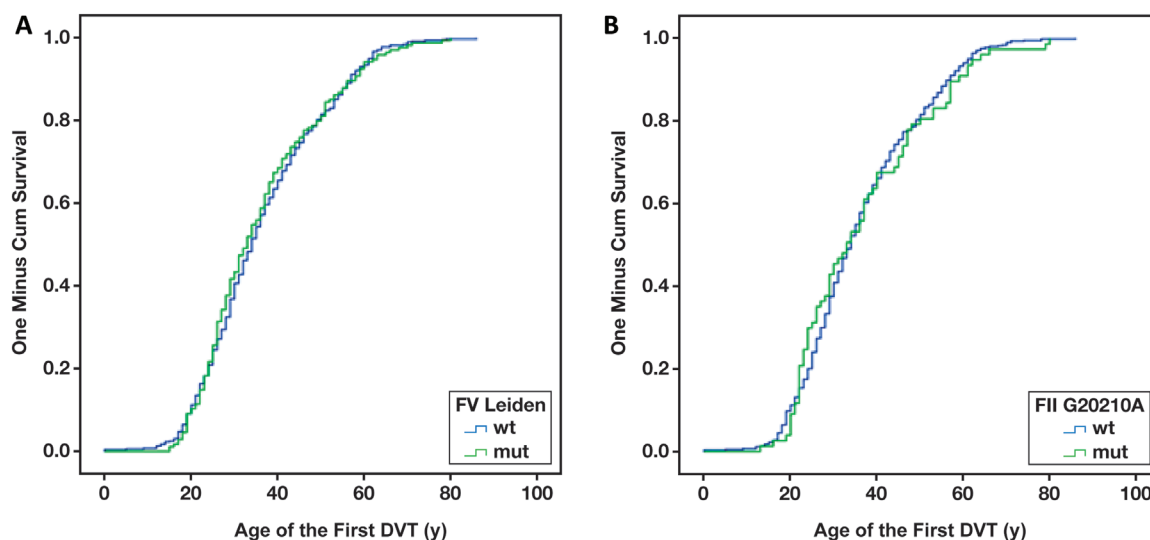
(range, 0–86 years; IQR, 19). Time-to-event analysis of the first DVT occurrence revealed no statistically significant difference between carriers and noncarriers for both mutations (*P* = .86 for FV Leiden and *P* = .53 for FII G20210A mutations) (**Figure 2**).

Risk assessment in time of first DVT occurrence was calculated for both mutations and for sex as age-related markers. The results showed that these mutations are not relevant for age at first DVT onset (**Table 2**). However, age at onset of first DVT episode was shown to be statistically relevant by patient sex (*P* = .03).

To determine the nature of this influence, patients were stratified according to the sex and prothrombotic, FV Leiden and FII G20210A, mutation carrier status (**Table 3**). Differences in age at first DVT occurrence were statistically significant between the sexes regardless of tested mutations (*P* = .01). No statistical significant differences in age at first DVT occurrence between the sexes for patients with the same prothrombotic mutation status (including noncarriers) were detected. The median age at first DVT does not differ significantly between males with different prothrombotic mutation status (range of median age 36–37 years). Also, similar results were observed for female patients (range of median age 26–32 years) (**Table 3**).

## Discussion

Thrombophilia is a frequent, age-related, multifactorial disease with environmental and genetic risk factors. However, only a limited number of studies report the impact of genetic risk factors on first DVT occurrence in relation to



**Figure 2**

Onset of first deep venous thrombosis (DVT) occurrence depending on presence of mutation (wt represents noncarriers of mutation; mut, carriers of mutation). **A**, FV Leiden mutation. **B**, FII G20210A mutation.

**Table 2. Risk Assessment for FV Leiden and FII G20210A Mutations and Sex as Age-Related Markers for First-Time Deep Vein Thrombosis**

Risk Factors	P value <sup>a</sup>	OR	95% CI	
Sex	.03	1.176	1.013	1.365
FV Leiden mutation	.76	1.027	0.865	1.220
FII G20210A mutation	.61	0.940	0.740	1.193

FV, Factor V; FII, Factor II; OR, odds ratio; CI, confidence interval.

<sup>a</sup>Determined via Cox regression analysis.

age. Advancing age represents a risk factor, and there is evidence of higher risk for elderly individuals for developing thrombotic events.<sup>19-21</sup> According to the Physician's Health Study, the thrombotic risk increases at a greater rate with advancing age in men with a Factor V Leiden mutation, which indicates that determination of Factor V Leiden mutation status should not be limited to young patients.<sup>22</sup> However, there is a tendency to exclude older patients from genetic thrombophilia screening because acquired risk factors are considered more important in these patients.<sup>23</sup> Taking this tendency into account, one might expect that frequencies of prothrombotic mutations in patients experiencing their first DVT would decrease with aging.

Detected prevalence of FV Leiden and FII G20210A mutations in patients with DVT as the first thrombotic event

(25% and 11%, respectively) corresponds to published data for the Serbian population.<sup>24</sup> Analysis of the effects of prothrombotic mutations as prognostic markers for onset of first DVT occurrence revealed that age of thrombotic event in the Serbian population is not influenced by studied mutations (**Table 2**). Our results show there is no statistically significant difference in mutation prevalence regarding age of patients in Serbia with developed first thrombotic event. FV Leiden and FII G20210A mutations are a prothrombotic risk factors equally contributing during the entire lifespan with no preference for DVT as the first thrombotic event developing at any age (**Figure 2**). Data from the current literature regarding this issue are inconsistent;<sup>25-27</sup> the clinical relevance of these findings still remains to be assessed.

The findings of recent studies suggest that risk factors for thrombosis in a young population are also risk factors for the elderly population. These findings are in concordance with those of our study.<sup>28</sup>

Previous studies, examining age at first DVT occurrence, were focused on patient groups stratified according to defined age cut-off values. However, there is no consensus in the literature regarding age cut-off values.<sup>7,9,29,30</sup> Results in our study cohort, using different age cut-off values, showed no statistically significant differences in prevalence of these mutations among groups (data not shown).

**Table 3. Age at First Occurrence of Deep Vein Thrombosis by Sex and the Prothrombotic Gene Mutations**

Sex	FV Leiden Mutation	FII G20210A Mutation	Patients, No.	Age, y (Median)	Age, y (range)	IQR	P Value <sup>a</sup>
Female	Regardless to Mutational Status		358	32	0–86	18	.01
Male			343	36	0–79	23	
Female	Noncarrier	Noncarrier	238	32	0–86	19	.24
Male			229	36	0–70	20	
Female	Noncarrier	Carrier	31	30	13–61	22	.09
Male			28	36.5	19–79	32	
Female	Carrier	Carrier	9	26	22–80	19	.49
Male			9	37	20–66	28	
Female	Carrier	Noncarrier	80	31	17–68	13	.05
Male			77	36	15–78	25	

*FV, Factor V; FII, Factor II; IQR, interquartile range.*  
<sup>a</sup>Determined via Mann-Whitney testing.

Beside mutations, sex could impact the age at development of DVT as the first thrombotic event. In our study, sex is a statistically relevant factor in onset of the first DVT occurrence (Table 2). Our study shows that females develop DVT as the first thrombotic event earlier than males (median age, 32 years vs 36 years). Calculation of prothrombotic mutation status as an additional factor leads to lack of statistical significance. Thus, this difference is not influenced by prothrombotic mutations status and probably is connected to other factors (such as genes or hormonal status).

This single-center study was conducted in a large group of patients with DVT selected from a large database. Our study group was selected from the database of 4700 patients referred for routine thrombophilia testing in Serbia. Because our group has a balanced sex ratio and wide age range (from 0–86 years), it represents a strong study group for testing the influence of prothrombotic genetic markers in age at first onset of DVT. To our knowledge, this study is the largest cohort of patients in Serbia with a first DVT occurrence in a wide age range. However, triage of elderly patients by physicians represents a limitation of our study because a large number of these patients are not referred for routine genetic thrombophilia testing.

## Conclusions

Our results indicate that FV Leiden and FII G20210A mutations are equally contributing risk factors of developing DVT as the first thrombotic event in a population in Serbia of a

wide range of ages. Nevertheless, these mutations cannot be used as prognostic marker of age at development of DVT as the first thrombotic event. Generally, females develop first DVT earlier, compared with males, regardless of prothrombotic mutation status. Also, we performed this study in a population of patients living in Serbia; the results may not apply to other population groups. For complete information regarding the influence of prothrombotic mutations, further larger studies in different populations will be required. **LM**

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