The 3'End Prothrombin Gene Variants in Patients With Different Thrombotic Events

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ABSTRACT

Background: Prothrombin (FII) *A19911G* and *C20221T* gene variants are associated with increased prothrombin levels and potentially represent thrombotic risk factors.

Objective: To determine the frequency of *A19911G* and *C20221T* FII gene variants in patients with thrombotic disorders and in women who have experienced pregnancy loss (PL).

Methods: We determined the frequency of these variants in 133 patients with deep venous thrombosis (DVT), 80 patients with isolated pulmonary embolism (PE), 101 patients with idiopathic PL, and 180 control individuals.

Results: The FII *A19911G* variant was more prevalent in patients with DVT and with PL compared with controls; however, these differences were not statistically significant. The *19911GG* genotype was associated with increased risk of PE (odds ratio, 1.91; 95% confidence interval, 1.04-3.51). We did not detect carriers of the FII *C20221T* gene variant in this study.

Conclusions: This is the first study, to our knowledge, that demonstrates the FII *19911GG* genotype may represent a risk factor for isolated PE. Also, our results show that the FII *C20221T* is a rare variant in this population and therefore, routine thrombophilia screening should not include screening for this genotype.

Keywords: FII *A19911G*, FII *C20221T*, venous thrombosis, pulmonary embolism, pregnancy loss, 3'end of the prothrombin gene

The most common causes of thrombophilia in the white population are 2 functional mutations in the genes that code for coagulation factor V (FV) and prothrombin (FII).[1] The first is a G to A substitution at gene position 1691 (FV Leiden), resulting in resistance of mutant FV to the activated protein C.[2] The FII *G20210A* gene variant is located in the 3′-untranslated region of the FII gene and is associated with increased plasma levels of FII.[3]

Heterozygous carriers of the *G20210A* variant have a 3-fold increased risk of venous thrombosis.[1,3] However, it has been shown[4] that some patients with a history of thrombosis and who have increased FII levels are not carriers of the *G20210A* gene variant. This finding could imply the presence of other variants in the 3′-region of the FII gene that affect the level of FII and, consequently, the prothrombotic risk.

Ceelie et al[5] reported that the *A19911G* variant, located in the last intron of the FII gene, is associated with a slightly increased plasma FII level, such that homozygous carriers of the *19911G* allele had 8 U/dL higher plasma FII level compared with carriers of the *19911A* allele.[5] In an in vitro functional study, von Ahsen and Oellerich[6] reported that the presence of the *19911G* variant leads to approximately 30% higher splicing efficiency of the final FII intron.[6] To date, the role of the *A19911G* gene variant in the pathology of thrombotic disorders and recurrent pregnancy loss (PL) has not been thoroughly studied.

Another variant in the 3′ region of the FII gene was discovered in a 9-year-old-boy after an acute vascular rejection of renal transplant due to arterial thrombosis.[7] Further investigations revealed novel C to T substitution at

Abbreviations

FV, factor V; FII, prothrombin; PL, pregnancy loss; bp, base pair(s); mRNA, messenger RNA; DVT, deep venous thrombosis; PE, pulmonary embolism; PCR-RFLP, polymerase chain reaction–restriction fragment length polymorphism; *Pc*orr, corrected *P* value; OR, odds ratio; CIs, confidence intervals; MEGA, Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis; NA, not applicable; RFPL, restriction fragment length polymorphism

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position 20221, only 11 base pairs (bp) downstream from the *G20210A* gene variant. One year later, researchers[8] described the case of a 28-year-old patient who was a heterozygous carrier of the *C20221T* variant with FII activity of 115%, who experienced Budd-Chiari syndrome and thrombocytosis. The third reported case of the *C20221T* variant was in a 40-year-old South Asian woman with a family history of thrombosis, a poor obstetric history (2 PL, pre-eclampsia and intrauterine growth restriction), and FII activity of 134%.[9] To date, no data are available regarding the frequency of the *C20221T* variant in patients with thrombotic disorders. However, it has been shown in vitro[10] that *C20221T* is a functional gene variant that enhances 3′ end formation of FII messenger RNA (mRNA), leading to an increased plasma FII level.[10]

The aim of our study was to determine the frequency of *A19911G* and *C20221T* FII gene variants in patients with thrombotic disorders. We also aimed to determine the frequency of these variants in women who experienced PLs.

Materials and Methods

Our study included 314 patients and 180 healthy individuals from the geographic region of Serbia. Patients were divided into 3 study groups: the first group included 133 patients (mean [SD] age, 37 [11.01] years, 82 men and 51 women) with deep venous thrombosis (DVT); the second group included 80 patients (mean [SD] age, 41 [13.15] years, 46 men and 34 women) with isolated pulmonary embolism (PE); and the third group consisted of 101 women with idiopathic PL (mean [SD] age, 33 [5.24] years). All patients had been referred between 2000 and 2010 to the Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia, for thrombophilia screening. They were referred for genetic testing after experiencing thrombotic events (diagnosed via color duplex, ultrasonography, and/or scintigraphy) or PL. We gathered anamnestic data from all the participants; next, we excluded patients with malignant neoplasms, diabetes mellitus, antiphospholipid antibodies, and deficiency of natural coagulation inhibitors (antithrombin, protein C, and protein S). After genetic thrombophilia testing, carriers of FV Leiden and FII *G20210A* mutations were also excluded from the study. The study groups were age and sex matched with control group, which consisted of 180 healthy blood donors (mean [SD] age, 39 [10.39] years, 86 men and 94 women) and 74 healthy women (mean [SD] age, 38 [9.42] years) with at least 1 live birth and without PL. This study was approved by the ethics committee of Blood Transfusion Institute of Serbia.

Peripheral blood was collected in tubes containing 3.8% sodium citrate as the anticoagulant. We purified genomic DNA from 200 µL of whole blood using the QIAamp DNA Blood Mini Kit (QIAGEN N.V., Venlo, Netherlands) according to the protocol provided by the manufacturer.

The *A19911G* and *C20221T* variants were detected by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis. The primer sequences, annealing temperatures for PCR reactions, restriction enzymes, and fragments lengths are given in **Table 1**. We distinguished wild type and mutated alleles by the size of the restriction fragments, using electrophoresis on 10% polyacrylamide gels, visualized by silver staining (**Figure 1**).

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software, version 13.0 for Windows (SPSS Inc., Chicago, IL). Hardy-Weinberg equilibrium for patient and control groups were compared using the χ^2 test. Fisher exact test was used to compare the prevalence of each mutation between patient and control groups. For multiple comparisons, we used the p.adjust function from the R software package (version 2.15.3) to perform Holm posthoc analysis.[11] In those cases, we report the *P* values of the Fisher exact test and its corrected value (*P*corr). We estimated the odds ratio (OR) and the 95% confidence intervals (CIs) separately for each mutation. We considered a *P* value of .05 or less to be statistically significant.

Results

Among 213 patients with a history of thrombotic events, 133 had DVT and 80 had developed isolated PE. Of the 133 patients with a history of DVT, 85 had had 1 or more DVT episodes (63.9%), 32 patients had DVT that was complicated with PE (24.1%), 10 patients had DVT and PL (7.5%), 1 patient had DVT and myocardial infarction (0.8%), 1 patient experienced DVT and cerebral vascular insult (0.8%), and 4 patients had DVT complicated with 2 or more other thrombotic events (3.0%). In the PE group of 80 patients, 71 had had 1 episode of PE and 9 had recurrent isolated PE. Among 101 female patients who had experienced PL, 93 had recurrent PL; more than 291 cases of PL were reported overall by this group.

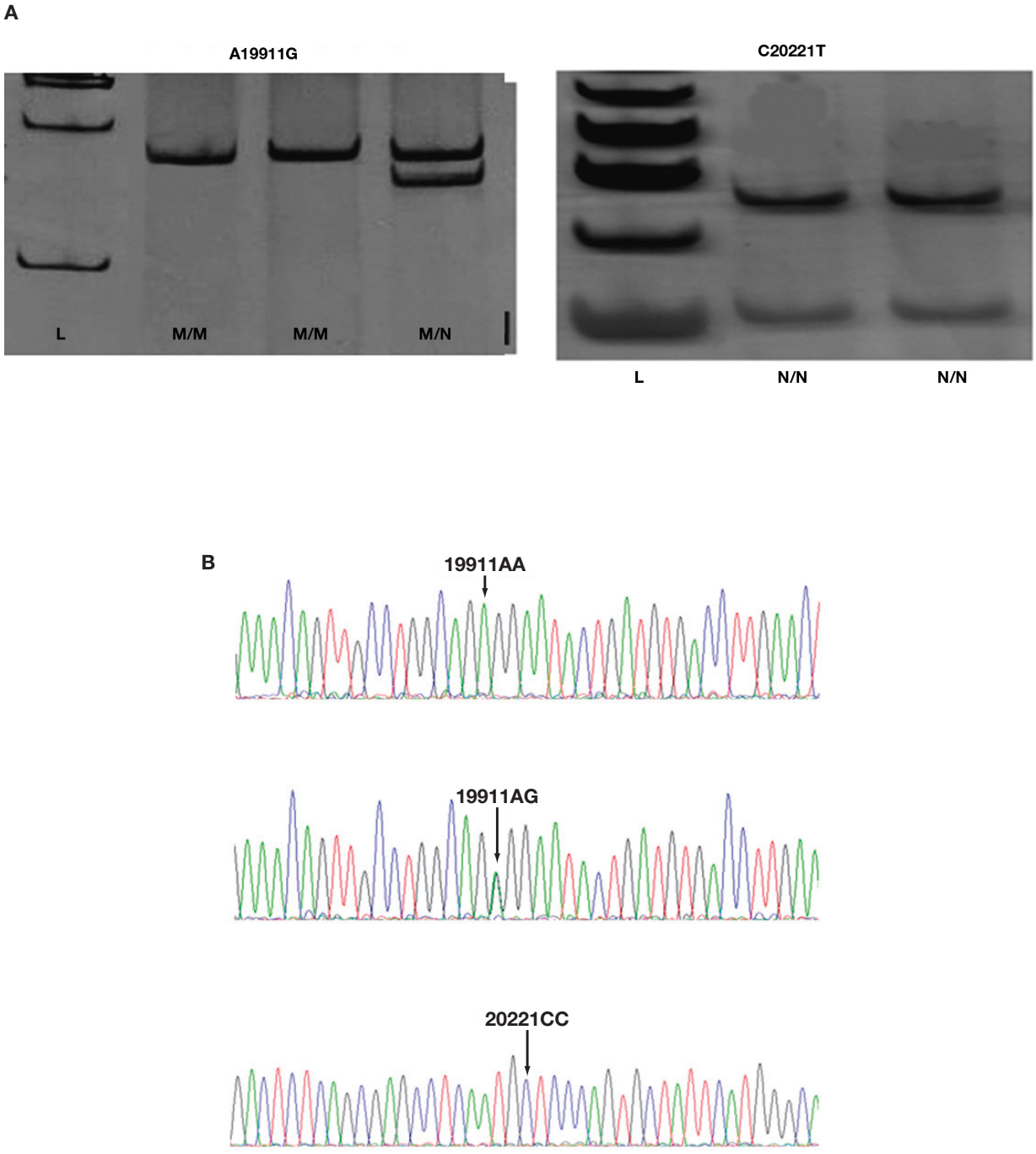

Figure 1

Validation of polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) methods for detection of prothrombin (FII) *A19911G* and FII *C20221T* gene variants. L indicated DNA ladder; N, wild type; M, mutant. **A**, Restriction fragment length polymorphism (RFPL) analysis conducted on the 10% polyacrylamide gels. **B**, Sequence analysis conducted on the 3130 Genetic Analyzer (Carlsbad, CA, Life Technologies).

Table 1. Primer Properties and Restriction Enzymes Used to Detect FII *A19911G* and FII *C20221T* Variants

Gene Variant	Primer (5′-3′)	Annealing Temperature (°C)	PCR Product Size (bp)	Restriction Fragment Size (bp)		Restriction Enzyme
				Wild type	Mutant	
A19911G	TCTAGAAACAGTTGCCTGGC ATGCACTGGGAGCATTGAAGC	56	345	326, 19	345	EcoNI
C20221T	GGATGGGAAATATGGCTTCTAC GGCTGAGGTGGGAGGAT	60	323	195, 108, 20	215, 108	MwoI

FII, prothrombin; PCR, polymerase chain reaction; bp, base pair.

Table 2. Frequency of FII *A19911G* and FII *C20221T* Gene Variants in Control Individuals and Women With Idiopathic PL

FII Genotype	Controls (n = 74) No. (%)	PL Group (n = 101) No. (%)	OR (95% CI) *P* Value
19911AA	24 (32.43)	22 (21.78)	0.58 (0.29-1.14) 0.12
19911AG	38 (51.35)	54 (53.47)	1.09 (0.60-1.98) 0.78
19911GG	12 (16.22)	25 (24.75)	1.69(0.79-3.65) 0.17
20221CT or 20221TT	0	0	NA

FII, prothrombin; PL, pregnancy loss; OR, odds ratio; CI, confidence interval; NA, not applicable.

Table 3. Frequency of FII *A19911G* and FII *C20221T* Gene Variants in Control Individuals and Patients With Thrombotic Events[a]

FII Genotype	Control Individuals (n = 180) No. (%)	DVT Group (n = 133) No. (%)	PE Group (n = 80) No. (%)	DVT Group vs Controls *P (Pcorr)* OR (95% CI)	PE Group vs Controls *P (Pcorr)* OR (95% CI)
19911AA	57 (31.67)	36 (27.07)	23 (28.75)	0.38 (0.76) 0.80 (0.49-1.31)	0.64 (0.76) 0.87 (0.49-1.55)
19911AG	90 (50)	66 (49.62)	33 (41.25)	0.95 (0.95) 0.99 (0.63-1.54)	0.19 (0.38) 0.70 (0.41-1.20)
19911GG	33 (18.33)	31 (23.31)	24 (30)	0.28 (0.28) 1.35 (0.78-2.35)	0.04 (0.08) 1.91 (1.04-3.51)
20221CT or 20221TT	0	0	NA	NA	NA

FII, prothrombin; DVT, deep venous thrombosis; PE, pulmonary embolism; Pcorr, corrected P value; OR, odds ratio; CI, confidence interval; NA, not applicable
[a]Pcor values were determined after Holm posthoc analysis.

The screening results for FII *A19911G* and FII *C20221T* gene variants are given in **Table 2** and **Table 3**. Data from the observed groups of patients (DVT $\chi^2 = 0$; PE $\chi^2 = 2.45$; PL $\chi^2 = 0.5$) and control groups were consistent with Hardy-Weinberg equilibrium predictions.

In our study, we did not observe a statistically significant difference in the frequency of FII *A19911G* genotypes between patients with DVT and control individuals. However, carriers of the GG genotype had a slightly increased risk for DVT (OR, 1.35; 95% CI, 0.78–2.35) (**Table 3**).

Among 101 women with idiopathic PL, the 19911GG genotype was overrepresented in the patients vs the control group (24.8% and 16.2%, respectively; OR, 1.69; 95% CI, 0.79–3.65). However, the difference was not statistically significant (**Table 2**).

In the PE group, carriers of the *19911GG* genotype had an increased risk for isolated PE (OR, 1.91; 95% CI, 1.04–3.51). Increased FII *19911GG* genotype frequency in the PE group vs the control group was statistically significant by Fisher exact test (*P* = .04). However, after correction for multiple

comparisons, the *P*corr value was .08 (**Table 3**). We did not detect the FII *C20221T* variant in the patients or control individual included in our study (**Table 2** and **Table 3**).

Discussion

In this study, we examined the frequency of the FII *A19911G* and *C20221T* variants in 3 groups of patients: 133 patients with DVT, 80 patients with isolated PE, and 101 women with PL. Several studies[5,12,13,14] have investigated the frequency of the FII *A19911G* variant in various populations; however, to date, the population from geographic area of Serbia has not been investigated, to our knowledge. Our results show that the FII *A19911G* variant does not represent a significant risk factor for the occurrence of DVT in the Serbian population. Pérez-Ceballos et al,[14] in a study of the Spanish population, also found no statistical difference in the prevalence of the FII *A19911G* polymorphism between 204 patients and 204 controls.[14] The prevalence of the FII *A19911G* variant was similar in ethnic Dutch and Italian populations,[5,13] which suggests that the FII *A19911G* variant does not represent a major risk factor for thrombotic disorders. The results of a recent study by Martinelli et al,[15] which investigated the risk of cerebral sinus-venous thrombosis in patients with *A19911G* polymorphism, also showed that this polymorphism does not represent a significant risk factor for thrombotic disorders. However, a report[16] on the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study, which included more than 5000 patients and 5000 controls, showed that presence of the *19911GG* genotype represents a 1.43-fold greater risk for thrombotic occurrence (95% CI, 1.27–.67) compared with the *19911AA* genotype.

In the group of women with PL, we observed a slightly higher frequency of the *19911GG* genotype. This finding is in agreement with the results reported by Cao et al,[17] who had observed no difference in the genotype and allelic frequencies between patients with PL and healthy controls selected from an Chinese population.

This is the first study, to our knowledge, in which the presence of the *19911GG* gene variant appears to be a risk factor for isolated PE. These results contribute to those of previous studies[18,19,20] in which 2 main manifestations of venous thromboembolism, PE and DVT, are shown to have different risk factors.

The FII *C20221T* gene variant was first described in 2001 in a 9-year-old patient with thrombosis.[7] To date, several sporadic cases have been described;[7,8,9] however, the frequencies of the *C20221T* variant in patients with thrombotic disorders and in healthy individuals remain unquantified. To our knowledge, ours is the first study that aimed to determine the frequency of the FII *C20221T* gene variant. We did not detect any carriers of the FII *C20221T* gene variant in the patient or control groups; this finding suggests that the entity is a rare variant, with a prevalence of less than 1% in the population from geographic area of Serbia. Although this is a functional variant that leads to increased FII plasma levels, its low frequency indicates that this *C20221T* variant should not be included in routine thrombophilia screening. However, screening for the *C20221T* gene variant as a potential risk factor may be helpful when other genetic risk factors have been ruled out.

In conclusion, we did not observe a statistically significant increase of FII *A19911G* variant frequency in patients with DVT and PL. However, this is the first study to demonstrate an association between the FII *19911GG* genotype and an increased risk for isolated PE. We did not detect the FII *C20221T* variant in our study; to elucidate the role of this FII variant in thrombotic disorders, further research with a larger cohort will be necessary.

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