

## FV LEIDEN MUTATION AND DEEP VEIN THROMBOSIS IN VOJVODINA: A CASE-CONTROL STUDY

FV LEIDEN MUTACIJA I TROMBOZA DUBOKIH VENA U VOJVODINI: STUDIJA ASOCIJACIJE

Iva Salatić<sup>1</sup>, Katarina Kiralj<sup>2</sup>, Gorana Mitić<sup>3</sup>, Igor Veselinović<sup>1</sup>, Dušan Vapa<sup>1</sup>

<sup>1</sup>Center of Forensic Medicine

<sup>2</sup>Clinic for Infectious Diseases

<sup>3</sup>Center for Laboratory Medicine, Clinical Center of Vojvodina, Novi Sad, Serbia

**Summary:** Between September 2007 and February 2010, the occurrence of symptomatic deep vein thrombosis (DVT) was investigated in a cohort of 79 consecutive patients. A case-control study included 71 healthy controls matched with cases for sex and age. The prevalence of factor V G1691A mutation genotype was analyzed. Eighteen cases (22.79%; 95% confidence interval (CI) 13.53% to 32.03%) and four controls (5.63%; 95% CI 0.27% to 10.99%) were heterozygous carriers of FV Leiden ( $p = 0.025$ ). The odds ratio for DVT was 4.94 (95% CI 1.58 to 15.42) and the relative risk 4.04 (95% CI 1.44–11.38) compared with FV 1691G carriers. Four cases were homozygous carriers of FV Leiden, giving a prevalence of 5.06% (95% CI 0.23 to 9.89%) and no controls, therefore OR and RR calculation was based on the prevalence of homozygotes in the general Caucasian population. The OR for DVT was 47.28 (95% CI 0.04 – 52167.3) and the RR 45.57 (95% CI 0.04 to 49540.77;  $p = 0.025$ ) compared with FV 1691 G carriers. Our study confirms that factor V Leiden carriers in Vojvodina, as in similar studies previously carried out in other populations, have an increased risk of developing DVT. The evaluated risk of DVT in heterozygous carriers of the mutation is four- to five-fold higher, whereas for homozygous carriers it is 45- to 48-fold higher than in non-carriers. These results confirm that patients with DVT and their relatives should undergo screening for FV Leiden mutation.

**Keywords:** factor V Leiden, deep vein thrombosis

**Kratak sadržaj:** U periodu od septembra 2007 do februara 2010. ispitana je pojava duboke venske tromboze u kohorti od 79 pacijenata. Prospektivna studija je obuhvatila kontrolnu grupu koja se po odnosu polova i starosti poklapa sa grupom pacijenata. Analizirano je prisustvo mutacije faktor V G1691A u grupi obolelih i kontrolnoj grupi. Osamnaest obolelih (22,79%; 95% interval poverenja (CI) 13,53% do 32,03%) i četiri kontrole (5,63%; 95% CI 0,27–10,99%) bili su nosioci FV Leiden mutacije u heterozigotnom stanju ( $p = 0,025$ ). Odnos verovatnoća (OR) za nastanak DVT iznosio je 4,94 (95% CI 1,58–15,42) a relativni rizik (RR) 4,04 (95% CI 1,44–11,38) u poređenju sa nosiocima neizmenjenog gena. Četiri obolela bili su nosioci FV Leiden mutacije u heterozigotnom stanju a nijedna zdrava osoba i stoga su OR i RR računati na osnovu opšte učestalosti homozigota u beloj populaciji. OR za DVT bio je 47,28 (95% CI 0,04–52167,3) a RR 45,57 (95% CI 0,04–49540,77;  $p = 0,025$ ) Naša studija potvrđuje da nosioci FV Leiden mutacije u Vojvodini, kao u studijama rađenim na drugim populacijama, imaju povećan rizik za nastanak DVT. Procenjen rizik od nastanka DVT za nosioce faktor V Leiden mutacije u heterozigotnom stanju je četiri do pet puta veći a za homozigotne nosioce čak oko 45 do 48 puta veći u odnosu na nosioce neizmenjenog gena za FV. Ovi rezultati potvrđuju da kod pacijenata sa DVT, kao i njihovih rođaka, treba ispitati prisustvo FV Leiden mutacije.

**Ključne reči:** faktor V Leiden, duboka venska tromboza

### Introduction

Inherited thrombophilia represents a variety of genetically determined abnormalities of haemostatic mechanisms that are generally associated with increased predisposition to blood coagulation and thrombosis development. In a patient with the genetic anomaly linked to venous thrombosis, another inherited or acquired risk factor can produce this clinical disorder (1, 2).

Address for correspondence:

Iva Salatić  
Center of Forensic Medicine, Clinical Center of Vojvodina  
Novi Sad, Serbia  
Phone/fax: +381 21 6624141  
e-mail: ivadnk@gmail.com

Inherited thrombophilia most commonly manifests itself as venous thromboembolism (VTE). It is usually present as deep venous thrombosis (DVT) or pulmonary embolism (PE) (3). DVT includes blood clot formation in the deep veins of extremities that accompany the arteries and their branches in the muscle layer close to the bones. Prevalence of DVT is high, approximately 0.1% in the general population (4), therefore the testing for inherited prothrombotic conditions is now done in healthcare institutions worldwide. In patients with inherited thrombophilic disorders thrombosis usually occurs at a young age; before 45 years of age (3). It is frequently recurrent and in a lot of cases causes an embolism. The progress in genetic research using PCR techniques revealed genetic polymorphisms in coagulation factor genes or their inhibitors, which represent a genetic predisposition to venous thrombosis development. Inherited activated protein C resistance (APC-R) is present with the highest prevalence compared to the other inhibitor deficiencies in the human population and therefore this is the most important of all inherited thrombophilic states. FV Leiden has been identified as the most prevailing autosomal dominantly inherited factor influencing the pathogenesis of DVT – more than 95% of patients with APC-R carry a factor V Leiden mutation (6, 7). A neomorphic mutation that changes the protein structure of coagulation factor V results in this prothrombotic condition. Activated protein C resistance may also be noticed in some cases of elevated homocysteine level, in some malignant diseases and in pregnancy – this acquired APC resistance is present in less than 5% of cases. Factor V is activated by thrombin into FVa which is later inactivated by APC proteolytic degradation. Resistance to the anticoagulant action of activated protein C is due to the synthesis of a mutant factor V protein. A single missense nucleotide polymorphism in the factor V gene substitutes guanine (G) for adenine (A) at nucleotide 1691, which results in a change in the amino acid sequence at position 506, from Arginine to Glutamine (Arg → Gln) and the loss of one cleavage site of FV protein. This change in the anticoagulation mechanism in most cases results in high FVIII and thrombin levels, thereby raising the risk of VTE due to the induction of the state of hypercoagulability (3). Factor V Leiden mutation is the most frequent inherited risk factor for VTE, with a prevalence of 5% in the general Caucasian population and a much higher frequency in the Greek Cypriot and southern Sweden population, up to 13.3% (7, 8). In the African and Asian populations this mutation is very rare (9). In the general Caucasian population the prevalence of FV Leiden mutation is 20–50% among patients with DVT. In heterozygous carriers of the mutation the estimated risk of DVT is 5- to 10-fold higher, while for homozygous carriers it is 80- to 100-fold higher than in non-carriers (10, 11).

Assessing the prevalence of FV Leiden mutation among DVT patients and healthy subjects is important because of the lack of data on the role of FV Leiden in DVT disease in the population of Vojvodina.

## Patients and Methods

Between September 2007 and February 2010, the testing for presence of thrombophilia was carried out at the Center of Forensic Medicine and the Center for Laboratory Medicine of the Clinical Center of Vojvodina. A case-control study included a cohort of 79 consecutive patients with documented symptomatic DVT and 71 healthy controls matched with cases for sex and age. Informed consent of each participant was obtained. Capillary blood samples were obtained from each participant as previously described (12) and processed shortly afterwards. Genomic DNA was extracted and purified from dried blood spots using the Chelex100®Molecular Grade Resin reagent (Bio-Rad, Hercules, CA) following the manufacturer's instructions. Detection of the FV 1691 G or FV 1691 A allele in the patients and controls was performed through real-time PCR on an ABI PRISM 7000 Sequence Detection System instrument using the allelic discrimination assay. For the amplification reactions, a set of primers and specific probes for the differentiation between wild alleles and FV Leiden, designed and synthesized by the TaqMan SNP Genotyping service (Part Number: 4351379) from the part of the normal FV gene and FV Leiden gene sequence sent to Applied Biosystems was used. Thereby diluted samples and the Taqman Universal PCR Master Mix (Applied Biosystems, Foster City, CA) set of reagents (Part Number: 4304437) were added. The allele specific oligonucleotide for FV 1691 G was 5'- VIC dye -TCAAGGACAAAATACCTGTATTCTCTGCCTGTCCAGGGATCTGCTCTTACA- NFQ - 3', and for FV 1691 A was 5'- FAM dye -TCAAGGACAAAATACCTGTATTCTCTGCCTGTCCAGGGATCTGCTCTTACA- NFQ - 3'. As a thrombosis risk estimation, in carriers of factor V Leiden mutation compared with non-carriers, unconditional logistic regression model was used to calculate the odds ratio (OR) and the relative risk (RR). The test was two-sided and statistical significance of the results where  $p \leq 0.5$ , was taken as referential.

## Results

Eighteen cases were heterozygous carriers of FV G1691A, giving a prevalence of 22.79%; (95% CI 13.53% to 32.03%), and four controls – a prevalence of 5.63%; (95% CI 0.27% to 10.99%). The OR for DVT was 4.94 (95% CI 1.58–15.42) and the RR 4.04 (95% CI 1.44–11.38) compared with individuals without FV Leiden mutation (FV 1691 G carriers). Four cases were homozygous carriers of FV Leiden, giving a prevalence of 5.06% (95% CI 0.23 to 9.89) and no controls, therefore OR and RR calculation was based on the prevalence of homozygotes in the general Caucasian population. The OR for DVT was 47.28 (95% CI 0.04 to 52167.30) and the relative risk 45.57 (95% CI 0.04 to 49540.77;  $p=0.025$ ) compared with FV 1691 G carriers.

**Table I** Prevalence of FV Leiden among patients with documented DVT and the control group

	Patients with DVT n=79	Controls n=71	OR	95% CI	RR	95% CI
FV Leiden Ht	18 (22.79%)	4 (5.63%)	4.94	1.58–15.42	4.04	1.44–11.38
FV Leiden Hm	4 (5.06%)	0 (0%)	47.28	0.04–52167.30	45.57	0.04–49540.77
FV WT	57 (72.15%)	67 (94.37%)				

Ht – heterozygous carriers, Hm – homozygous carriers, WT – wild type (normal gene) carriers

## Discussion

This is the first study of the prevalence of factor V Leiden mutation in the Vojvodina population. Our results are slightly above the range of the previously published results of FV Leiden prevalence in the general Serbian population (13) and this study shows a certain relationship between the DVT and FV Leiden mutation which is in an agreement with other similar studies. In order to provide the optimal prophylactic therapy it is very useful to test the patients for the presence of this mutation after the first episode of DVT. Our results confirm that patients with DVT, their relatives and also those with other risk factors for DVT, inherited or acquired, should undergo this screening. Treatment of a patient with factor V Leiden depends upon the individual patient's risk of recurrent thromboembolic disease. The administration of long-term anticoagulation is influenced by considering the risks of recurrent thrombosis and the risks associated with long-term anticoagulant therapy for each patient. Patients who have had more than one thromboembolic episode or are at high risk of recur-

rence (multiple deficiencies of haemostatic mechanisms or factor V Leiden homozygotes) are suitable candidates for long-term anticoagulation (14). Anticoagulant treatment should be prolonged for more than 6 months, as long as another risk factor is still effective (15). If permanent anticoagulant treatment of patients with FV Leiden is not possible, short-term anticoagulation in situations of an increased risk of venous thrombosis, such as immobilization, acute respiratory and urinary tract infections, or pregnancies, can be recommended.

*Acknowledgments.* We are grateful to Branka Smiljanić for the technical assistance and also to the staff members of the Forensic Center and the others who collaborated as the control group.

## Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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*Received: June 18, 2010*

*Accepted: August 26, 2010*