# Prevalence of 1691G>A *FV* mutation in females from Bosnia and Herzegovina - a preliminary report

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## Abstract

Factor V is the liver-synthesized multidomain glycoprotein encoded by a gene localised on chromosome 1q23. The point mutation 1691G>A in this gene results in formation of an altered protein of V Factor resistant to activated protein C (APC) cleavage. This mutation alone is the most frequent cause of inborn thrombophilia and the most widely acknowledged genetic risk factor for venous thrombosis in a Caucasian population. This study was designed to provide the first estimate of the frequency of the allele 1691A *FV* in the Bosnian female population. The 1691G>A *FV* mutation was examined by polymerase chain reaction-restriction fragment length polymorphism, in a group of 67 women, mean age of 58.6 years with no history of cardiovascural incident. Our findings revealed an absence of the mutated allele 1691A *FV* in the studied group.

This is the first report on the 1691G>A *FV* mutation in a population from Bosnia and Herzegovina. Further research is needed to establish prevalence of the mutated allele in the population from Bosnia and Herzegovina.



KEY WORDS: FV, Factor V Leiden, 1691A FV, thrombophilia, prevalence, recurrent miscarriages

## INTRODUCTION

Inherited resistance to activated protein C (APC) is one of the most common genetic defects associated with familial thrombophilia and is now known to be the most common genetic risk factor for venous thrombosis. It is an autosomal dominant disorder caused by a single point mutation in the Factor V gene, which predicts substitution of arginine (R) to a glutamine (Q) at aminoacid position 506. G>C transition ocures at nucleotide position 1691 located in exon 10. This mutation is known as Factor V Leiden (FVL) [1]. Factor V Leiden, in comparision with the active form, is inactivated by protein C at a much slower rate. Since mutated Factor V is resistant to the anticoagulative action of protein C, it cannot be activated by cleavage, causing inactivation of the antithrombotic regulatory pathway [2-3]. Carriers of 1691G>A *FV* are more likely to develop abnormal blood clots, which in extreme cases can cause deep vein thrombosis (DVT), pulmonary embolism and superficial thrombophlebitis. Furthermore, mutation 1691G>A of the gene coding for Factor V accounts for the most common cause of inherited predisposition to thrombosis. It has been reported that, presumably due to placental thrombosis, Factor V Leiden increases by 2- to 3- fold the risk for recurrent miscarriage and possibly other obstetrical complications such as intrauterine growth restriction and placental abruption [4-8]. The 1691 G>A *FV*mutation in fertile women might be associated with deep vein thrombosis and pulmonary embolism during pregnancy, increased risk of preeclampsia, recurrent miscarriage and placental development. During postmenopausal period, estrogen replacement therapy is associated with imbalance in coagulation and fibrinolysis especially relevant for 1691G>A *FV* mutation carriers. It is known that the frequency of the 1691G>A *FV* allele varies worldwide and differences are observed between geographic locations and ethnic populations. Recent studies have concluded that the 1691G>A mutation is virtually nonexistent in Asia and in some regions of Africa; however, the mutation showed a higher prevalence in the Lebanese population (14.4%) and was extremely high among healthy Palestinians (20.1%) [9-12]. In Europe the 1691G>A *FV* provides a mosaic, among South-Eastern European countries the frequency of allele 1691A *FV* varied from 3.6% in Bulgaria, 3.5% in Macedonia, 2.2% in Serbia to 1.6% in Croatia, indicating an upward trend in Southeasterly direction

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[13]. Multiple data is available in most of Europe; however, no accurate data show any degree of prevalence for Bosnia and Herzegovina. Since women might be more susceptible to present with complications due to Factor V Leiden both during the fertile and postmenopausal period the purpose of this study was to show the prevalence of 1691G>A *FV* mutation in females from Bosnia and Herzegovina and to evaluate whether the prevalence of *FV* 1691G>A was consistent with the results from other studies carried on female population.

## MATERIALS AND METHODS

*Subjects*

For the study purposes 67 apparently healthy females (mean age 58.6 y, range: from 41 to 75 y) were enrolled. None of the subjects included in the study reported previous history of deep vein thrombosis, pulmonary embolism or superficial thrombophlebitis. Sample collection was focused on the oral cavity. Buccal swabs were collected at the Laboratory for Molecular Medicine, Center for Genetics, Faculty of Medicine, University of Sarajevo. Written consent was obtained from each individual enrolled in this study. The local ethical committee approved the protocol of the study.

*Methods*

Following DNA isolation from buccal swabs (PrepFilerTM Forensic DNA Extraction Kit, Life Technologies, USA) the polymerase chain reaction-restriction fragment length polymorphism 1691G>A *FV* was performed. For a 10 μL PCR, ~25 ng of genomic DNA was used. The PCR mixture contained 10x PCR Buffer (pH 8.3, 1.5 mM $MgCl_2$), 0.2 mM each of the deoxynucleoide triphosphates, 0.5 U AmpliTaq®DNA Polymerase (Roche) and 4 pmol each of the forward and reverse primers as designed by Gandrille et al. [14]. PCR reactions were performed in a Mastercycler® pro with vapo protect device (Eppendorf, Germany) with the following temperature profiles: initial denaturation at 94˚C for 5 min, 38 cycles of 20 s at 94˚ C, 40 s at 56˚ C and 40 s at 72˚C; and final extension step at 72˚C for 7 min. Amplification was followed by digestion of a 241 bp product with Hind III restriction enzyme (5'-A↓AGCTT-3') (MBI Fermentas, Lithuania) for 16 hours at 37˚C. The restriction fragments were visualized after electrophoresis in 3 % agarose gels, stained with Sybr® Green (Apllied Biosystems, USA). Hind III digestion yields fragment of 241 bp (homozygote GG), 241, 209 and 32 bp (heterozygote GA) and 209, 32 bp (homozygote AA).

## RESULTS

The results obtained in this study, which included 67 healthy women, reveal that the 1691A allele was absent.

## DISCUSSION

The 1691G>A *FV* variant has been described as a common genetic risk factor in thrombophilia and pulmonary embolism. Previously we provided a summary study of the frequency distribution of 1691G>A *FV* including the whole of Europe by addition of values from 11 Slavic countries [13]. Our findings revealed in upward trend Southeasterly direction in allele 1691A *FV*, from Croatia (1.6%), Serbia and Montenegro (2.2%), Slovenia (2.5%) to Bulgaria (3.6%) [13]. However, there were no data on the frequency of 1691A *FV* in Bosnia and Herzegovina. This has prompted us to investigate the prevalence of this mutation in this country. To our knowledge we give the first study on the subject. The results presented here reveal that in the group of 67 Bosnian woman the 1691A allele was absent. In Bulgarian women Factor V Leiden was more prevalent in the group of women with fetal loss (37.5%) compared to the healthy women (6.2%) [15]. In Polish women FVL in a cohort of 313 women with a history of at least two miscarriages was 3.2% while in the control group consisting of 200 women without obstetric complications was found to be 3.0% [16]. The Hordaland homocysteine study from Norway reported the 1691G>A *FV* to be 7.9% in females [17]. A study from Germany reported the prevalence of the Factor V Leiden to be equally high in the group of women with recurrent abortions (11/101; 10.9%) compared with the control group (9/122; 7.4%) [18]. In Greek women 15 of 80 recurrent miscarriage patients and 4 out of 100 controls carried the Factor V Leiden mutation (19% vs. 4%) [6]. A study from France reported the Factor V Leiden distribution was 27/260 (10.38%) which was significantly higher than the Factor V than in the control women (11/240 (4.6%) [19]. In North American women 1691G>A mutation was 6.7% in Caucasian women (N=560) and 0.8% (N=399) in African Americans women [20]. A study from US compared prevalence of *FV* mutation in 50 nonpregnant women with three or more pregnancy losses and 50 healthy nonpregnant controls. 1691G>A *FV* mutation was observed in 1 (2%) women with pregnancy loss and was not statistically significantly different from controls [21]. A study on Israeli women with recurrent miscarriages revealed the 1691G>A mutation in 4/108 women (3.7%) and 5/82 (6.1%) in healthy controls [22]. In Lebanese women with primary habitual abortion, 45 (40.91%) carried the FV Leiden, compared to 16.42% carrier rates among controls [23]. In Palestinian women the 1691G>A *FV* mutation was detected in 41 of the 145 women with recurrent miscarriages (28.2%), and in 24 of the 205 control healthy women (11.7%) [24]. The prevalence of Factor V Leiden in Turkish women with recurrent pregnancy loss was 7.9% (9/114) compared with 7%

(13/185) in the control group [25]. In Iranian women the prevalence of FVL in the healthy women group was 0.0% [26]. These preliminary results of the presence of 1691G>A mutation in the Bosnia and Herzegovina raise the question of future screening for this mutation, including both genders.

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## DECLARATION OF INTEREST

The authors state no conflict of interest.

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