Prevalence of Rare F5 Variants in General Population From Bosnia and Herzegovina

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Research Article

Keywords: Bosnia and Herzegovina, Factor V Asp2194Gly, Factor V Leiden, Factor V Met2120Thr, rare Factor V variants, thrombophilia

Posted Date: May 5th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-456810/v1>

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Abstract

Background: Human gene *F5*, encoding coagulation factor V, was previously reported to be highly polymorphic. Apart from FV Leiden, an array of other rare variants has been detected in clinical practice and associated with thrombotic events, especially in cases when patient's phenotype and FV Leiden genotype were not in agreement.

Methods and Results: In this study, the prevalence of 17 rare *F5* variants has been studied on a sample of 130 healthy adult individuals from the general Bosnian-Herzegovinian population. DNA was isolated from buccal swab samples, while genotyping was performed using MALDI-TOF MS method. The results have shown that Asp2194Gly and Met2120Thr are polymorphic in the study population with minor allele frequencies of 0.077 and 0.073, respectively. Additionally, these two variants were found to be mutually exclusive with FV Leiden and none of them was positively associated with participants' family history of cardiovascular or cerebrovascular diseases.

Conclusions: While the obtained results are in agreement with previously reported data for the general Caucasian populations, it is worth noting that only two rare *F5* variants were detected in the study population, albeit at considerable frequencies. Still, scientific information on rare *F5* variants is rather scarce and further studies aiming to assess functional importance of these variants, as well as their role as prothrombotic factors are necessary.

Introduction

Coagulation factor V (FV) is encoded by *F5* gene whose mRNA product is 6.8 kb long and has 25 exons, while the corresponding polypeptide is 2,224 amino acids long. Activated FV (FVa) is inactivated in a coagulation cascade by the peptide cleavage at arginine residues 306, 506 and 679 by anticoagulant protein C (APC) through its serine protease activity [1].

FV Leiden (FVL; c.1601G>A, p.Arg506Gln, rs6025) is a pathogenic variant that was first identified in individuals with APC resistance, since it disables APC in recognizing the major cleavage site on FV peptide, causing unchecked continuation of prothrombin activation [2, 3]. FVL is a major predisposing factor for venous thrombosis, with approximations that heterozygous genotype increases the risk of venous thromboembolism (VTE) 5- to 10-fold, while homozygosity increases the risk up to 40- to 80-fold [3, 4]. It was also reported that FVL mutation is present in 18-25% of all VTE cases [5, 6].

However, *F5* gene is highly polymorphic, even considering its size. There have been several hypotheses aiming to explain this phenomenon. The most probable one relates the frequency of *F5* DNA variants with its dual role as procoagulant and anticoagulant protein, whereby this gene is usually described as Dr. Jekyll and Mr. Hyde of human coagulation [7]. According to the previously published literature [8], rare *F5* variants have been detected in clinical practice when the results of FVL genotyping and observed patient's phenotype were not in alignment, thus implying other underlying mutations. An overview of the most significant rare *F5* variants is given in Table 1, along with their clinical significance.

Most of these variants were reported as isolated clinical cases and are usually not systematically studied to determine their prevalence in general population. Therefore, the aim of this study was to perform genotyping for 17 rare *F5* variants in the general population from Bosnia and Herzegovina and identify which variants are present and at what frequency. In addition, it was aimed to assess whether statistically significant correlation between rare *F5* variants and family history of cardiovascular diseases can be detected.

Materials And Methods

Sample collection and DNA isolation

This study has been performed on 130 buccal swab samples collected from unrelated adult individuals of both genders from different parts of Bosnia and Herzegovina without previous history of cardiovascular diseases. Sample collection has been performed between August and October 2017. Prior to volunteering to give a sample, all participants signed an informed consent form. Ethical clearance for conducting this study was obtained from the Ethical Committee of the International Burch University (Sarajevo, Bosnia and Herzegovina).

DNA was isolated using modified Miller's protocol [13] with an overnight incubation step at 60°C. The quality and quantity of DNA isolates was tested spectrophotometrically using BioSpec-nano spectrophotometer (Shimadzu Corp., Kyoto, Japan).

DNA genotyping

Factor V Leiden mutation has been analyzed along with 17 other rare variants in *F5* gene. PCR amplification has been carried out according to Storm and Darnhofer-Patel [14] in two multiplexes as follows:

1. Multiplex reaction for 14 rare *F5* variants: Casablanca, Met2120Thr, 2952 DelT Ex13, 4bp Dup Ex13, Arg1133Ter, Tyr1702Cys, Arg2075Cys, Ile359Thr, G>A Ex18, Cambridge, InsA Ex13, Asp2194Gly, 5493 InsG Ex16 and Glu119Ter, as well as Leiden mutation
2. Multiplex reaction for three rare *F5* variants: G>A Ex16, Seoul-1 and Hong Kong.

Multiplex reactions were designed and appropriate primers selected in Assay Design Suite v2 (Agena Bioscience, San Diego, CA, USA).

Excess unreacted dNTPs were removed from reaction mixtures via shrimp alkaline phosphatase (SAP) treatment [14], followed by a general iPLEX reaction for SNP genotyping using single-base extension chemistry [14]. Thermal cycling and sample incubation in these three reactions have been performed using GeneAmp PCR System 9700 (Applied Biosystems, Waltham, MA, USA) in 384-well plates.

Samples were subsequently treated with Clean Resin as a means of ion exchange conditioning to remove excess cations. SNP genotyping was performed using 15-nl samples utilizing MALDI-TOF MS approach

through two software packages, namely SpectroACQUIRE for raw data collection and MassARRAY Typer for plate design and accessing genotype data, both manufactured by Agena Bioscience (San Diego, CA, USA) [14].

Statistical analyses

To confirm whether tested loci are in Hardy-Weinberg equilibrium (HWE), we have used GenAIEx v6.5 and chi-square statistics at significance level of 0.05 [15, 16]. Linkage disequilibrium (LD) between loci was tested for in pairwise manner using online version of GenePop v4.2 at significance level of 0.05 [17, 18]. In order to test for possible correlation between rare *F5* variants and participants' family history of cardiovascular diseases, IBM SPSS Statistics for Windows v23.2 (IBM Corp., Armonk, NY, USA) has been used, whereby Fisher's exact test was performed at significance level of $p < 0.05$.

Results

In this study, the prevalence of 17 rare *F5* variants, in addition to Factor V Leiden (previously published in [19]), has been studied in 130 individuals from the general Bosnian-Herzegovinian population. Females accounted for 63.8% of the study population. Mean participants' age was 33.8 ± 14.3 with the youngest participant being 19 and the oldest 82 years old at the time of sample collection.

Out of 18 investigated loci, 13 of them were defined as SNP changes, along with two deletions, two insertions and one duplication. Apart from Factor V Leiden, two more loci were found to be polymorphic in Bosnian-Herzegovinian population, namely *F5* Asp2194Gly and *F5* Met2120Thr (Figure 1).

In terms of Factor V Leiden, six study participants were heterozygous (4.6%), while the rest of them carried wild-type genotype, thus making minor allele frequency (MAF) present at frequency of 0.023. Heterozygous genotype was found in 16 and mutant homozygous in two study participants on locus Asp2194Gly, while at locus Met2120Thr, 15 heterozygous and two mutant homozygous genotypes were detected. MAFs for Asp2194Gly and Met2120Thr were 0.077 and 0.073, respectively. It is interesting to note that Factor V Leiden mutation was mutually exclusive with two rare *F5* variants. Moreover, one participant was found to be compound heterozygous for Asp2194Gly and Met2120Thr, while the rest of them had mutated allele(s) at only one of these two loci.

Three polymorphic loci were found to be in agreement with Hardy-Weinberg equilibrium with p -values of 0.788, 0.128 and 0.091 for Factor V Leiden, *F5* Asp2194Gly and *F5* Met2120Thr, respectively. LD analysis at significance level of $p = 0.05$ revealed no indication of LD between loci Asp2194Gly and Met2120Thr.

Fisher's exact test was used to check if variant distribution is correlated with the family history of cardiovascular and cerebrovascular diseases. At $p = 0.05$, neither Asp2194Gly nor Met2120Thr were significantly correlated with participants' family histories.

Discussion

Rare *F5* variants have previously been detected and studied in human populations from different parts of the world. Furthermore, many *F5* variants have their common names reflecting cities in which they were first detected [10, 11, 20-22]. It is, however, necessary to conduct systematic and more elaborate studies in order to extend our understanding of the prevalence of *F5* variants, and especially their functional and clinical significance.

In the general Bosnian-Herzegovinian population, two rare *F5* variants have been detected, namely Met2120Thr and Asp2194Gly. The importance of Met2120Thr SNP has been demonstrated using recombinant FV protein. It has been shown that this variant is significantly associated with decreased FV levels *in vivo* and *in vitro*. MAF for Met2120Thr is 3% in Caucasians and it seems to be restricted to this population only since African and East Asian populations do not exhibit this variant in previously published studies [7]. The present study identified higher MAF of 7.3% with two individuals being homozygous carriers of 2120Thr allele.

On the other hand, Asp2194Gly variant constitutes HR2 haplotype that was first described in 1998 in terms of four linked missense variants with His1299Arg being functionally the most significant. It was immediately associated with lower FV levels in plasma [7, 23]. This haplotype was later characterized in more detail and is now believed to be consisted of 13 linked polymorphisms in exons 8 through 25 of *F5* gene with seven of them being amino acid changes related to functional modifications of the protein [1, 23, 24]. The function of this haplotype is now mainly associated with an increased factor V1 to V2 ratio, thus increasing prothrombotic activity, as well as with decrease in APC-mediated degradation of FV, including individuals with wild-type Leiden genotype [23, 24]. Asp2194Gly is described as one of the major functional determinants of FVHR2 haplotype [25], interfering with the transport of mutant protein from ER to Golgi complex [26]. When compared to other rare variants, HR2 is relatively common with MAF of 5 to 17% in Europe, Asia and Africa, reaching its peak value of 50% in Indian tribes from Costa Rica [23].

There has been an ongoing debate of HR2 haplotype role in predisposition to thrombotic events with previous studies reporting opposing results. In their meta-analysis, Castaman and colleagues [23] found a statistically significant differences in VTE frequency in FV Leiden carriers alone when compared to FV Leiden/HR2 compound carriers. The authors have also concluded that HR2 haplotype can only be observed as a mild prothrombotic factor since this variant alone did not significantly increase the risk of VTE compared to wild-type individuals [23]. On the other hand, in one of the most extensive and detailed meta-analyses on this topic, Gohil and colleagues [27] identified, among others, *F5* variants representing risk factors for VTE. FVHR2 haplotype was analyzed by comparing the results of 14 studies in Caucasian populations. Population attributable risk (PAR) for VTE was 2.6% in the presence of this variant, while MAF was 6% in both patients and healthy controls. This meta-analysis identified a significant risk of VTE in the presence of HR2 haplotype mutant allele [27].

Asp2194Gly SNP alone corroborates lower FV levels *in vivo*, which has been shown to contribute to increased APC resistance and, therefore, increased probability of thrombotic event. However, it is expected

that this variant has a prothrombotic character if accompanied by FV Leiden mutation. MAF for this SNP is 0%, 7% and 5% in Africans, Caucasians and Asians, respectively [7]. This finding is in agreement with the present study, in which 2194Gly allele had frequency of 7.7%.

In the most detailed study of Met2120Thr and Asp2194Gly variants in Caucasian populations so far, 1013 Italians were genotyped and variant alleles were detected for both polymorphisms. The results report that 4.4% and 13.2% participants were Met2120Thr and Asp2194Gly carriers, respectively [25]. The same study offers extensive information on functional changes associated with these two variants. FV Leiden/Met2120Thr compound heterozygotes had significantly decreased APC ratios, while this difference was not significant for FV Leiden/Asp2194Gly compound heterozygotes. The prevalence of FV Leiden/Asp2194Gly genotype was significantly higher in symptomatic VTE patients than in asymptomatic controls. Higher frequency of FV Leiden/Met2120Thr genotype was also observed in symptomatic patients than in controls, but the difference was not statistically significant. Additional analysis confirmed that both variants lead to decrease of recombinant FV activity and antigen levels *in vitro* and protein levels in plasma *in vivo*. In heterozygous state, Met2120Thr decreased Factor V levels by 25% *in vivo* and 34% *in vitro* in expression studies. Comparable to this, heterozygous Asp2194Gly decreased FV levels by 20% and 45% *in vivo* and *in vitro*, respectively [25].

Human coagulation cascade is a delicate and fine-tuned pathway, while thrombophilia is a heterogenous condition being associated with many acquired and hereditary variants. Aside from FV Leiden, several other variants in *F5* gene have been detected in clinical practice. We have here shown that Met2120Thr and Asp2194Gly are present in healthy population from Bosnia and Herzegovina and that the variant distribution is not significantly correlated with participants' family history of cardiovascular and cerebrovascular diseases. In order to fully assess clinical significance of these and other *F5* variants, it is necessary to design and implement studies that will aim at comparing general and patient population and assessing potential prothrombotic nature of rare *F5* variants. Furthermore, functional consequences of rare *F5* variants, including varying FV levels in blood and impaired protein secretion are to be investigated in order to get better understanding of their roles.

Declarations

Funding: No funding was received for conducting this study.

Competing interests: The authors have no competing interests to disclose that are relevant to the contents of this article.

Availability of data and material: The datasets generated and/or analyzed during the study are available from the corresponding author on reasonable request.

Code availability: Not applicable

Authors' contributions: AA performed sample collection, experimental work, data analysis and participated in manuscript writing. RS and NS performed experimental work and participated in manuscript preparation. SD performed statistical analyses and participated in manuscript drafting. WH and DM supervised the project and critically revised the manuscript. DP designed and supervised the project and critically revised the manuscript. All authors approved the final version of the manuscript for publication and agree to be held accountable for all aspects of the work.

Ethics approval: Ethical clearance for conducting this study was obtained from the Ethical Committee of the International Burch University (Sarajevo, Bosnia and Herzegovina), protocol number 04-172-1/17. The study was conducted according to the 1964 Helsinki Declaration.

Consent to participate: Informed consent was obtained from all individual participants included in the study prior to sample collection.

Consent for publication: All participants signed an informed consent agreeing to publication of anonymized genotype data.

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Tables

Table 1 An overview of alternative HGVS and dbSNP [12] designations for the most significant rare *F5* variants, along with the summary of clinical relevance and/or phenotypes associated with them

Variant	Nucleotide change	Amino acid change*	dbSNP entry	Related phenotype	References
Hong Kong	c.1000A>G	p.Arg334Gly	rs118203905	Possibly pathogenic but clinical relevance not proven Mildly reduced APC sensitivity <i>in vitro</i>	[1, 8, 9]
Cambridge	c.1001G>C	p.Arg334Thr	rs118203906	APC resistance	[8-10]
Stanford (4bp Dup Ex13)	2805ATTG	Frameshift	N/A	Factor V deficiency	[8, 9]
Seoul-1 (8bp Del Ex7)	1131AAGAGGTG	Frameshift	N/A	Factor V deficiency	[8, 9, 11]
Seoul-2	c.5189A>G	p.Tyr1702Cys	rs118203907	Factor V deficiency	[8, 9, 11]
Casablanca	c.2401C>T	p.Gln801Ter	rs118203908	Factor V deficiency	[8, 9, 11]
Arg1133Ter	c.3481C>T	p.Arg1133Ter	rs118203909	Factor V deficiency	[8, 9]
2952 Del Ex13	2952T	Frameshift	N/A	Factor V deficiency	[8, 9]
5493 Ins Ex16	5493G	Frameshift	N/A	Factor V deficiency	[8, 9]
Arg2074Cys	c.6304C>T	p.Arg2074Cys	rs118203910	Factor V deficiency	[8, 9]
Liverpool	c.1160T>C	p.Ile359Thr	rs118203911	APC resistance	[7-9]
Glu119Ter	c.439G>T	p.Glu119Ter	rs118203912	APC resistance	[8, 9]
Asp2194Gly	c.6665A>G	p.Asp2194Gly	rs6027	Lower factor V levels	[7]
Met2120Thr	c.6443T>C	p.Met2120Thr	rs9332701	Lower factor V levels	[7]

*Ter stands for termination codon.

Figures

Figure 1

Genotype frequencies of 18 analyzed F5 variants in general Bosnian-Herzegovinian population