Vidović et al. Scr Med 2023 Jun;54(2):175-9. DOI:10.5937/scriptamed54-43841

ORIGINAL ARTICLE

The Prevalence of *VKORC1* Alleles in the Population of the Republic of Srpska, Bosnia and Herzegovina

Vanja Vidović,[1, 2] Jelena Bećarević,[1] Žana Radić Savić,[1, 3] Aljoša Marić,[1] Stojko Vidović,[1, 2] Irina Milovac,[1, 2] Nela Maksimović[4]

1. Centre for Biomedical Research, Faculty of Medicine, University of Banja Luka, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina.
2. Department of Human Genetics, Faculty of Medicine, University of Banja Luka, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina.
3. Department of Medical Biochemistry, Faculty of Medicine, University of Banja Luka, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina.
4. Institute of Human Genetics, Faculty of Medicine, University of Belgrade, Belgrade, Serbia.

Correspondence:
VANJA VIDOVIĆ
E: vanja.vidovic@med.unibl.org

Abstract

Background/Aim: Warfarin is one of the most common orally prescribed anticoagulant in patients with deep venous thrombosis, myocardial or cerebral infarctions. The main side effects of non-adequate dose of these drugs are prolonged peripheral or internal bleeding. *VKORC1* 1173C>T polymorphism (rs9934438) is of particular importance, since carriers of non-wild type allele correlates with the lower dosage of warfarin therapy. Thus, the aim of the research was to determine the distribution of 1173C>T polymorphism in population of the Republic of Srpska, Bosnia and Herzegovina (RS) and to compare results with frequencies in other populations.
Methods: A total of 124 healthy participants of both genders were enrolled in the study, from all parts of the RS. Molecular genotyping was performed by real-time PCR, using drug metabolism assays according to the manufacturer's instructions.
Results: Of the total number, 22 subjects (17.74 %) were genotyped as CC, 69 subjects (55.65 %) as CT and 33 subjects (26.61 %) as TT. The frequencies of alleles C and T were 45.18 % and 54.82 %, respectively. No statistical significance was found among allele distribution between genders (χ^2 = 0.236; p = 0.627). All observed genotype frequencies were in Hardy-Weinberg equilibrium. No statistical significance was observed among the frequency of minor T allele between presented findings and other European countries, besides Russia (p = 0.021).
Conclusion: This was the first study analysing the distribution of rs9934438 alleles in population of the RS. These findings will be helpful in better and more precise drug prescribing in patients who require anticoagulant therapy.

Key words: *VKORC1*; Allele distribution; Polymorphism; Warfarin.

ARTICLE INFO
Received: 5 April 2023
Revision received: 20 April 2023
Accepted: 20 April 2023

Introduction

Vitamin K epoxide reductase enzyme is encoded by homonymous *VKORC1* gene located on the chromosome 16p.[1] *VKORC1* enzymatic complex catalyses reduction of vitamin K 2,3 epoxide to the metabolically active forms of vitamin K in two steps, first to vitamin K quinone and afterwards vitamin K hydroquinone.[2] Thus, vitamin K hydroquinone acts as a cofactor for the gamma-carboxylation of glutamic acid residues of vitamin K-dependent proteins (VKDP) which include coagulation factors II, VII, IX, X, haemostatic proteins C, S and Z.[3] The essential role of this enzyme complex in the coagulation process represents the drug target of oral anticoagulants,

This article should be cited as follows: Vidović V, Bećarević J, Radić Savić Ž, Marić A, Vidović S, Milovac I, et al. The prevalence of *VKORC1* alleles in the population of the Republic of Srpska, Bosnia and Herzegovina. Scr Med 2023 Jun;54(2):175-9.

notably warfarin.[4] Warfarin binds to the VKOR and lowers the concentration of vitamin K hydroquinone, which decreases the amount of biologically active vitamin K to produce the factors of coagulation.[5] The main issue with prescribing anticoagulant therapy are adverse drug reactions, such as prolonged gastrointestinal, intracranial, or peripheral bleeding. For prescribing anticoagulant therapy, genetic variations among *VKORC1* and CYP2C9 are of particular importance, since genotype characteristics among these genes could explain the individual differences in response to warfarin.[6]

Numerous studies have reported that there is a firm connection between *VKORC1* SNPs and sensitivity to warfarin which varies due to inter-individual and inter-ethnic differences. According to data obtained from pharmacogenetic studies, two SNPs - 1639G>A and 1173C>T, are commonly genotyped. The 1173C>T rs9934438 is located in the first intron of *VKORC1* gene.[7] It is in near linkage disequilibrium with G3673A. The 1173C>T was the first known SNP to correlate with the low-dosage warfarin therapy.[8] This indicates a lower activity of the coagulation system, which is partly related to a decreased activity of key enzyme.[9] Accordingly, individuals with CT or TT genotype require lower warfarin doses in comparison to the carriers of the CC genotype.[7, 10]

The unpredictable pharmacodynamics and pharmacokinetics represent a big challenge in choosing the right dose of a drug.[11] Nevertheless, the patient's response to the recommended dosage of warfarin is inter-individual, assigned to genetics polymorphisms within the genes responsible for the pharmacokinetics and pharmacodynamics of warfarin.[12]

The aim of the research was to investigate the frequencies and distribution of *VKORC1* 1173C>T polymorphism in population of the Republic of Srpska, Bosnia and Herzegovina, as well as to compare results with the obtained frequencies in other populations.

Methods

This study included 124 healthy participants from the Republic of Srpska, of which 74 (61.29 %) participants were men and 48 (38.71 %) were women aged between 18 and 86 (median: 59). Participants were randomly selected from all parts of the Republic of Srpska. Exclusion criteria were mental and physical illness.

The study was conducted in accordance with the Declaration of Helsinki. Ethical Committee of the Faculty of Medicine at the University of Banja Luka approved this study (No 01-19-521-2/20). Also, all participants signed a statement providing their written informed consent.

The molecular genetic analysis was performed at the Laboratory for Molecular Biology and Genetics of the University of Banja Luka, Faculty of Medicine, Centre for Biomedical Research. Total genomic DNA was isolated using PureLink® gDNA Blood Kit (Invitrogen, Carlsbad, CA, USA). The Real-Time PCR instrument 7500 by Applied Biosystems and TaqMan® Drug Metabolism Genotyping Assay (C_30204875_10) were used for determination of genotypes for *VKORC1* (1173C>T, rs9934438). The real-time polymerase chain reaction was proceeded according to the manufacture's protocol.

Statistics

The χ^2 test was used to detect if the gene distribution accorded with the Hardy-Weinberg equilibrium and to compare genotype and allele frequency between different ethnic groups. For all analyses the Social Science Statistics online calculator was used (https://www.socscistatistics.com/tests/).[32]

Results

The alleles and genotypes distribution of the investigated gene in the population of the Republic of Srpska are summarised in Table 1. Observed genotype frequencies were in Hardy-Weinberg equilibrium ($\chi^2 = 1.837$; $p = 0.175$).

For the *VKORC1* 1173C>T polymorphism, 22 subjects (17.74 %) were genotyped as CC, 69 subjects (55.65 %) as CT and 33 subjects (26.61 %) as TT. The frequencies of alleles C and T in *VKORC1* 1173C>T in a total of 124 subjects were 45.18 % (113 alleles) and 54.82 %.

Table 1: Frequencies of the VKORC1 1173C>T genotypes and alleles in a sample of the population of the Republic of Srpska

Gene	Genotype	N	%	CI (95 %)	Allele	N (%)
VKORC1 1173C>T	CC[a]	22	17.74	11.47 – 25.62	C	113 (45.18)
	CT	69	55.65	46.45 – 64.56	T	135 (54.82)
	TT	33	26.61	19.08 – 35.30		

a - referent genotype; CI - 95 % confidence interval; N – number of participants;

The allele frequencies of *VKORC1* gene were compared across several different populations as shown in Table 2.

Table 2: The prevalence of the VKORC1 allelic variants in the population of the Republic of Srpska (this study) compared to other populations

Gene	Allele	Alleles frequencies in different populations (%)[a]								
		This study	Croatian	French	Italian	Slovenians	German	Arabian	Austrian	Chinese
VKORC1 1173C>T	C	0.409	0.593	0.587	0.602	0.567	0.585	0.573	0.570	0.084
	T	0.591	0.407	0.413	0.398	0.433	0.415	0.427	0.430	0.916

a – references 1, 13-21;

The data for potential gender differences were also analysed. However, there were no statistically significant differences in *VKORC1* 1173C>T polymorphisms distribution in relation to gender in study group ($\chi^2 = 0.236$; $p = 0.627$).

Discussion

Warfarin inhibits the enzyme *VKORC1*, which leads to the reduction of vitamin K and the production of hypofunctional coagulation factors.[22] It is estimated that the influence of *VKORC1* genetic variants on warfarin dose determination is approximately 25 %.[23] A narrow therapeutic range of warfarin is known with high inter-individual sensitivity to the drug. The presence of *VKORC1* gene polymorphism affects the pharmacodynamics of warfarin. One of the most common single nucleotide polymorphisms (SNP) *VKORC1* is 1173C>T where cytosine is replaced by thymine.[24] It was estimated that due to the presence of the T allele, the dose of warfarin should be reduced by 20-28 %. Therefore, the carriers of the *VKORC1* CC genotype require a higher daily dose of warfarin, the heterozygous genotype involves a medium dose and the carriers of the *VKORC1* TT genotype the lowest dose of the drug.[25]

In authors' previous research, the prevalence of pharmacologically most important allelic variants of the *CYP2C9* in the general population of the Republic of Srpska was analysed. CYP2C9 metabolises around 15 % of all drug in modern use, involving oral anticoagulants.[26] The two most common allelic variants are *CYP2C9*2* and *CYP2C9*3*, where the **2* allele is classified as a loss-of-function variant and the **3* allele as a no-function variant.[26, 27] According to the Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Pharmacogenetics-Guided Warfarin Dosing, individuals with *CYP2C9*2* and *CYP2C9*3* have decreased warfarin maintenance dose.[28] Thus, the prescription of warfarin therapy depends not only on *VKORC1*, but also on *CYP2C9*.

This is the first study performed in the population of the Republic of Srpska. Study showed that the *VKORC1* gene polymorphism differs between ethnicities. The frequency values for polymorphic alleles and genotypes corresponded to the frequencies for other European populations. The prevalence of *VKORC1* 1173T allele found in presented study are similar to the respective ones reported in other counties such as the French population (40.7 %; $p = 0.55$),[13] Italian (39.8 %; $p = 0.059$),[14] Slovenian (43.3 %; $p = 0.134$),[15] German (41.5 %; $p = 0.074$),[16] Arabic (42.7 %; $p = 0.13$)[17] and Austrian (43 %; $p = 0.115$).[18]

The *VKORC1* 1173T frequency was significantly different in comparison with Russian population (38.2 %; $p = 0.021$),[19] Chinese (91.6 %; $p < 0.001$)[20] and Egyptian (72.3 %; $p = 0.001$).[21]

The *VKORC1* 1173T allele is present in Caucasians

with a frequency of about 40 % and the rarest in African-Americans (14 %).[9, 29] Following, allele T is mostly present in the East Asian population (90 %) and this explains the use of significantly lower doses for Asians compared to Caucasians.[8, 30] In the rest of the Asian countries (South East, West and Central Asia) the prevalence of this allele oscillated between 14 and 80 %.[31]

Conclusion

In conclusion, this study showed that *VKORC1* gene was polymorphic in population of the Republic of Srpska, with a similar distribution as noticed in other European populations (about 40 %). Opposite, there was a statistically significant difference in other populations like Chinese (1 %) where the prevalence of *VKORC1* polymorphic allele was lower compared to studied population or Egyptian (72 %) where the frequency of T allele was higher than in this study. The importance of population-genetic studies, such as this one is to have a precise knowledge of the prevalence of specific genetic variants in population, thus the prescribing of particular therapy could be as precise as possible.

Acknowledgements

None.

Conflict of interest

None.

References

1. Mandic D, Mandic S, Horvat V, Samardzìja M, Samardìja M. Vitamin K epoxide reductase complex 1 (VKORC1) gene polymorphisms in population of eastern Croatia. Coll Antropol 2013;37(4):1321–6.
2. Oldenburg J, Marinova M, Müller-Reible C, Watzka M. The Vitamin K cycle. Vitam Horm 2008;78:35–62.
3. Aquilante CL, Langaee TY, Lopez LM, Yarandi HN, Tromberg JS, Mohuczy D, et al. Influence of coagulation factor, vitamin K epoxide reductase complex subunit 1, and cytochrome P450 2C9 gene polymorphisms on warfarin dose requirements. Clin Pharmacol Ther 2006;79(4):291–302.
4. Cavallari LH, Momary KM. Pharmacogenetics in cardiovascular diseases. In: Pharmacogenomics: Challenges and Opportunities in Therapeutic Implementation. Amsterdam, Netherlands: Elsevier; 2018. p. 133–79.
5. Makar-Ausperger K, Krželj K, Benčić ML, Aumiler MR, Turk VE, Božina N. Warfarin dosing according to the genotype-guided algorithm is most beneficial in patients with atrial fibrillation: A randomized parallel group trial. Ther Drug Monit 2018;40(3):362–8.
6. Akdeniz CS, Cevik M, Canbolat IP, Yurdakul S, Cagatay P, Ciftci C, et al. The effects of and VKORC1 gene polymorphisms on warfarin maintenance dose in Turkish cardiac patients. Future Cardiol 2020;16(6):645–54.
7. Li Y, Zhu J, Ding J. VKORC1-1639G/A and 1173 C/T Genetic Polymorphisms influence individual differences in warfarin maintenance dose. Genet Test Mol Biomarkers 2015;19(9):488–93.
8. Kosaki K, Yamaghishi C, Sato R, Semejima H, Fuijita H, Tamura K, et al. 1173C>T polymorphism in VKORC1 modulates the required warfarin dose. Pediatr Cardiol 2006;27(6):685–8.
9. Owen RP, Gong L, Sagreiya H, Klein TE, Altman RB. VKORC1 pharmacogenomics summary. Pharmacogenet Genomics 2010;20:642–4.
10. Soltani Banavandi MJ, Satarzadeh N. Association between VKORC1 gene polymorphism and warfarin dose requirement and frequency of VKORC1 gene polymorphism in patients from Kerman province. Pharmacogenomics J 2020;20(4):574–8.
11. Biswas M, Bendkhale SR, Deshpande SP, Thaker SJ, Kulkarni DV, Bhatia SJ, et al. Association between genetic polymorphisms of CYP2C9 and VKORC1 and safety and efficacy of warfarin: Results of a 5 years audit. Indian Heart J 2018;70:S13–9.
12. al Ammari M, AlBalwi M, Sultana K, Alabdulkareem IB, Almuzzaini B, Almakhlafi NS, et al. The effect of the VKORC1 promoter variant on warfarin responsiveness in the Saudi WArfarin Pharmacogenetic (SWAP) cohort. Sci Rep 2020 Jul 15;10(1):11613. doi: 10.1038/s41598-020-68519-9.
13. Moreau C, Pautas E, Gouin-Thibault I, Golmard JL, Mahé I, Mulot C, et al. Predicting the warfarin maintenance dose in elderly inpatients at treatment initiation: Accuracy of dosing algorithms incorporating or not VKORC1/CYP2C9 genotypes. J Thromb Haemost 2011 Apr;9(4):711-8.
14. Lucia RD, di Perna P, Chetta M, Santacroce R, Brancaccio V, Grandone E, et al. A polymorphism in the VKORC1 gene is associated with an interindividual variability in the dose-anticoagulant effect of warfarin. Blood 2005;105(2):645-9.
15. Herman D, Peternel P, Stegnar M, Breskvar K, Dolzan V. The influence of sequence variations in factor VII, γ-glutamyl carboxylase and vitamin K epoxide reductase complex genes on warfarin dose requirement. Thromb Haemost 2006;95(5):782–7.
16. Geisen C, Watzka M, Sittinger K, Steffens M, Daugela L, Seifried E, et al. VKORC1 haplotypes and their impact on the inter-individual and inter-ethnical variability of oral anticoagulation. Thromb Haemost 2005;94(4):773–9.
17. Alzahrani AM, Ragia G, Hanieh H, Manolopoulos VG. Genotyping of CYP2C9 and VKORC1 in the arabic population of Al-Ahsa, Saudi Arabia. Biomed Res Int 2013;2013:315980. doi: 10.1155/2013/315980.
18. Cadamuro J, Dieplinger B, Felder T, Kedenko I, Mueller T, Haltmayer M, et al. Genetic determinants of acenocou-

marol and phenprocoumon maintenance dose requirements. Eur J Clin Pharmacol 2010;66(3):253–60.
19. Panchenko E, Kropacheva E, Dobrovolsky A, Titaeva E, Zemlyanskaya O, Trofimov D, et al. CYP2C9 and VKORC1 genotyping for the quality of long-standing warfarin treatment in Russian patients. Pharmacogenomics J 2020;20(5):687–94.
20. Miao L, Yang J, Huang C, Shen Z. Contribution of age, body weight, and CYP2C9 and VKORC1 genotype to the anticoagulant response to warfarin: Proposal for a new dosing regimen in Chinese patients. Eur J Clin Pharmacol 2007;63(12):1135–41.
21. El Din MS, Amin DG, Ragab SB, Ashour EE, Mohamed MH, Mohamed AM. Frequency of VKORC1 (C1173T) and CYP2C9 genetic polymorphisms in Egyptians and their influence on warfarin maintenance dose: Proposal for a new dosing regimen. Int J Lab Hematol 2012;34(5):517–24.
22. Au N, Rettie AE. Pharmacogenomics of 4-hydroxycoumarin anticoagulants. Drug Metab Rev 2008;40:355-75.
23. Schwarz UI, Stein CM. Genetic determinants of dose and clinical outcomes in patients receiving oral anticoagulants. Clin Pharmacol Ther 2006;80(1):7–12.
24. Nakai K, Tsuboi J, Okabayashi H, Fukuhiro Y, Oka T, Habano W, et al. Ethnic differences in the VKORC1 gene polymorphism and an association with warfarin dosage requirements in cardiovascular surgery patients. Pharmacogenomics 2007;8(7):713–9.
25. Vidovic S, Skrbic R, Stojiljkovic MP, Vidovic V, Becarevic J, Stoisavljevic-Satara S, et al. Prevalence of five pharmacologically most important CYP2C9 and CYP2C19 allelic variants in the population from the Republic of Srpska in Bosnia and Herzegovina. Arh Hig Rada Toksikol 2021:28;72(3):129–34.
26. Theken KN, Lee CR, Gong L, Caudle KE, Formea CM, Gaedigk A, et al. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC) for CYP2C9 and nonsteroidal anti-inflammatory drugs. Clin Pharmacol Ther 2020;1;108(2):191–200.
27. Johnson JA, Caudle K, Gong L, Whirl-Carrillo M, Stein CM, Scott SA, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for pharmacogenetics-guided warfarin dosing: 2017 update. Clin Pharmacol Ther 2017 Sep;102(3):397-404.
28. McClain MR, Palomaki GE, Piper M, Haddow JE. A Rapid-ACCE review of CYP2C9 and VKORC1 alleles testing to inform warfarin dosing in adults at elevated risk for thrombotic events to avoid serious bleeding. Genet Med 2008;10(2):89–98.
29. Li S, Zou Y, Wang X, Huang X, Sun Y, Wang Y, et al. Warfarin dosage response related pharmacogenetics in chinese population. PLoS One 2015 Jan 16;10(1):e0116463. doi: 10.1371/journal.pone.0116463.
30. Gaikwad T, Ghosh K, Shetty S. VKORC1 and CYP2C9 genotype distribution in Asian countries. Thromb Res 2014 Sep;134(3):537-44.
31. 31. Gaikwad T, Ghosh K, Shetty S. VKORC1 and CYP2C9 genotype distribution in Asian countries. Thromb Res 2014 Sep;134(3):537-44.
32. Social Science Statistics [Internet]. [Cited: 1-Jan-2021]. Available at: https://www.socscistatistics.com/tests/.