ORIGINAL RESEARCH

Vitamin K epoxide reductase complex subunit 1 gene promoter polymorphism - a potential genetic basis for survival from thromboembolism in COVID-19

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Abstract

Introduction. The coronavirus induced disease 2019 (COVID-19), caused by the severe acute respiratory coronavirus 2 (SARS-CoV-2), which was identified in patients in China in 2019, was pronounced a pandemic in March 2020. It resulted in more than 7 million deaths worldwide. As hypercoagulation emerged as its key pathological hallmark, the objective of this study was to investigate if a polymorphism within the *VKORC1* gene, which plays a role in the vitamin K-dependent blood coagulation pathway, contributed to the survival from thrombosis in individuals who developed some form of it during their COVID-19.

Methods. This was an observational, case-control study. Characterization of the *VKORC1* -1639G>A (rs9923231) polymorphism-associated genotypes was carried out in cases (N=16), volunteers who developed some form of thromboembolism during COVID-19, but who survived from it, and controls (N=32), volunteers who did not develop any form of thromboembolism during COVID-19, by using polymerase chain reaction restriction fragment length polymorphism method, followed by Sanger sequencing of the *VKORC1* gene promoter-specific, polymerase chain reaction-amplified products.

Results. Our preliminary data indicate that the variant or A allele, which is associated with intermediate or low blood coagulability, is more frequently present within the *VKORC1* gene of individuals who developed some form of thromboembolism during their COVID-19, but who survived from it, than the wild-type or G allele, which is associated with standard or high blood coagulability.

Conclusion. These results warrant further studies into the role of the *VKORC1* promoter-associated polymorphism in the COVID-19-associated coagulopathy, as the specific *VKORC1* genotypes could become genetic biomarkers for prediction of a thrombotic state during COVID-19, and possibly, other thrombosis-associated diseases and disorders.

Keywords: COVID-19, Hypercoagulability, Thrombosis, Venous thromboembolism, Vitamin K epoxide reductase

INTRODUCTION

The coronavirus induced disease 2019 (COVID-19), caused by the severe acute respiratory coronavirus 2 (SARS-CoV-2), which was identified in patients in China in 2019 [1], was pronounced a pandemic in March 2020. It resulted in more than 776 million confirmed cases [2] and 7 million deaths worldwide [3]. Early during the pandemic, it was recognized that COVID-19's pathophysiology was not restricted to the lungs, as it could progress to a systemic disease and affect other organs. In addition, hypercoagulation termed COVID-19-associated coagulopathy (CAC) has emerged as its key pathological hallmark, exhibiting either as venous thromboembolism (pulmonary embolism and deep vein thrombosis), or arterial thromboembolism (ischemic stroke, systemic arterial embolism, acute coronary syndrome, limb artery thrombosis, mesenteric artery thrombosis, etc.) [4]. Various systematic reviews and meta-analyses reported high prevalence of venous thromboembolism: 28%, 22.7% and 24.1% among the intensive care units' (ICU) patients, and 10%, 7.9% and 7.7% among general wards' patients, respectively [5] [6] [7]. However, a sensitivity analysis showed that the prevalence of venous thromboembolism was probably lower, i.e., 15.7% in ICU patients. Furthermore, a reported overall prevalence of arterial thromboembolism in patients diagnosed with COVID-19 was 1-5%, i.e., lower than that of venous thromboembolism [8].

Biomarkers measured in hospitalized COVID-19 patients were used to estimate the severity of COVID-19 pathogenesis and predict the course of the disease: biomarkers of the inflammatory and immune system activation were interleukin (IL)-6 and C-reactive protein (CRP), whilst biomarkers of coagulation, and the consequent thrombosis risk, were platelet count, activated partial thromboplastin time (aPTT), prothrombin time, D-dimer, fibrinogen, von Willebrand (vWF) factor activity, vWF antigen, lupus anticoagulant, etc. It was established that the most severe clinical stage of CAC is characterized with high D-dimer and fibrinogen concentrations, prolonged prothrombin time and reduced platelet count, accompanied with high incidence of venous thromboembolism [9]. However, the reported incidence of thrombosis was different in different racial/ethnic groups, with a significant disparity in the incidence of thrombotic events between African Americans, Caucasians and Asians [10]. It was suggested that an inherent antithrombotic state in the Asian population, termed "East Asian Paradox" [11], was responsible for a significantly lower thrombosis rate of 1.86% in the examined 5,807 patients from the Japanese population [12] compared with a substantially higher thrombosis rate of 40.8% in the examined 184 patients from the European population [13].

One of the genetic factors, which contribute to the "East Asian Paradox", is the variant sequence within the vitamin K epoxide reductase complex subunit 1 (*VKORC1*) gene, which encodes the enzyme vitamin K epoxide reductase (VKOR) - the molecular target of warfarin, the most commonly prescribed oral anticoagulant drug worldwide. VKOR regulates the synthesis of the reduced form of vitamin K and, thus, indirectly controls the levels of the vitamin K-dependent procoagulant factors (II, VII, IX, X) and anticoagulant factors (proteins C and S). *VKORC1* is one of four genes whose variations in sequence give rise to large inter-individual variability to sensitivity to warfarin [14] [15] [16]. A variant sequence in the promoter region of the *VKORC1* gene (*-1639G>A*, rs9923231) is significantly associated with an individual's response to warfarin, with carriers of the variant or A allele, who are prevalent in East Asian populations, requiring reduced therapeutic doses of warfarin. Because the consequence of the presence of the variant or A allele in an individual is a phenotype of an enhanced endogenous anticoagulation, it was hypothesized that its presence could be, at least in part, responsible for the low incidence of CAC in East Asian populations [17].

In order to investigate if the *VKORC1 -1639G>A* polymorphism-associated genotypes/alleles may influence a survival from CAC, we examined them in 16 cases - individuals who developed some form of thromboembolism during their COVID-19 and survived from it, and 32 controls - individuals who did not develop any form of thromboembolism during their COVID-19.

METHODS

Patients and study design

This study was an observational, case-control study. For the case group of volunteers, inclusion criteria were age ≥ 18 years, history of COVID-19 diagnosis, and development of a thromboembolic event (either venous or arterial thromboembolism) during the course of COVID-19. Exclusion criterion was inability to provide written, informed consent. The cases were matched with volunteers who did not develop a thromboembolic event during their COVID-19. Two controls were randomly selected for each case, individually matched by biological sex and age (within 4 years age-range). The recruitment of volunteers took place after they were infection free, from January 2022 until January 2024. The study followed the ethical guidelines of the Declaration of Helsinki (2013) and was approved by the Bioethical Committee of the Sarajevo Medical School on the 23rd November 2021 (SMS-23112021). In addition, written, informed consent was obtained from all volunteers in the study.

Human, genomic DNA extraction and characterization of the *VKORC1 -1639G>A* (rs9923231) polymorphism-associated genotypes

Approximately 3ml of whole blood was obtained from all volunteers in ethylenediaminetetraacetic acid (EDTA)-containing tubes. Genomic DNA extraction and characterization of the *VKORC1 -1639G>A* (rs9923231) polymorphism-associated genotypes was described in a protocol deposited on Protocols.io (https://www.protocols.io/) which can be accessed through the following digital object identifier (DOI): dx.doi.org/10.17504/protocols.io.j8nlko8mdv5r/v1 [18].

Statistical methods

Descriptive statistics was used to summarize the results of the study (Table 1). In addition, a Chi-squared test was used to compare allele frequencies between cases and controls. Statistical significance (p) was set at 0.05.

Availability of data and materials

The dataset generated and analyzed during the current study was deposited in the Harvard Dataverse repository, which can be accessed through the following digital object identifier (DOI): https://doi.org/10.7910/DVN/JCCWCO [19]. In addition, this dataset was deposited in a database associated with clinical variations titled ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). The results are public and can be accessed through the accession numbers: SCV004807478 and SCV004812937 [20]. In addition, details of individual human, genomic DNA samples, which were generated in this study, were also deposited on the National Centre for Biotechnology Information data platform within a BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/). They can be accessed through the accession number: PRJNA1095829 [21]. Human, genomic DNA sample material, which was generated in this study, is available on request from the corresponding author.

RESULTS

In this study, an association between the *VKORC1 -1639G>A* (rs9923231) polymorphism-associated genotypes and survival from COVID-19-associated thrombosis was examined. In the case group, 13 volunteers

(81%) had venous thromboembolism and 3 volunteers (19%) had arterial thromboembolism. In this group, 3 volunteers (19%) had the wild-type, *VKORC1-GG* or the most thrombogenic *VKORC1* genotype, 7 volunteers (44%) had *VKORC1-AG* or the *VKORC1* genotype with an intermediate thrombogenic effect, and 6 volunteers (37%) had *VKORC1-AA* or the least thrombogenic *VKORC1* genotype. In the case group, the wild-type, G allele frequency was 0.41 or 41%, while the variant or A allele frequency was 0.59 or 59% (Table 1, Fig. 1). In contrast, in the control group, 11 volunteers (34%) had the wild-type, *VKORC1-GG* genotype, 14 volunteers (44%) had *VKORC1-AG* genotype, and 7 volunteers (22%) had *VKORC1-AA* genotype. In the control group, the wild-type, G allele frequency was 0.56 or 56%, while the variant A allele frequency was 0.44 or 44% (Table 1, Fig. 1). In order to establish whether a statistically significant difference exists between cases and controls, with regard to allele frequencies, a Chi-squared test was performed. With a degree of freedom of 1, a value of 2.0625 was obtained (p=0.1510).

DISCUSSION

Multiple patient characteristics (e.g. age, biological sex, race, existing medical condition, prescribed pharmacological agents, different biomarkers, etc.) were previously studied in order to understand which determining factors affect patients' outcomes following COVID-19 diagnosis. We examined an association between a genetic factor with an established effect on blood coagulation, the *VKORC1 -1639G>A* polymorphism, and survival from a thromboembolism in COVID-19. A trend towards a decrease in the frequency of the wild-type or G allele (0.41) and an increase in the frequency of the variant or A allele (0.59), in cases - individuals who developed a thromboembolic event during their COVID-19, but who survived it, was detected. In controls - individuals who did not de-

Table 1. Clinical, demographic and the VKORC1 -1639G>A (rs9923231) polymorphism-associated genotype characteristics of the study's volunteers.

Demographic and genetic characteristics of human volunteers	Survived cases Human volunteers with thromboembolic event during COVID-19 N=16	Controls Human volunteers without thromboembolic event during COVID-19 N=32
Age in years (Mean ± SD; Range in years)	55±14; 31-74	55±13; 29-77
Biological sex (N, % M; N, % F)	9, 56%; 7, 44%	18, 56%; 14, 44%
VKORC1-GG (N, %)	3, 19%	11, 34%
VKORC1-AG (N, %)	7, 44%	14, 44%
VKORC1-AA (N, %)	6, 37 %	7, 22%
G allele frequency (%)	41%	56%
A allele frequency (%)	59%	44%

F = female biological sex; N = number of human volunteers; M = male biological sex; SD = standard deviation; VKORC1-GG = homozygous, wild-type genotype; VKORC1-AG = heterozygous, variant genotype; VKORC1-AA = homozygous, variant genotype.

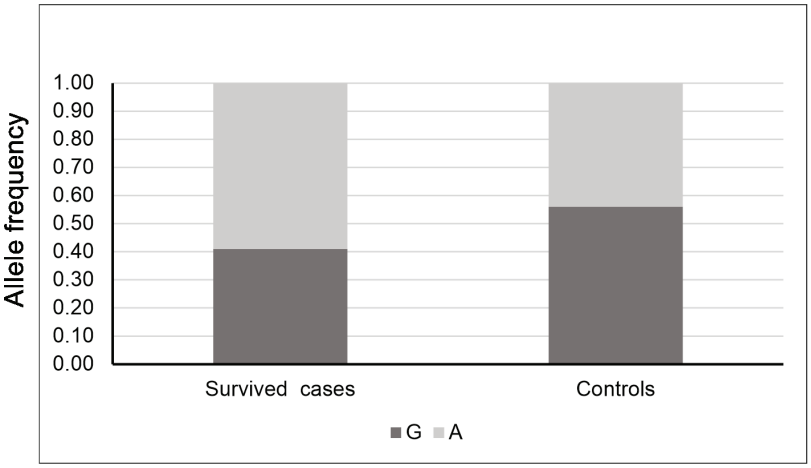

Figure 1. Allele frequencies at the VKORC1 -1639 locus in survived cases and controls.

Allele frequencies for the wild-type, VKORC1 -1639G (G) allele, and the variant, VKORC1 -1639A (A) allele were calculated and compared between survived cases (N=16), volunteers who developed a thromboembolic event during their COVID-19, and controls (N=32), volunteers who had no thromboembolic event during their COVID-19 disease. In the survived cases, the wild-type or G allele frequency was 0.41 or 41%, while the variant or A allele frequency was 0.59 or 59%. In the controls, the wild-type or G allele frequency was 0.56 or 56%, while the variant or A allele frequency was 0.44 or 44%.

velop a thromboembolic event during their COVID-19, we detected the opposite trend: the wild-type or G allele at 0.56 and the variant or A allele at 0.44. Although these differences in allele frequencies between cases and controls were not statistically significant (p=0.1510), the results of this study indicate that having the A allele during a thromboembolic event in COVID-19 contributed, at least in part, to survival from it (Fig. 2).

To our knowledge, this is the first study on this topic and its results warrant further investigations of the G/A alleles within the *VKORC1* gene promoter in individuals who survived a thromboembolic event in COVID-19, as the knowledge of the intrinsic *VKORC1* polymorphism effects on blood coagulability could improve prediction of thrombosis, monitoring, treatment and overall clinical outcomes for future patients. As data and samples, which have been generated through this study, will be made available to other researchers, we hope that this study will contribute to future research on this topic.

Limitations of the study are small sample size and unavailability of detailed information on the volunteers who were analyzed (e.g. date of their COVID-19 disease, which would indicate an infection with a specific SARS-CoV-2 variant, pharmacological agents that were prescribed/administered to them during their thrombosis and/or after it, and the presence of any comorbidities). However, in the control group of volunteers, the obtained allele frequencies (G: 0.56; A: 0.44) were similar to the average allele frequencies reported for the population of the Americas (G: 0.586; A: 0.414) [16], suggesting that the results of this study could have implications for a wider, global populations and their health outcomes.

CONCLUSION

In conclusion, our preliminary results indicate that, in the individuals who develop COVID-19-associated thrombosis, the presence of the variant or A allele within the *VKORC1* gene promoter, at least in part, offers a better chance of survival, due to its association with an intermediate or low blood coagulation. Our results also suggest that individuals with the wild-type or G allele within the *VKORC1* gene promoter, due to its association with high or standard blood coagulation, should be carefully monitored for early signs of thromboembolic events and/or pre-emptively treated when necessary.

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Declaration of Patient Consent: Written, informed consent was obtained from all volunteers in the study.

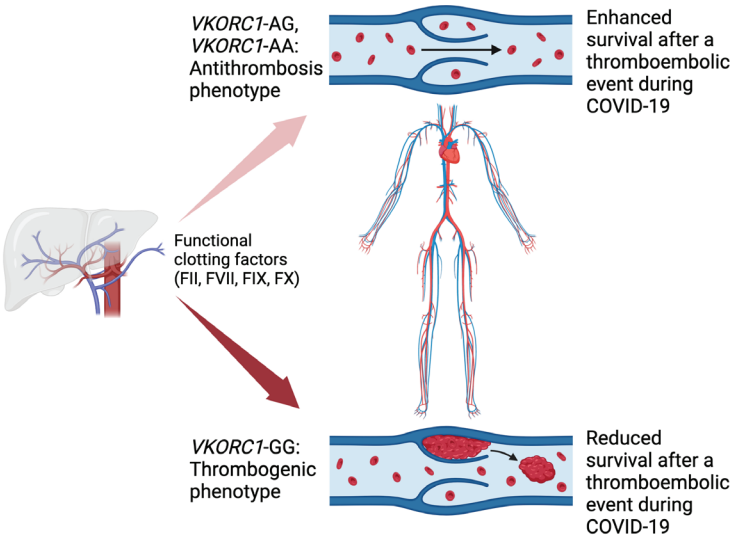

Figure 2. A hypothesized effect of the VKORC1 -1639G>*A* polymorphism on survival from COVID-19-associated thromboembolism.
Our results indicate that individuals with the variant or A allele within the VKORC1 -1639 locus, giving rise to the VKORC1-AG and VKORC1-AA genotypes, survived a thromboembolic event during COVID-19, compared with individuals with the wild-type or G allele, giving rise to the VKORC1-GG genotype. Image created with BioRender.com.

Authors' Contribution: Mirsada Causevic: Conceptualization, Funding acquisition, Investigation, Project administration, Methodology, Writing - original draft, reviewing & editing. Amina Sahbaz: Investigation, Methodology, Validation, Visualization, Writing - reviewing & editing; Nedim Galijasevic: Data curation, Methodology, Resources; Lamija Sikalo: Data curation, Methodology, Resources; Slobodan Jankovic: Funding acquisition, Formal analysis, Methodology, Writing - reviewing & editing; Edin Begic: Conceptualization, Funding acquisition, Methodology, Resources.

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Conflict of Interest: None.

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