RESEARCH ARTICLE

Prediction of Warfarin Dosage Based on Genotype (VKORC1) - 1639 G>A - rs9923231 in South Indian Populations

Ankani Bala[1*], Sivaraj R[2]

[1]*Department of Pharmacology, AVMC & Hospital, Vinayaka Mission's Research Foundation, Salem, Tamil Nadu, India.*
[2]*Department of Pharmacology, Aarupadai Veedu Medical College & Hospital, Puduccherry. Vinayaka Mission's Research Foundation, Salem, Tamil Nadu India.*

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ABSTRACT

Background: Venous thromboembolism (VTE), characterized by thrombus formation in veins, ranks as the third most prevalent vascular disease globally after myocardial infarction (MI) and stroke. Deep vein thrombosis (DVT) and pulmonary embolism (PE) are common clinical manifestations of VTE, with potentially fatal consequences. In this study, the mean daily warfarin dose using VKORC1 (-1639 G>A) rs9923231 genotyping will be established in order to categorize warfarin treatment into high, moderate, and low-dose groups within the South Indian population.

Methods and Materials: A cohort of 192 patients was enrolled, and genotyping was conducted using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique.

Results: In our study sample, the A allele frequency was 23.9%, and the AA genotype frequency was 12.5%. The mean daily doses required by AA homozygous (1.54 ± 1.05 mg/day) and GA heterozygous (2.93 ± 2.03 mg/day) genotype carriers were considerably lower to attain the optimal international normalized ratio (INR) than those of GG genotype carriers (4.07 ± 1.75 mg/day), with a *p-value* of 0.866.

Conclusion: Our findings strongly advocate for incorporating VKORC1 polymorphism analysis in South Indian patients with DVT to guide the initial warfarin dosage genetically.

Keywords: Warfarin, Venous thromboembolism, Polymerase chain reaction, Restriction fragment length polymorphism.

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INTRODUCTION

Venous thromboembolism (VTE), which includes pulmonary embolism (PE) and deep vein thrombosis (DVT), is a major global health concern. VTE is a major burden on healthcare systems, ranking third in prevalence among cardiovascular diseases after myocardial infarction and stroke.[1] While anticoagulants like warfarin have been used for VTE management for decades, their effectiveness varies considerably between individuals. This variation results from a complicated interaction between environmental and inherited factors that affect the anticoagulant effect of warfarin as well as its metabolism. Warfarin's therapeutic action lies in inhibiting the synthesis of clotting factors that depend on vitamin K. However, it has a narrow therapeutic window, necessitating careful monitoring and dose adjustments to balance effectiveness with bleeding risk.[2] Individual responses to warfarin can differ substantially due to variations in how the body processes the drug (pharmacokinetics) and how it interacts with its targets (pharmacodynamics).[3] The CYP2C9 enzyme significantly influences warfarin metabolism in the liver. Individuals with certain genetic variations in CYP2C9 may metabolize warfarin more slowly, requiring lower doses for optimal effect. Conversely, those with different variations may metabolize warfarin more rapidly, necessitating higher doses.[4] VKORC1 is directly inhibited by warfarin, which has the anticoagulant effect via lowering the activity of factors II, VII, IX, and X as well as proteins C and S.[5,6] Extensive research has revealed that genetic polymorphisms within the VKORC1 gene significantly impact warfarin treatment response across diverse populations.[7,8] This gene exhibits polymorphism, meaning it exists in multiple variants, with three main haplotypes (*2, *3, and *4) encompassing the majority of identified single-nucleotide polymorphisms (SNPs). The most notable of these is the haplotype VKORC 1*2, which is related to five important SNPs in the promoter area, which is the part of a gene that regulates its expression.[9,10] Notably, the 1639G>A variant appears to be the most influential among these polymorphisms, as it directly correlates with abnormal

**Author for Correspondence: sundu329@gmail.com*

enzyme expression and the warfarin dosage required for optimal treatment.[11] In essence, a patient's genetic makeup within the VKORC1 gene significantly influences their warfarin sensitivity.[12-14] This highlights the importance of pharmacogenetics, a field that explores how genetic variations affect drug response. By incorporating pharmacogenetic data into treatment plans, clinicians can tailor warfarin dosing to individual needs, optimizing therapeutic outcomes and minimizing the risk of complications. This is particularly relevant in diverse populations like those found in India, where significant variations in VKORC1, CYP2C9, and CYP4F2 genes have been documented across subpopulations.[15] By accounting for these genetic differences, clinicians can achieve more precise and personalized treatment strategies for VTE patients using warfarin.

Pharmacogenetics holds immense promise for personalized medicine and improving patient care. By leveraging genetic information, clinicians can not only optimize warfarin therapy but also tailor treatment strategies for a wider range of medications. This approach can enhance treatment effectiveness, reduce side effects, and improve patient outcomes for a wide range of conditions.

MATERIALS AND METHODS

A total of 196 patients were enrolled in this study. Of these, 96 were diagnosed with DVT and in the test group and were on warfarin for at least a 3-month were included in the test, and 96 were healthy individuals in the control group. This study has been approved by the IEC and done at the GVPIHC&MT, Visakhapatnam, Andhra Pradesh. This study included patients with venous thrombosis (DVT) and participants aged 18 to 65 years with a stable maintenance warfarin dose. People who took inducers/inhibitors had pre-existing risk factors (e.g., diabetes mellitus/hypertension/liver disorders), or did not give consent were excluded.

Lab Investigations

Hematological tests like a complete hemogram, prothrombin time, and estimated D-dimer were done by standard methods.

Sample Collection and Processing

A blood sample of 2 mL was taken from each patient and transferred into the EDTA tube for clotting prevention. After running these samples for 15 minutes at 4°C at 4000 rpm, the plasma was separated, and a PT-INR test was performed. The pellet was kept cold (-20°C) in order to examine its DNA.

DNA Isolation and PCR-RFLP Method

Blood DNA was extracted using Sajja *et al*., (2014) with minor modifications.

PCR amplification

The PCR-RPLP method was used to find the corresponding SNP, namely- VKORC1-1639 G>A. To amplify 150 ng of g DNA, 2.5 µL of 10XTaq buffer were added to a 25 µL PCR reaction mixture. About 100 µM of dNTPs (Takara Bio Inc., India), 250 $MgCl_2$ 0.5 U Taq polymerase forward and reverse primers 10 µM thermal cycler (Biorad MJ Mini TM). The PCR cycle was denatured at a temperature of 95°C for 5 minutes, annealed at 59°C for 45 seconds, and extended at 72°C for 45 seconds. The primers used for the PCR cycle were 5'-F and 5'-R primers. The PCR products were digested with 2 units of restriction enzyme Misspl (NEB). The 2% agarose gel stained with ethidium bromide was used for visualizing the results (Figure 1). A single uncut band measuring 290 base pairs (bp) was found to indicate the homozygous GG genotype. Two bands measuring 168 and 122 bp each were used to score the AA homozygous genotype, while three bands measuring 290, 168, and 122 bp each were used to identify the GA heterozygous genotype. 10% or so of the samples underwent additional analysis. To determine whether genotyping concurred 100% of the time, about 10% of the samples underwent further analysis.

Lane -M: 500 bp DNA ladder; Lane- 1: AA homozygous mutant; Lane -2, 3, 4: GG homozygous genotype ; and Lane- 5, 6: GA heterozygous genotype.

Statistical Analysis

Descriptive statistics were calculated using the chi-square test and Fisher's exact test in Graph Pad using Prism 8 software. Statistics were considered statistically significant when $p > 0.05$ for allelic frequency deviation from HWE. The predicted daily dose based on genotype is represented in terms of standard deviation. ANOVA and coefficient of variation were used to estimate the genotypic-based difference in mean daily dose (MDD) among patients with three genotypes (GG, GA, AA) of the polymorphism (VKORC1 - 1639 G>A - rs9923231)[12,13]

RESULTS

There were 96 individuals in the study, comprising the test and control groups. There were 45 (46.9%) men and 51 (53.1%) women in the test group, compared to 53 (53.1%) men and 45 (46.9%) women in the control group. Demographic information (refer to Table 1) and genotype frequencies were subjected to analysis.

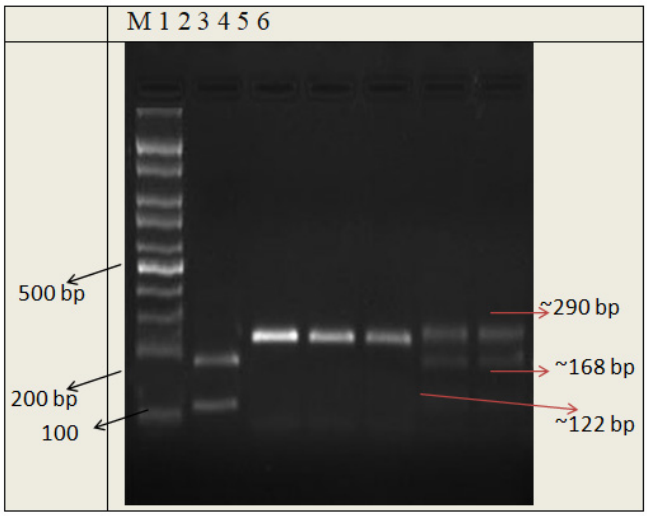

Figure 1: MspI PCR-RFLP analysis of the VKORC1 (1639G>A) polymorphism

Table 1: Demographic profile of patients

Characteristic	*Test group*	*Control group*
Gender - Male	(46.9%) – 45	(53.1%) - 51
Female	(53.1%) -51	(46.9%) - 45
Age	Median: 47	Median: 36
Weight	Median: 45.5	Median: 60.5

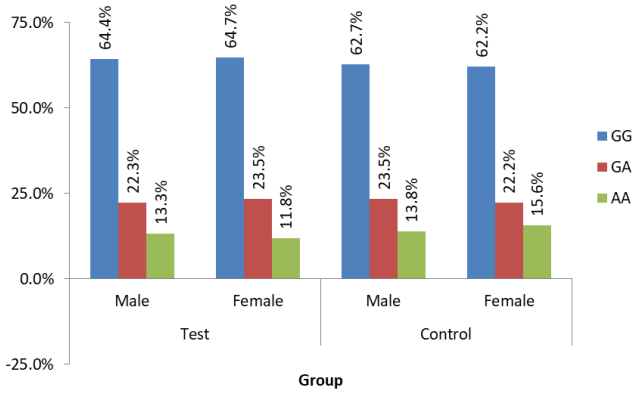

Figure 2: Male and female genotyping frequencies in both groups

The VKORC1 (1639G>A) genotype frequencies of GG, GA, and AA among 192, 62 (64.6%), 22 (22.9%), and 12 (12.5%) were in the test group, and 61 (63.5%), 22 (2.9%), and 13 (13.5%) (*p-value* of 0.96) were in the control group (Table 2).

The male and female frequencies are shown in Figure 2, with a *p-value* of 0.968 and 0.863, respectively.

- Genotype distribution: In both the patient and control groups, the most frequent genotype is GG-wild type, followed by GA-heterozygous and AA-homozygous mutant. This suggests that the G allele is more common than the A allele in this population.
- Allele frequency: The frequency of the G allele is slightly higher than the A allele in both groups. This is consistent with the genotype distribution.
- Hardy-weinberg equilibrium: The *p-value*s for both groups (0.967 for patients and 0.744 for controls) are greater than 0.05, indicating that the genotype frequencies are in Hardy-Weinberg equilibrium (HWE). HWE is a principle of population genetics that states that the number of alleles in a population will be the same from generation to generation under certain conditions. The VKORC1 (-1639G>A), GG is the wild genotype and requires a higher warfarin dose; GA is a heterozygous mutant genotype that requires an intermediate dose; and AA is a homozygous mutant that requires a lower dose of warfarin.

Mean estimated daily dose (MDD) by genotype (test group *vs.* control group) (Table 3).

The dose variations were found to be as follows: In the test group, the GG genotype was 4.07 ± 1.75, GA was 2.93 ± 2.03, and AA was 1.54 ± 1.05, while the controls were 4.33 ± 1.93, GA was 1.61 ± 1.31, and AA was 0.96 ± 0.13.AA and GA genotypes carry a lower dose than the GG genotype.

- In both the test and control groups, the AA genotype (homozygous mutant) has the lowest average daily warfarin dose).
- Those who have the GG genotype (wild type) usually need larger doses of warfarin than people who have the AA genotype. This is consistent with what was mentioned in the text you provided earlier.
- The CV measures variability in relation to the mean. A higher CV indicates greater variability in the data. In this table, the CV is highest for the GA genotype in the test group. This suggests that there is more variability in the daily dose requirement among individuals with the GA genotype in the test group compared to the other genotypes in the test group or the control group.

DISCUSSION

DVT risk factors are a complex topic that has received extensive research. Pharmacogenetic data assists clinicians in improving patient health by reducing side effects or reducing treatment costs by reducing hospital admissions by reducing side effects, treatment costs or hospital admissions.[16] The most important predictor for warfarin intake is thought to be the gene called VKORC1.[17] The present genotyping study was done on 196 patients from the south Indian population for VKORC1 gene polymorphisms. This study looked at the prevalence of VKORC1 gene variants in patients with deep vein thrombosis and how they related to the warfarin maintenance dosage.[7] The variants of the VKORC1 gene are GG, GA, and AA. GG from VKORC1 is regarded as homozygous wild type; heterozygous and homozygous are GA and AA, respectively. From VKORC1 GG: The variants of VKORC1 that depend on sensitivity to warfarin are categorized into three types.[18,19] When wild-

Table 2: Genotype and allelic distribution of VKORC1 1639G>A polymorphism

Study group	*Genotype*	*Frequency (%)*	*Allele*	*Frequency (%)*	*HWE (p-value)*
Patient	GG	62 (64.6%)	G	146 (76.1%)	0.967
	GA	22 (22.9%)	A	46 (23.9%)	
	AA	12 (12.5%)			
Control	GG	61 (63.5%)	G	143 (74.6%)	0.744
	GA	22 (22.9%)	A	49 (25.4%)	
	AA	13 (13.5%)			

Table 3: An ANOVA test has significance at $p < 0.05$, b.The variation coefficient (CV = SD/mean)

Genotype	*Test*	*Dose*	*CV*	*Control*	*Dose*	*CV*	*p-value*
GG	62	4.07 ± 1.75	0.42	61	4.33 ± 1.93	0.44	0.866
GA	22	2.93 ± 2.03	0.62	22	1.61 ± 1.31	0.3	
AA	12	1.54 ± 1.05	0.68	13	0.96 ± 0.13	0.13	

type homozygous GG individuals take warfarin, warfarin sensitivity is higher and requires a higher dose. Warfarin sensitivity is lower in heterozygous mutants (GA) and requires intermediate dosage. Warfarin susceptibility is higher in homozygous mutants (AA) and requires an intermediate dose.

The present study is similar to those observed by other studies like Shalia *et al*. (GG 75.5%, GA 22.4%, AA 2.0%) and Gaikwad *et al*. (GG 76.6%, GA 21.4%, AA 2.0%) where GG required a higher warfarin dose.[20,21] In several studies conducted in the Indian population, the frequency of the genotype VKORC1 was found to be significantly higher than in other world populations, particularly in the populations of Japan and China[4,22-27]

In this study, patients with homozygous mutant genotype VKORC1-(1639 G>A) AA needed to modify their warfarin dosage. When compared to the average daily dose needed by the patient with homozygous wild-type genotype, the mean daily dose of warfarin per day for patients with homozygous mutation VKORC1 (1639) G>A was significantly lower. The daily dose requirement of heterozygous genotype GA is 2.93 in the test group and homozygous wild type VKORC1-(1639 G>A) GG (4.33 mg, GA 1.61, and AA 0.96 mg with a *p-value* of 0.866). The relationship between variant alleles and warfarin dosage has been demonstrated to be statistically significant in several investigations.[28-32] However, differences in results were observed in a few other studies due to sample size variation and genetic and non-genetic determinants play an important role in certain populations.

CONCLUSION

Warfarin dose variability is influenced solely by the VKORC1 polymorphism, and individuals with the variant genotype need less medication. Thus, VKORC1 genotyping prior to starting warfarin medication can enhance the patient's response to therapy and reduce the risk of complication-related bleeding. The current study clearly demonstrates that VKORC1 gene genotyping would help with precise dosage prediction to stabilize INR, and it likely presents a strong argument for routine pharmacogenomic testing that should be performed on our population.

AUTHORS CONTRIBUTION

Data Analysis and Interpretation by SR and prepared and designed the manuscript, Analysis of Statistics by BTS.

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