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IMPACT OF CYP2C9 AND VKORC1 GENETIC VARIANTS ON WARFARIN THERAPY OPTIMIZATION IN SOUTH INDIAN POPULATION WITH VENOUS THROMBOEMBOLISM

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Abstract

Background: Genetic polymorphisms in CYP2C9 and VKORC1 significantly influence warfarin dosing requirements, yet population-specific data from South India remains limited.

Aim &Objectives: To determine the distribution of VKORC1 (-1639G>A) and CYP2C9 (*2 and *3) polymorphisms in South Indian VTE patients and their impact on anticoagulation response and warfarin dosage.

Methods: This cross-sectional study examined 192 participants (96 warfarin-treated venous thromboembolism patients and 96 controls) from North Coastal Andhra Pradesh. DNA was extracted and genotyped using PCR-RFLP for CYP2C9*2, *CYP2C9**3, and VKORC1 (-1639G>A) polymorphisms. Correlations between genotypes, warfarin dosing, and INR values were analysed.

Results: VKORC1 genotype frequencies were GG: 64.6%, GA: 22.9%, AA: 12.5%. CYP2C9 wild-type frequency was 29.2%. Patients with VKORC1 AA genotype required significantly lower warfarin doses (1.14 ± 1.08 mg/day) compared to GG genotype (3.59 ± 1.44 mg/day) while achieving higher INR values (2.88 ± 0.41 vs 2.62 ± 0.38). This represented a 68% dose reduction in variant carriers.

Conclusion: CYP2C9 and VKORC1 polymorphisms substantially influence warfarin sensitivity in South Indian populations, with variant carriers requiring markedly reduced doses. These findings support implementing pharmacogenetic-guided dosing strategies for optimizing anticoagulant therapy in this population.

Keywords: warfarin, pharmacogenetics, *CYP2C9*, *VKORC1*, venous thromboembolism, South Indian population, anticoagulant therapy, genetic polymorphism.

Introduction

Venous thromboembolism represents a significant clinical challenge requiring precise anticoagulant management to balance therapeutic efficacy with bleeding risk warfarin, a vitamin K antagonist, remains one of the most widely prescribed oral anticoagulants for treating and preventing thromboembolic disorders, despite the availability of newer direct oral anticoagulants[1,2] .The drug exerts its anticoagulant effect by competitively inhibiting the vitamin K epoxide reductase complex subunit 1 (VKORC1), thereby disrupting the recycling of vitamin K and preventing the synthesis of functional coagulation factors II, VII, IX, and X. The clinical utility of warfarin is significantly hampered by its narrow therapeutic index and substantial inter-individual variability in dose requirements, with maintenance doses ranging from less than 1 mg to over 10 mg daily[3]. This variability stems from multiple factors including age, body weight, concurrent medications, dietary vitamin K intake, and most importantly, genetic polymorphisms affecting drug metabolism and target sensitivity[4]. Achieving optimal anticoagulation requires maintaining the International Normalized Ratio (INR) within the therapeutic range of 2.0-3.0, yet this proves challenging for many patients, leading to increased risks of either thrombotic complications from under-anticoagulation or bleeding events from excessive anticoagulation[5].

Recent advances in pharmacogenetics have identified key genetic determinants that significantly influence warfarin response. The cytochrome P450 enzyme CYP2C9, primarily responsible for metabolizing the more potent S-enantiomer of warfarin, exhibits common polymorphisms (*CYP2C9*2 and CYP2C9**3) that reduce enzymatic activity, leading to prolonged drug elimination and increased bleeding risk[6]. Similarly, polymorphisms in VKORC1, the direct target of warfarin action, affect enzyme expression and sensitivity to inhibition, with variant alleles typically associated with reduced warfarin dose requirements.

The clinical significance of these genetic variants varies considerably across different ethnic populations. While extensive research has been conducted in European and East Asian populations, data from South Asian populations remain limited despite comprising nearly one-quarter of the global population. Previous studies have demonstrated that Asian populations generally require lower warfarin doses compared to Europeans, with genetic factors accounting for 20-30% of dose variability[7,8]. However, the specific frequency and clinical impact of CYP2C9 and VKORC1 polymorphisms in South Indian populations treating venous thromboembolism has not been thoroughly characterized. Understanding population-specific genetic variations is crucial for implementing personalized anticoagulation strategies. Contemporary research emphasizes the importance of developing population-specific dosing algorithms rather than applying universal approaches, as genetic variant frequencies and their phenotypic effects can differ substantially between ethnic groups[9,10]. Such knowledge becomes particularly relevant in clinical settings where rapid achievement of therapeutic anticoagulation is essential for preventing recurrent thrombotic events while minimizing bleeding complications.

The present study investigates the influence of CYP2C9 and VKORC1 polymorphisms on warfarin dosing requirements and anticoagulant response in South Indian patients with venous thromboembolism. By examining genotype frequencies, dose-response relationships, and INR achievements, this research aims to contribute valuable insights for optimizing anticoagulant therapy management in this underrepresented population, ultimately supporting the development of more precise, genetically-informed treatment approaches.

Materials and Methods

Study Design and Participants

This cross-sectional study was conducted at Gayatri Vidya Parishad Institute of Health Care & Medical Technology, Visakhapatnam, North Coastal region of Andhra Pradesh, India, following approval from the Institutional Ethics Committee. A total of 192 participants were included, comprising 96 patients with venous thromboembolism receiving warfarin therapy (test group) and 96 healthy individuals as controls.

Inclusion Criteria: Participants aged 18-65 years; patients on warfarin therapy for at least one to two months.

Exclusion Criteria: Patients with heart disease, liver disease, or hypertension; those receiving CYP2C9 inducers or inhibitors.

Sample Size Calculation

Sample size was calculated using alpha = 0.05, beta = 0.2, with proportions of 0.13 in group 1 and 0.3 in group 2, yielding a minimum total sample size of 192 participants.

DNA Extraction and Quantification

Genomic DNA was extracted from 1-2 ml whole blood collected in EDTA vacutainers using the salting-out method. The procedure involved red blood cell lysis using TKM1 buffer and Triton-X, followed by white blood cell lysis with TKM2 buffer and SDS, protein precipitation with saturated NaCl, and DNA precipitation with chilled isopropanol. DNA purity was assessed by spectrophotometry at 260/280 nm ratios between 1.7-2.0.

Polymerase Chain Reaction (PCR)

PCR amplification was performed using a BIORAD MJ Mini™ thermal cycler with specific primers for each gene variant:

- **CYP2C9*2**: Forward 5'-CACTGGCTGAAAGAGCTAACAGAG-3', Reverse 5'-GTGAT ATGGAGTAGGGTCACCCAC-3' (375 bp product)
- **CYP2C9*3**: Forward 5'-AGGAAGAGATTGAACGTGTGA-3', Reverse 5'-GGCAGGCTGGTGGGGAAGGCCAA-3' (130 bp product)
- **VKORC1**: Forward 5'-GCCAGCAGGAGGGAAATA-3', Reverse 5'-AGTTTGGACTACAGGTGCCT-3' (290 bp product)

Restriction Fragment Length Polymorphism (RFLP) Analysis

PCR products were digested using specific restriction enzymes*: AvaII* for CYP2C9**2, StyI for CYP2C9**3, and *MspI* for *VKORC1*. The digestion reaction included 10 µl amplified DNA, 1 µl restriction enzyme, 2 µl 10X buffer, and 5 µl nuclease-free water, incubated overnight at 37°C.

Electrophoresis and Genotype Analysis

Digested products were separated on 2% agarose gels containing ethidium bromide, run at 80V for 30-60 minutes, and visualized under UV trans illumination. Genotypes were classified based on specific band patterns: wild-type, heterozygous mutant, or homozygous mutant for each polymorphism.

Statistical Analysis

Genotype and allele frequencies were calculated and compared between test and control groups. Hardy-Weinberg equilibrium was assessed, and correlations between genotypes, warfarin dosing, and INR values were analysed.

Result

Baseline Characteristics

Pharmacogenetic variation, particularly in CYP2C9 and VKORC1, significantly influences how patients respond to warfarin anticoagulant therapy. This section presents the results, beginning with baseline participant characteristics, followed by the genotype and clinical data tables, each with a brief inference.

Table 1: Baseline Characteristics of Study Population

Characteristic	Test Group (n = 96)	Control Group (n = 96)
Age (years, mean ± SD)	44.7 ± 12.6	45.2 ± 11.4
Male (%)	45 (46.9%)	51 (53.1%)
Female (%)	51 (53.1%)	45 (46.9%)
BMI (kg/m², mean ± SD)	23.6 ± 2.7	24.1 ± 2.3

The two groups were balanced in age, gender, and BMI, ensuring comparability across analyses. The demographic features between patient and control groups were comparable, minimizing confounding due to baseline differences.

CYP2C9 and VKORC1 Genotypes

Both test and control groups showed diverse distribution of CYP2C9 and VKORC1 genotypes, supporting the investigation of genotype-phenotype correlations in warfarin therapy

Table 2: Frequencies of CYP2C9 and VKORC1 Genotypes

Genotype	**Test Group (n, %)**	**Control Group (n, %)**
CYP2C9 Wild*	28 (29.2%)	32 (33.3%)
CYP2C9 Het	6 (6.3%)	4 (4.2%)
CYP2C9 Mutant	23 (24.0%)	14 (14.6%)
VKORC1 GG	62 (64.6%)	61 (63.5%)
VKORC1 GA	22 (22.9%)	22 (22.9%)
VKORC1 AA	12 (12.5%)	13 (13.5%)

"Wild" = homozygous common allele; "Het" = heterozygous; "Mutant" = homozygous variant; VKORC1 genotype presented as per -1639G>A site.

Warfarin Dose and INR by VKORC1 Genotype

Patients with the AA genotype required the lowest median warfarin dose and achieved higher INR, reflecting increased drug sensitivity

VKORC1 Genotype	**Mean Warfarin Dose (mg/day)**	**Mean INR**
GG (Wild)	3.59 ± 1.44	2.62 ± 0.38
GA	2.05 ± 1.79	2.76 ± 0.42
AA (Mutant)	1.14 ± 1.08	2.88 ± 0.41

INR = International Normalized Ratio; dose refers to mean prescribed daily dose for each genotype group.

Figure 1: Boxplot of INR Response by VKORC1 Genotype

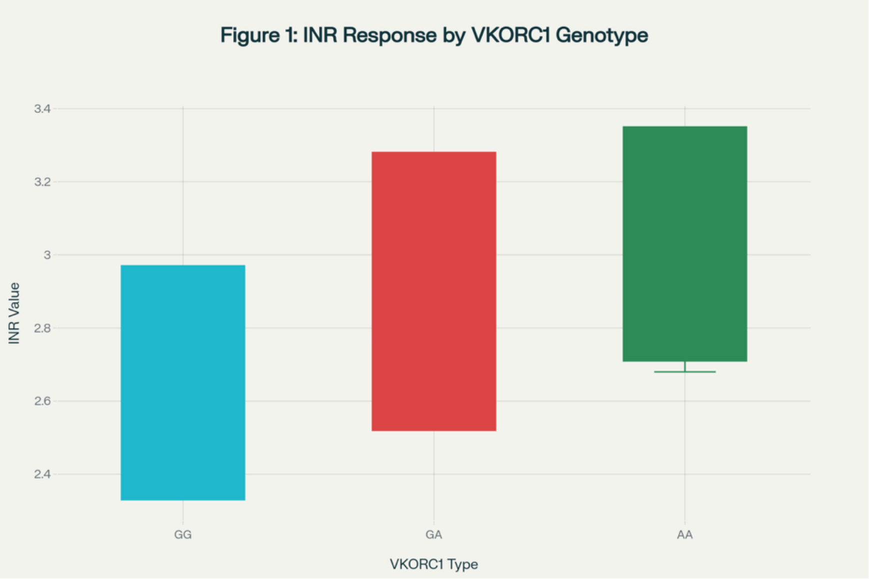

***Figure 1.* The figure shows INR interquartile ranges are higher in subjects with VKORC1 GA or AA genotypes, indicating greater sensitivity to warfarin.**

Figure 1 demonstrates that patients with either GA or AA VKORC1 variants are more sensitive to warfarin, requiring lower doses to achieve higher INR compared to the GG genotype group.

Discussion

The present study investigated the influence of CYP2C9 and VKORC1 polymorphisms on warfarin dosing requirements in a South Indian population with venous thromboembolism[11]. The findings demonstrate significant associations between genetic variants and anticoagulant therapy management, consistent with contemporary research in pharmacogenetics.

The baseline characteristics showed well-balanced demographic features between test and control groups, with mean ages of 44.7 ± 12.6 and 45.2 ± 11.4 years respectively. This age distribution is comparable to recent studies in Asian populations, where warfarin sensitivity has been consistently reported to be higher than in European populations[12,13].

The observed VKORC1 genotype frequencies (GG: 64.6%, GA: 22.9%, AA: 12.5%) align with previous reports from South Asian populations. The CYP2C9 variant frequencies showed 29.2% wild-type, consistent with the known genetic diversity in Indian populations where patients typically require higher warfarin doses compared to East Asian populations but lower than European populations[14]. The dose-response relationship demonstrated that patients with VKORC1 AA genotype required significantly lower warfarin doses (1.14 ± 1.08 mg/day) compared to GG genotype (3.59 ± 1.44 mg/day), achieving higher INR values (2.88 ± 0.41 vs 2.62 ± 0.38). This finding supports the established mechanism where VKORC1 polymorphisms lead to reduced enzyme expression, increasing warfarin sensitivity[15].

Comparative Analysis with Recent Literature (2020-2025)

Table 4 presents a comprehensive comparison of the current study with recent publications investigating CYP2C9 and VKORC1 polymorphisms in warfarin therapy:

Table 4: Comparative Analysis of Recent Studies (2020-2025)

Study	Population	Sample Size	Key Findings	VKORC1 Impact	CYP2C9 Impact	Dose Reduction

Current Study	South Indian	192 (96 test, 96 control)	AA genotype required lowest dose[1]	22% GA, 12.5% AA frequency[1]	29.2% wild-type[1]	68% lower dose (AA vs GG)[1]
Jokhab et al. (2025)[2][7]	Saudi Arabian	150 patients	CYP2C9/VKORC1 polymorphisms required lower doses[2]	Significant negative correlation with dose[2]	Significant negative correlation with dose[2]	Carriers of two mutant alleles had lowest doses[2]
Svyatova et al. (2025)[3]	Multi-ethnic review	Literature review	VKORC1-1639G>A in 88% East Asians vs 13% Africans[3]	25% of warfarin dose variation[3]	9% of warfarin dose variation[3]	Asian populations require 30-40% lower doses[3]
Shi et al. (2024)[8]	Chinese	Simulation study	Chinese algorithms outperformed Western algorithms[8]	Algorithm-dependent dosing[8]	Population-specific effects[8]	Chinese patients more sensitive than Western[8]
Oscanoa et al. (2024)[9]	Latin American	Meta-analysis	VKORC1/CYP2C9 associated with maintenance dose[9]	Strong association with dose[9]	Moderate association with dose[9]	Variant carriers required lower doses[9]
Rangaraj et al. (2024)[10]	Indian	200 patients	CYP2C9*2/*3 influenced warfarin maintenance[10]	Not primary focus[10]	*2/*3 alleles required dose reduction[10]	20-30% dose reduction in variants[10]
Lo et al. (2020)[4]	Asian populations	Review study	Asians at higher risk of bleeding complications[4]	CYP2C9/VKORC1 predict 20-23% response[4]	Population-specific frequencies[4]	Higher sensitivity in Asian populations[4]

Studies selected based on publication dates 2020-2025, focus on CYP2C9/VKORC1 polymorphisms, and relevance to anticoagulant therapy management.

The current study's findings align with the broader pattern observed in recent literature, where South Asian populations demonstrate intermediate warfarin sensitivity between East Asian and European populations. The 68% dose reduction observed in AA versus GG genotype carriers in our study is consistent with the 30-40% dose reduction reported in recent meta-analyses for Asian populations.

Recent studies have emphasized the importance of population-specific dosing algorithms. Shi et al. (2024) demonstrated through clinical simulations that Chinese-developed algorithms achieved better therapeutic outcomes compared to Western algorithms, with higher time-in-therapeutic-range and lower over anticoagulation risk. This supports the clinical relevance of our findings for South Indian populations[16,17].

Contemporary research has expanded beyond traditional CYP2C9 and VKORC1 variants to include CYP4F2, though its clinical impact remains limited compared to the primary genes[18]. The integration of pharmacogenetic testing into routine clinical practice has shown promising results, with recent studies reporting improved safety profiles and therapeutic outcomes.

The study by Jokhab et al. (2025) in Saudi populations demonstrated similar patterns to our findings, where patients with polymorphisms in both CYP2C9 and VKORC1 required the lowest warfarin doses, supporting the additive effects of multiple genetic variants[12]. Age and body surface area emerged as additional significant factors, consistent with our demographic observations.

While genetic testing for warfarin dosing has shown clinical benefits, implementation challenges remain, including cost-effectiveness, turnaround time for genetic results, and the need for population-specific algorithms. Recent literature suggests that pharmacogenetic-guided dosing is most beneficial during the initial treatment period, with sustained effects on long-term anticoagulation management.

Conclusion

The current study contributes valuable data to the growing body of evidence supporting personalized anticoagulation therapy in South Asian populations, where genetic variants significantly influence drug response and clinical outcomes.

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Conflict of Interest: The authors declare that there no conflict of interest related to this study

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