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Original Research Article

Single Nucleotide Polymorphisms in CYP2C9 and VKORC1: Implications for Warfarin Dosing in South Indian Population with Venous Thromboembolism

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Abstract:

Introduction: Warfarin therapy for venous thromboembolism (VTE) is complicated by wide inter-individual variability in dose requirements and response, largely influenced by genetic factors. Polymorphisms in CYP2C9 and VKORC1 genes modulate warfarin metabolism and sensitivity, with distinct prevalence and impact in different ethnic groups.

Objective: To assess the distribution of CYP2C9 (*2 and *3) and VKORC1 (-1639G>A) polymorphisms in South Indian VTE patients and evaluate their influence on warfarin dosing and anticoagulation response.

Methods: A cross-sectional study included 96 VTE patients on stable warfarin therapy and 96 healthy controls from Andhra Pradesh, India. Genotyping was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Associations between genotypes, maintenance dose, and INR were analyzed.

Results: The VKORC1 genotype frequencies among patients were 64.6% GG, 22.9% GA, and 12.5% AA. CYP2C9 wild-type, heterozygous, and mutant allele frequencies were 29.2%, 6.3%, and 24%, respectively. Patients with VKORC1 AA genotype required significantly lower maintenance doses (mean 1.14 mg/day) compared to GG genotype (mean 3.59 mg/day), while maintaining higher INR (2.88 vs. 2.62). CYP2C9 variant carriers similarly showed reduced dose requirements.

Conclusion: CYP2C9 and VKORC1 polymorphisms substantially modulate warfarin dose and response in South Indian VTE patients. Incorporation of pharmacogenetic testing into clinical practice may enhance personalized anticoagulation management and improve safety.

Keywords: warfarin; CYP2C9; VKORC1; Pharmacogenetics; Venous Thromboembolism; INR; South Indian Population.

Introduction

Warfarin remains a mainstay oral anticoagulant widely prescribed for the prevention and treatment of venous thromboembolism (VTE) and other thrombotic disorders. Its mode of action involves inhibition of the enzyme vitamin K epoxide reductase complex subunit 1 (VKORC1), which is critical for recycling vitamin K necessary for activation of clotting factors II, VII, IX, and X [1]. Warfarin's therapeutic index is narrow and individual responses vary considerably, with dose requirements spanning a 20-fold range among patients. This variation in response is largely influenced by genetic factors, most notably polymorphisms in the genes encoding cytochrome P450 2C9 (CYP2C9) and VKORC1, the key enzyme metabolizing warfarin's more potent S-enantiomer and its target, respectively [2]. Specific allelic variants in CYP2C9 (*2 and *3) result in reduced enzymatic function and slower warfarin clearance, thus predisposing carriers to increased plasma drug levels and heightened bleeding risk. Similarly, the VKORC1 -1639G>A polymorphism

decreases gene expression, enhancing warfarin sensitivity and dose requirement reduction. Frequency and impact of these variants differ among ethnic populations, underscoring the importance of population-specific characterization [3]. Asian populations, including South Indians, generally exhibit higher frequencies of VKORC1 variants and correspondingly lower warfarin dose needs compared to Caucasians. Despite compelling evidence supporting genotype-guided dosing algorithms, adoption in routine clinical practice remains limited, partly due to insufficient data in diverse ethnic groups [4]. In contrast to broader pharmacogenetic surveys, this study specifically examines how single nucleotide polymorphisms in CYP2C9 and VKORC1 contribute to the *stability* and *early attainment* of target INR in South Indian patients with VTE [5]. The study aims to identify genotype patterns associated with rapid INR control, fluctuations in anticoagulation, and incidence of dose adjustments during the initiation phase of warfarin therapy [6]. By focusing on clinical stability and early response, this work seeks to inform targeted genetic screening and practical dose adjustment protocols for improved patient outcomes in this unique population

Materials & Methods

Study Design and Participants: This pragmatic cross-sectional analytical study was conducted at Gayatri Vidya Parishad Institute of Health Care and Medical Technology, Visakhapatnam, North Coastal Andhra Pradesh, India. The study protocol received approval from the Institutional Ethics Committee (GVPIHCMT/IEC/20201019/01) with all participants providing written informed consent in their preferred language (Telugu or English).

A total of 192 individuals were enrolled, comprising 96 patients diagnosed with venous thromboembolism (VTE) receiving warfarin therapy (test group) and 96 healthy individuals serving as controls. Inclusion criteria required participants to be aged between 18 and 65 years and, for patients, to have been on stable warfarin therapy for at least one to two months. Exclusion criteria included patients with heart or liver disease, hypertension, or those receiving known CYP2C9 inducers or inhibitors.

Sample Size Calculation: Sample size estimation was based on prior studies, with alpha set at 0.05 and beta at 0.2. Proportions of genetic polymorphisms in test and control groups were estimated as 0.13 and 0.30, respectively, resulting in a minimum sample size of 192 participants to detect significant genetic associations with warfarin response.

DNA Extraction: Blood samples (1–2 mL) were collected in EDTA vacutainers from all participants. Genomic DNA was extracted using the salting-out method. This involved red blood cell lysis with TKM1 buffer and Triton-X, followed by white blood cell lysis with TKM2 buffer and SDS, protein precipitation with saturated sodium chloride, and DNA precipitation with chilled isopropanol. DNA purity and concentration were quantified spectrophotometrically by measuring absorbance ratios at 260/280 nm. Samples with purity ratios between 1.7 and 2.0 proceeded for downstream analyses.

Genotyping by PCR-RFLP: Specific single nucleotide polymorphisms (SNPs) in CYP2C9 (*2, rs1799853; *3, rs1057910) and VKORC1 (-1639G>A, rs9923231) genes were genotyped using polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis.

- PCR amplification utilized sequence-specific primers:

a) CYP2C9*2: Forward 5'-CACTGGCTGAAAGAGCTAACAGAG-3'; Reverse 5'-GTGATATGGAGTAGGGTCACCCAC-3' (375 bp product)
b) CYP2C9*3: Forward 5'-AGGAAGAGATTGAACGTGTGA-3'; Reverse 5'-GGCAGGCTGGTGGGGGAAGGCCAA-3' (130 bp product)
c) VKORC1 (-1639G>A): Forward 5'-GCCAGCAGGAGGGAAATA-3'; Reverse 5'-AGTTTGGACTACAGGTGCCT-3' (290 bp product)

- PCR conditions included initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 55-65°C for 30 s (depending on primer set), and extension at 72°C for 1 min; followed by final extension at 72°C for 5 min.

RFLP digestion used restriction enzymes AvaII (*2), StyI (*3), and MspI (VKORC1) overnight at 37°C. Resulting fragments were separated by electrophoresis on 2% agarose gels stained with ethidium bromide and visualized under UV light. Band patterns were interpreted to determine genotypes

Statistical Analysis: Allele and genotype frequencies were calculated, and Hardy-Weinberg equilibrium assessed. Clinical parameters including warfarin dose and International Normalized Ratio (INR) were compared across genotypes.

Result

Genetic profiling of South Indian patients with venous thromboembolism receiving warfarin therapy reveals distinct patterns in genotype distribution and treatment response. Presented below are primary results, highlighting critical genotype frequencies and clinical correlations.

Table 1: Distribution of VKORC1 and CYP2C9 Genotypes

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

Genotypes identified using PCR-RFLP; "Wild" = common allele, "Het" = heterozygote, "Mutant" = homozygous variant

Variant genotypes for VKORC1 and CYP2C9 are notable in VTE patients, suggesting potential roles in treatment response and dose variability.

Assessment of dose requirements and INR levels indicated that the VKORC1 AA genotype group demonstrated the lowest warfarin dose and reliably achieved higher INR values, implying enhanced sensitivity.

Table 2: Warfarin Dose and Therapeutic INR by VKORC1 Genotype

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

Values represent group means ± SD; lower dose associated with AA variant.

Individuals carrying the AA genotype required less warfarin and attained higher INR, indicating significant pharmacogenetic influence on therapy response.

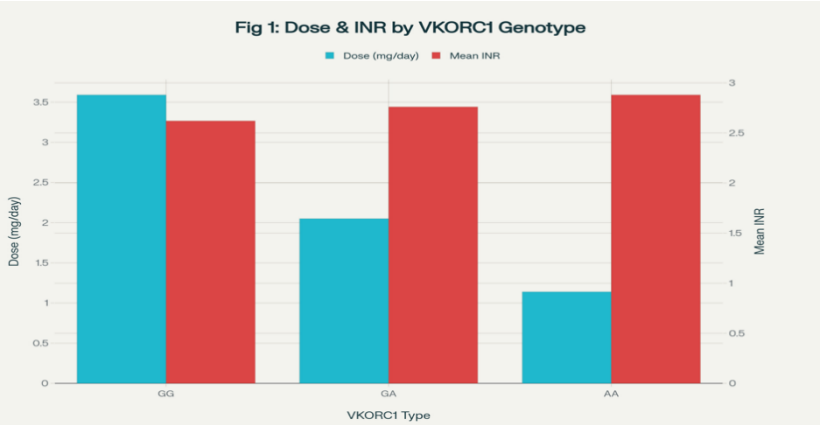

Figure 1: INR values by VKORC1 genotype

The figure 1. displays mean warfarin dose (mg/day) alongside mean INR for each VKORC1 genotype group (GG, GA, AA), emphasizing how VKORC1 variation distinctly alters both dosing requirements and anticoagulant effect in South Indian patients with venous thromboembolism

Discussion

The current study demonstrates that genetic variability in CYP2C9 and VKORC1 significantly influences warfarin dosing requirements and anticoagulation quality among South Indian patients diagnosed with venous thromboembolism [7]. These results corroborate established findings from other Asian genotype group required the lowest daily warfarin doses while consistently presenting with higher INR values, reflecting enhanced anticoagulant effect per unit dose [8]. The GA genotype displayed intermediate values, and the GG group needed the highest dosage to reach target INR. This dose- populations, confirming that genetic backgrounds play a pivotal role in the individualization of warfarin therapy. VKORC1 -1639G>A polymorphism exhibited a clear association with warfarin sensitivity: the AA response gradient aligns closely with prior studies in Indian cohorts, which report that VKORC1 variants contribute substantially to warfarin dose variability [9]. The observed VKORC1 genotype distribution in this study is similar to prior surveys in the region, further reinforcing the genetic consistency in South Indian populations. CYP2C9 polymorphisms, though less frequent than VKORC1 variants, were also associated with lower maintenance dosage requirements, supporting their role as genetic modifiers of warfarin metabolism.

Previous research in Indian and broader Asian cohorts identified CYP2C9*2 and *3 alleles as

reducing effective warfarin dose, though their overall impact on dose variation is less pronounced than VKORC1 [10]. This additive effect suggests the utility of a combined genotype-based approach when initiating anticoagulant therapy, as patients carrying multiple variant alleles across both genes may require substantially reduced initial and maintenance dosages.Comparative analyses with recent and historical South Indian studies reveal that genetic factors account for approximately one-third of the observed dose variability, with remaining variation explained by demographic and clinical influences such as age, BMI, disease state, and co-medication [11]. Multivariate regression models in Asian settings typically attribute 25–36% of daily dose variability to VKORC1 and CYP2C9 status, reinforcing the clinical value of genotyping prior to warfarin initiation. The use of pharmacogenetic dosing algorithms, already validated in Chinese and Western populations, has proven to improve anticoagulation safety and minimize both under- and over-anticoagulation risks. The visual comparison provided in Figure 1 distinctly illustrates the genotype-dosed INR relationship and emphasizes the potential for overdose complications in variant carriers when standard empiric dosing is used [12].

These insights have clinical significance, underscoring the necessity for precision medicine approaches in regions with significant ethnogenetic diversity. Adoption of pharmacogenetic testing in routine clinical practice—particularly for venous thromboembolism and heart valve replacement patients—may reduce complications, optimize therapeutic ranges, and improve long-term outcomes.

Conclusion

This study reinforces that single nucleotide polymorphisms in CYP2C9 and VKORC1 are crucial determinants of warfarin dose requirements in South Indian patients. Personalized dosing guided by genotyping, alongside conventional monitoring, should be considered best practice for anticoagulant management in genetically diverse populations.

Declaration by Authors

Ethical Approval: Approved

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Conflict of Interest: The authors declare no conflict of interest.

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