Specialty Journal of Pharmacognosy, Phytochemistry, and Biotechnology
ISSN: 3062-441X
2022, Volume 2, Page No: 81-90

Available online at: www.galaxypub.co/page/journals

Galaxy Publication

Galaxy Publication

Influence of CYP2C9, VKORC1, and CYP4F2 Genetic Variants on Warfarin Dosage Needs in Saudi Patients

Erik Johansson[1], Andreas Nilsson[1], Johan Andersson[1], Mattias Holm[1]*

[1]Department of Pharmaceutical Biosciences, Faculty of Pharmacy, Uppsala University, Uppsala, Sweden.

***E-mail** ✉ mattias.holm.pb@yahoo.com

Received: 12 October 2021; **Revised:** 08 February 2022; **Accepted:** 09 February 2022

ABSTRACT

There is a scarcity of data regarding determinants of warfarin dose requirements among Saudi patients, an ethnic group largely underrepresented in pharmacogenetic studies of warfarin. This study aimed to evaluate the prevalence of CYP2C9*2 and *3, CYP4F2 (G1347A), and VKORC1 –1639G>A genotypes and their influence on warfarin dose requirements in a cohort of Saudi individuals undergoing anticoagulation therapy. A total of 193 patients receiving long-term warfarin therapy with stable anticoagulation were enrolled. Genotyping for VKORC1 1639G>A, CYP4F2 G1347A, CYP2C92 430C>T, and CYP2C93 1075A>C was performed using TaqMan assays. Analysis of variance assessed the relationship between CYP2C9, CYP4F2, and VKORC1 genotypes and warfarin dose across two target INR groups. Backward linear regression was applied to identify both genetic and clinical factors influencing dose requirements. Individuals carrying CYP2C9 and VKORC1 variants required significantly lower warfarin doses compared to wild-type carriers, with those harboring two variant alleles needing lower doses than single-allele carriers. CYP4F2 variants did not affect warfarin dosing. Age and the presence of CYP2C9 or VKORC1 variants were inversely associated with dose requirement, whereas body surface area (BSA) showed a positive correlation. In Saudi patients, polymorphisms in CYP2C9 and VKORC1 are linked to reduced warfarin dose requirements, whereas CYP4F2 variants have no impact. Combining genetic information with clinical parameters such as age and BSA offers the most accurate prediction of warfarin dose needs in this population.

Keywords: Polymorphisms, Warfarin, CYP2C9, CYP4F2, VKORC1

How to Cite This Article: Johansson E, Nilsson A, Andersson J, Holm M. Influence of CYP2C9, VKORC1, and CYP4F2 Genetic Variants on Warfarin Dosage Needs in Saudi Patients. Spec J Pharmacogn Phytochem Biotechnol. 2022;2:81-90. https://doi.org/10.51847/ZgsgukmoDp

Introduction

Warfarin, a widely used vitamin K antagonist (VKA), has long served as a cornerstone for preventing and managing both arterial and venous thromboembolic disorders. Its anticoagulant effect arises from inhibition of the vitamin K epoxide reductase (VKOR) enzyme, which blocks the regeneration of active vitamin K and thereby decreases the production of functional vitamin K–dependent clotting factors. Variations in the VKORC1 gene are key determinants of warfarin dose, with the VKORC1-1639A allele linked to lower dose requirements and a heightened risk of excessive anticoagulation [1-3]. The VKORC1 gene is located on chromosome 16 and encodes the VKOR enzyme.

Warfarin therapy involves a 1:1 mixture of R- and S-enantiomers, with the S form exhibiting roughly fivefold greater anticoagulant potency [4]. The cytochrome P450 enzyme CYP2C9 is primarily responsible for metabolizing S-warfarin [5, 6]. To date, over 57 CYP2C9 variants have been described [7], among which CYP2C9*2 and *3 are the most clinically significant, reducing enzymatic activity, slowing drug metabolism, lowering clearance, and increasing sensitivity and bleeding risk [3, 4, 8-10].

Due to the narrow therapeutic window of VKAs, careful monitoring is essential to prevent both thrombotic and hemorrhagic complications [11]. The International Normalized Ratio (INR) is the standard tool for assessing

anticoagulation intensity, and deviations from the target range, especially during early treatment, significantly elevate the risk of adverse outcomes [12].

Individual response to warfarin varies widely, with daily dose requirements differing up to 20-fold, reflecting the complex interplay of demographic, clinical, and genetic factors [9, 13]. Among genetic contributors, CYP2C9 and VKORC1 are consistently the strongest predictors of warfarin dose across populations [14-16], while CYP4F2 variants have a smaller influence. Combined, VKORC1 -1639G>A and CYP2C9*2/*3 genotypes, along with clinical parameters like age and sex, can account for over half of the variability in warfarin dosing [17-20].

Ethnic background also affects warfarin requirements: Asian populations typically need lower doses, whereas African Americans often require higher doses compared to Caucasians [9, 17]. Data on Saudi patients are limited, with only a few studies assessing CYP2C9 and VKORC1 prevalence and their impact on dosing and anticoagulation outcomes [20-24]. No research has yet evaluated the role of CYP4F2 polymorphisms in warfarin dosing among Saudis.

This study aimed to (a) determine the frequency of CYP2C9*2/*3, CYP4F2 G1347A, and VKORC1 –1639G>A genotypes in Saudi patients, (b) assess how these genetic variants affect warfarin dose requirements, and (c) explore the combined effects of genetic and non-genetic factors, including demographic and clinical characteristics, on warfarin dosing.

Materials and Methods

Study design

A cross-sectional investigation was carried out at the anticoagulation clinic of King Khaled University Hospital (KKUH). Ethical clearance was granted by the Institutional Review Board in November 2017, and all participants provided informed consent. DNA extraction and genotyping followed standard procedures conducted at King Faisal Specialist Hospital and Research Centre (KFSHRC).

Study population

Between March 2018 and October 2019, 193 patients on long-term warfarin therapy were enrolled from the KKUH anticoagulation clinic. Inclusion criteria included age ≥18 years, at least 12 months of continuous warfarin therapy, and stable anticoagulation, defined as a consistent warfarin dose over three consecutive clinic visits spanning a minimum of three months with INR values within the target range. Patients with hepatic or renal dysfunction or those taking medications known to interfere with warfarin metabolism were excluded.

Data collection

Patient information was collected using both a structured self-administered questionnaire and review of hospital medical records. The questionnaire gathered demographic details (age, sex, height, weight, marital status, education, and residence) and warfarin-specific information, including indication for therapy, treatment duration, and the target INR range. Clinical data from medical records included coexisting medical conditions, concurrent medications, history of stroke, and prior bleeding events.

DNA extraction and genotyping

Genomic DNA was isolated at KFSHRC using the PureGene DNA extraction kit (Qiagen Sciences, Germantown, MD, USA) following the manufacturer's instructions. DNA quantity and quality were evaluated using a Nanodrop ND-1000 spectrophotometer (Wilmington, DE, USA). Genotyping of VKORC1 1639G>A (rs9923231; C_30403261_20), CYP4F2 G1347A (rs2108622; C_16179493_40), CYP2C9*2 430C>T (rs1799853; C_25625805_10), and CYP2C9*3 1075A>C (rs1057910; C_27104892_10) was conducted with TaqMan real-time PCR assays in accordance with Applied Biosystems' protocols. To ensure the accuracy of the genotyping, a subset of 96 samples was reanalyzed using Sanger sequencing. PCR primers were custom-designed by the Oligonucleotide Synthesis Unit at the Genetics Department of KFSHRC, with specific sequences and details provided in **Table 1**.

Table 1. Designed primers used for PCR.

Rs number-product size-annealing temperature	Forward primer	Reverse primer
*CYP2C9*3* Rs1057910 404 bp 56°C	TGGCAGAAACCGGAGCCCCT	GCACCTAAGAGTAGCCAAACCAATCTT
VKORC1 Rs9923231 372 bp 55°C	GGGAGGAGCCAGCAGGAGAGG	AGCGGTGCCATCTCGGC

Data analysis

Statistical analyses were performed using SPSS version 27 (IBM Corp., Chicago, IL, USA). Continuous variables were summarized using means, medians, and standard deviations, whereas categorical variables were presented as counts and percentages. The Hardy-Weinberg equilibrium (HWE) was evaluated using the equation $p^2 + 2pq + q^2 = 1$, where p^2 represents the frequency of homozygous wild-type genotypes, 2pq denotes heterozygous genotypes, and q^2 corresponds to homozygous mutant genotypes. Observed genotype distributions were compared with expected frequencies using the chi-square test.

To assess the relationship between CYP2C9, VKORC1, and CYP4F2 genotypes and warfarin dose requirements, analysis of variance (ANOVA) was conducted separately for two target INR groups. Warfarin doses were log-transformed to approximate a normal distribution. Backward linear regression modeling was then employed to identify both genetic and clinical predictors of warfarin dose. In these models, the log-transformed warfarin dose served as the dependent variable, while age, sex, body surface area (BSA), smoking status, comorbidities (hypertension, diabetes mellitus, myocardial infarction, heart failure, hypothyroidism, renal impairment), concurrent medications (beta-blockers, antiplatelets), and genotypes (CYP2C9, VKORC1, CYP4F2) were included as independent variables.

Results and Discussion

Baseline characteristics

From an initial pool of 207 patients receiving long-term warfarin therapy, 193 individuals (93.2%) fulfilled all inclusion criteria, provided informed consent, completed the questionnaire, and donated a 5 mL blood sample for genetic analysis. A flowchart detailing patient recruitment is presented in **Figure 1**.

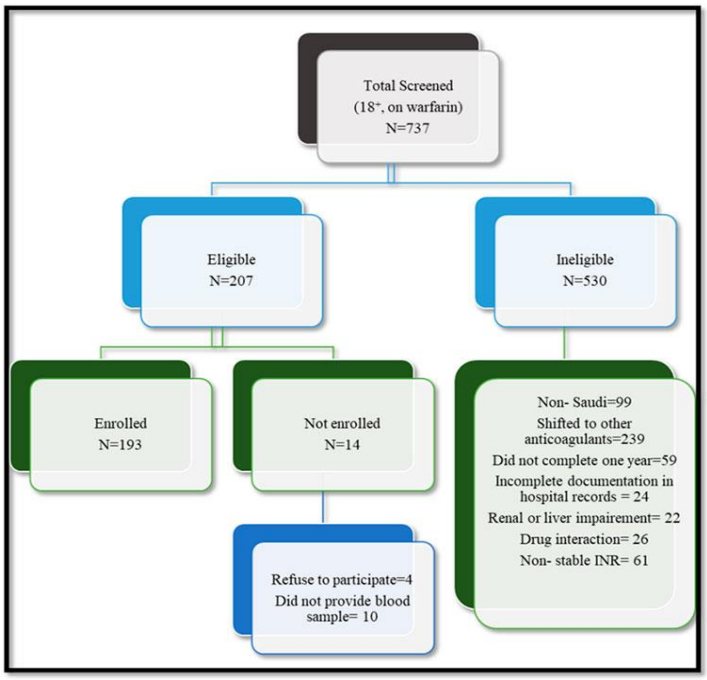


Figure 1. Flow diagram illustrating the recruitment process of patients receiving warfarin therapy for this study.

Table 2 summarizes the demographic and clinical characteristics of participants, stratified according to their target INR range. Among the 96 patients with mechanical prosthetic heart valves and a target INR of 2.5–3.5, the mean age was 53 ± 13 years (median 54; range 19–92), with females representing 52.1% of the group. Approximately 40.6% of these patients had been on warfarin therapy for over a decade. The average daily warfarin dose in this subgroup was 5.86 mg (±3.06, median 5, range 1–18 mg).

The remaining 97 patients were managed with a target INR of 2.0–3.0. Their mean age was 51.6 ± 12.75 years (median 52; range 22–88), and 68% were female. The most frequent indication for anticoagulation was deep vein thrombosis or pulmonary embolism, accounting for 45.4% of cases, followed by valvular replacement (24.7%), atrial fibrillation (17.5%), stroke (7.2%), and other indications (5.2%). About half of the patients in this group had been receiving warfarin for more than ten years, with a mean daily dose of 6.13 mg (±3.41, median 5, range 1–18 mg).

Table 2. Demographic and clinical characteristics of patients who received warfarin therapy.

Characteristics	2–3 INR group (97 patients)	2.5–3.5 INR group (96 patients)
	n (%)	n (%)
Age (years) Mean (±SD, range)	51.6 (±12.75, 22–88)	53 (±13, 19–92)
Gender		
Female	66 (68)	50 (52.1)
Male	31 (32)	46 (47.9)
BMI, mean (±SD) BMI = Weight (kg)/height(m)^2	31.7 (±6.6)	28.8 (±5.47)
Smoking status		
Smoker	5 (5.2)	9 (9.4)
Nonsmoker	92 (94.8)	87 (90.6)
Warfarin Indication		
Valve replacement	24 (24.7)	95 (99)
AF	17 (17.5)	
DVT or PE	44 (45.4)	
Stroke	7 (7.2)	
Other	5 (5.2)	1 (1)
Duration on warfarin (years)		
1–4	24 (24.7)	19 (19.8)
5–10	24 (24.7)	38 (39.6)
>10	49 (50.5)	39 (40.6)
Daily doses (years) Mean (±SD, median, range)	6.13 mg (±3.41, 5, 1–18 mg)	5.86 mg (±3.06, 5, 1–18 mg)

BMI, Body mass index; AF, Atrial fibrillation; DVT, Deep venous thrombosis; PE, Pulmonary embolism

CYP2C9, VKORC1, and CYP4F2 genotype distribution

The distribution of VKORC1, CYP2C9*2 and *3, and CYP4F2 genotypes among the Saudi cohort is presented in **Table 3**. All genotypes conformed to Hardy-Weinberg equilibrium. Additionally, genotyping results obtained via TaqMan PCR showed complete agreement (100% concordance) with Sanger sequencing validation.

Table 3. Genotype frequency in the Saudi general population and study patients.

Genotype	Patients on warfarin therapy (n = 193)		
	n-Frequency (%)	Genotype	n-Frequency (%)
CYP4F2 V433M		CYP2C9	
Homozygous wild CC	67–0.35 (34.7)	*1*1	99–0.51 (51.3)

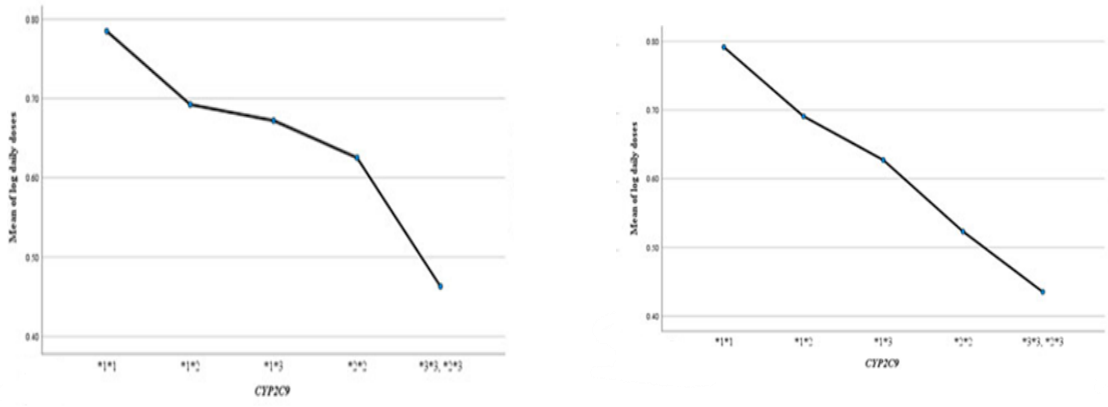
Heterozygous CT	95–0.49 (49.2)	*1*2	51–0.26 (26.4)
Homozygous mutant TT	31–0.16 (16.1)	*1*3	22–0.12 (11.4)
Wild (C)	0.59 (59.1)	*2*2	11–0.06 (5.7)
Mutant (T)	0.41 (40.9)	*3*3	4–0.02 (2.1)
		*2*3	6–0.03 (3.1)
VKORC1		Wild (*1)	0.7 (70.2)
Homozygous wild GG	49–0.25 (25.4)	Mutant (*2)	0.20 (20.5)
Heterozygous GA	83–0.43 (43)	Mutant (*3)	(9.3)
Homozygous mutant AA	61–0.32 (31.6)		
Wild (G)	0.47 (46.9)		
Mutant (A)	0.53 (53.1)		

*1 is the wild- type allele of CYP2C9, *2 and *3 are variant type alleles.

Among the study cohort, the CYP2C91/1 genotype was the most frequent, present in 51.3% of participants. The CYP2C92 allele (20.5%) was more commonly observed than CYP2C93 (9.3%). For VKORC1, approximately 43% of patients were heterozygous (GA), 31% carried homozygous mutant alleles (AA), and 25% had the homozygous wild-type genotype (GG). Regarding the CYP4F2 V433M variant, nearly half of the participants (49.2%) were heterozygous (CT), around 34% were homozygous wild-type (CC), and 16% were homozygous mutant (TT).

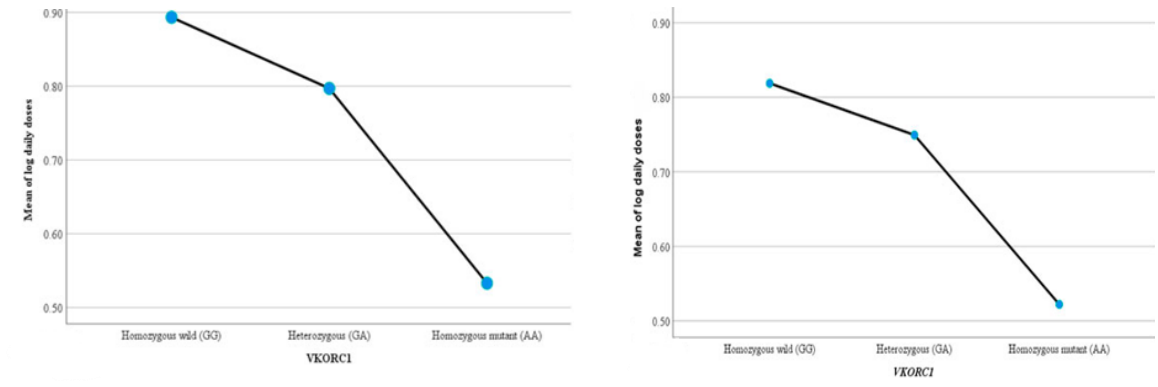
Influence of CYP2C9, VKORC1, and CYP4F2 on warfarin dose

Patients carrying CYP2C9 or VKORC1 variants required significantly lower daily warfarin doses compared with individuals with wild-type genotypes **(Figures 2 and 3)** (one-way ANOVA). Furthermore, individuals harboring two mutant alleles needed markedly lower doses than those with only one mutant allele. In contrast, CYP4F2 polymorphisms did not appear to influence warfarin dose requirements, as summarized in **Table 4**.



a) b)

Figure 2. Comparison of average daily warfarin doses between CYP2C9 wild-type and variant genotypes for (a) patients with a target INR of 2.0–3.0 and (b) patients with a target INR of 2.5–3.5.



a) b)

Figure 3. Average daily warfarin dose requirements for VKORC1 wild-type versus variant genotypes in (a) patients with a target INR of 2.0–3.0 and (b) patients with a target INR of 2.5–3.5.

Table 4. The median daily warfarin doses for polymorphisms in each gene.

Genotype	n	INR target 2–3	n	INR target 2.5–3.5
		Median warfarin daily dose in mg (IQR)		Median warfarin dose in mg (IQR)
CYP2C9 p-value		0.034		<0.001
*1*1	53	5.5 (4.29)	46	6 (2.5)
*1*2	26	5 (3.57)	25	5 (2.90)
*1*3	10	4.5 (3.38)	12	3.75 (3.75)
*2*2	5	3.64 (2.75)	6	3.61 (3.07)
*2*3 and *3*3[a]	3	3.5 (2.3)	7	3 (2)
VKORC1 p-value		(<0.001)		(<0.001)
GG	26	7.25 (6.88)	23	6.5 (3)
GA	37	6 (2.25)	46	5.25 (3)
AA	34	3.5 (1)	27	3.5 (1.29)
CYP4F2 p-value		0.31		0.986
CC	29	5 (2.43)	38	5 (3.09)
CT	51	5.5 (4.5)	44	5 (3.09)
TT	17	5 (3.65)	14	5.2 (2.48)

[a] Only a single patient in the 2.0–3.0 INR group and three patients in the 2.5–3.5 INR group carried the *3/*3 genotype. Consequently, data for this genotype were pooled with the *2/*3 genotype group. (INR, International Normalized Ratio)

Backward linear regression was applied to determine which genetic and clinical factors influence warfarin dosing, with the log of daily dose as the dependent variable and age, gender, smoking status, hypertension, diabetes, myocardial infarction, heart failure, hypothyroidism, renal impairment, beta-blocker use, antiplatelet therapy, and the genes CYP2C9, VKORC1, and CYP4F2 as independent variables **(Table 5)**. In patients targeting an INR of 2.0–3.0, age, BSA, CYP2C9, and VKORC1 together explained 54.6% of the variability in warfarin dose, whereas in those with a target INR of 2.5–3.5, BSA, CYP2C9, and VKORC1 accounted for 51.2% of dose variability. Age and CYP2C9 and VKORC1 polymorphisms were inversely associated with warfarin dose, while BSA showed a positive association with dose requirements.

Table 5. Linear regression analysis for predictors of warfarin dose requirements.

Predictor	B	Standardized coefficient beta	p-value	95% confidence interval
		INR 2–3		
CYP2C9	−0.045	−0.226	0.002	−0.073 – −0.017

Age	−0.004	−0.238	0.001	−0.007 – −0.002
VKORC1	−0.174	−0.618	<0.001	−0.214 – −0.135
BSA	0.241	0.225	0.002	0.093–0.388
INR 2.5–3.5				
CYP2C9	−0.066	−0.433	<0.001	−0.088 – −0.043
VKORC1	−0.127	−0.449	<0.001	−0.169 – −0.085
BSA	0.224	0.247	0.001	0.093–0.355

Warfarin therapy remains challenging due to its narrow therapeutic index and the risk of bleeding or thrombotic events, compounded by considerable interindividual variability in dose response. Multiple factors, including demographic characteristics, clinical conditions, concurrent medications, genetic variants, smoking habits, and diet-drug interactions, are known to influence patient response to warfarin [25]. Among these, clinical factors combined with genetic polymorphisms in VKORC1 (-1639G>A), CYP2C9 (*2 and *3), and CYP4F2 (V433M) account for approximately 55%–57% of variability in anticoagulation response in Caucasian populations, compared to roughly 25% in African Americans [17-19].

Variants in CYP2C9, VKORC1, and CYP4F2 have been widely reported to influence warfarin dose requirements across ethnic groups. Mutations in VKORC1 typically reduce expression of the VKOR enzyme, increasing the risk of bleeding in patients receiving vitamin K antagonists and necessitating lower warfarin doses. CYP2C9, the primary enzyme metabolizing warfarin, has two clinically significant variant alleles (2 and 3), which reduce enzymatic activity, with CYP2C93 having a stronger effect than CYP2C92. Individuals carrying these variants generally require lower warfarin doses compared to those with the wild-type allele [14-16]. Conversely, carriers of the CYP4F2 variant often need higher doses, although its overall impact on dose variability is smaller than that of CYP2C9 or VKORC1 [26].

Despite extensive research in other populations, data on warfarin pharmacogenetics in Saudi patients remain limited. To our knowledge, this study is the first to assess the prevalence of the CYP4F2 V433M variant and its influence on warfarin response in this population. Additionally, few studies have investigated the frequency of CYP2C9 and VKORC1 variants and their relationship with dose requirements and anticoagulation response in Saudi patients [20-24]. Accordingly, our study aimed to determine the distribution of VKORC1 –1639G>A, CYP2C9*2 and *3, and CYP4F2 (G1347A) polymorphisms, and their association with warfarin dosing.

The prevalence of CYP2C9, VKORC1, and CYP4F2 genotypes shows notable variation across ethnic groups. Caucasians and Americans have higher frequencies of CYP2C92 and 3 alleles compared to African Americans and Asians. Among Caucasians, CYP2C91, 2, and 3 are found in approximately 80%, 13%, and 7% of individuals, respectively. Southeast Asians exhibit a slightly higher prevalence of CYP2C93 (2%–10%) with almost no CYP2C92, whereas both 2 and 3 alleles are rare in African Americans (3%–5% and 1%–2%, respectively) [6, 8]. In our Saudi cohort, CYP2C911 was the most common genotype, while 33 was least frequent. Heterozygous genotypes (12 and 13) were more prevalent than homozygous mutant genotypes (22 and 33), with CYP2C911 present in 51.3% of patients on warfarin. This is slightly lower than previous reports in Saudi populations, which ranged from 63.4% to 68.7% [21, 22, 24], while CYP2C91*2 frequency (26.4%) was consistent with prior findings (26.7%) by Alzahrani *et al*. [21]. Notably, no homozygous mutant alleles (22 or 33) were reported in their cohort.

Regarding VKORC1, the -1639A variant allele is present in approximately 67% of Asians, 40% of African Americans, and 11% of Caucasians. In this study, 43% of patients carried the heterozygous GA genotype, 31% were homozygous AA, and 25% had the wild-type GG genotype, closely matching the distribution reported by Al Ammari *et al*. [20] (GA 45.3%, AA 34%, GG 20.7%). For CYP4F2 V433M, 49.2% of participants were heterozygous (CT), 34% homozygous wild-type (CC), and 16% homozygous mutant (TT), with the mutant T allele frequency at 40.9%, compared to approximately 30% in white and Asian populations and 7% in Black individuals [26].

The relationship between CYP2C9 (*2 and 3), CYP4F2 (G1347A), and VKORC1 (–1639G>A) genotypes and warfarin dosing differs across populations. Compared with Caucasians, African Americans generally require higher warfarin doses, while Asians need lower doses, largely reflecting differences in allele frequencies among ethnic groups. Specifically, VKORC1 (–1639A), CYP2C92, and 3 variants are consistently linked to reduced

warfarin requirements. These alleles occur less frequently in African Americans than in Asians and Caucasians [17]. CYP4F2 variants have been shown to influence warfarin dosing in White and Asian populations but appear less relevant in Indian, Egyptian, Brazilian, and Black populations [27]. In the current study, carriers of CYP2C92, *3, or VKORC1 A alleles required lower warfarin doses compared with individuals carrying wild-type alleles. Notably, this is the first study assessing the impact of CYP4F2 polymorphism on warfarin dosing and response in Saudi patients, and unlike CYP2C9 and VKORC1, CYP4F2 variants did not significantly influence warfarin requirements in this population.

Research examining the combined effect of clinical and genetic factors on warfarin dosing among Saudi patients remains limited. Prior studies often focused on individual polymorphisms or specific therapy phases. For example, Al Ammari *et al*. [28] investigated VKORC1 variants during the first 10 days of treatment, whereas Al-Saikhan *et al*. [29] evaluated the effects of CYP2C9 and VKORC1 1173C>T on warfarin dose variability in separate cohorts of 112 and 164 patients, without assessing the interaction between genetics and clinical variables [30]. Saour *et al*. [24] studied CYP2C9 variants in patients receiving ≤2 mg of warfarin daily, leaving higher dose ranges unexplored.

In this study, age, BSA, CYP2C92 and 3, and VKORC1 (–1639G>A) variants were identified as significant determinants of warfarin dose. Age and CYP2C9/VKORC1 variant alleles were inversely correlated with warfarin requirements, whereas BSA showed a positive correlation. These findings align with prior observations across multiple ethnicities, though VKORC1 variants are less frequent in African Americans [17]. Consistent with earlier research in Caucasians, Kamali *et al*. [31] reported age and CYP2C92/3 as key contributors to warfarin dose, while Sconce *et al*. [2] found negative correlations between warfarin dose and age, body size, VKORC1, and CYP2C9 variants, collectively explaining 54% of dose variability. Similarly, Caldwell *et al*. [17] noted that clinical and genetic factors accounted for 54% of interindividual differences. Bourgeois *et al*. [1] observed that VKORC1 and CYP2C9 variants, combined with clinical factors, explained 57.9% of variation in mean weekly doses among 711 British patients. A meta-analysis by Jorgensen *et al*. [32] encompassing 117 studies confirmed the major contribution of CYP2C9 and VKORC1 variants to warfarin dosing across ethnic groups. Furthermore, older age has been consistently associated with lower dose requirements [33]. Gage *et al*. [34] quantified predictors of warfarin dose, highlighting the substantial roles of VKORC1 (−28%), CYP2C93 (−33%), CYP2C92 (−19%), age (−7% per decade), BSA (+11% per 0.25 m^2), INR target (+11% per 0.5 unit), amiodarone (−22%), smoking (+10%), race (−9%), and current thrombosis (+7%). Similar to prior studies [1, 35, 36], VKORC1 had a greater effect on dose variability than CYP2C9.

Strengths of the study include strict participant selection criteria, requiring stable INR for at least three months, and exclusion of patients with factors potentially affecting anticoagulation. Genotyping was validated through sequencing in randomly selected samples, showing 100% concordance with real-time PCR results, ensuring accurate identification of alleles.

The main limitation is that the study did not include a priori sample size calculation, which may limit the power to detect a significant effect of CYP4F2 on warfarin dosing. Future research with larger cohorts is warranted to clarify the role of CYP4F2 in Saudi patients.

Conclusion

This study expands knowledge on warfarin pharmacogenetics in the underrepresented Saudi population. It demonstrates that patients carrying CYP2C9 and VKORC1 variants require lower warfarin doses than those with wild-type alleles. Integrating clinical parameters such as age and BSA with genetic polymorphisms in CYP2C9 and VKORC1 provides the most accurate estimation of factors influencing warfarin dose in this population.

Acknowledgments: We acknowledge Dr. Peter Avery, Senior Lecturer in Statistics (School of Mathematics and Statistics, Newcastle University) for his assistance with the statistical analyses of the data.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Financial Support: The author(s) declare that no financial support was received for the research and/or publication of this article.

Ethics Statement: The studies involving humans were approved by King Khalid University Hospital Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

References

1. Bourgeois S, Jorgensen A, Zhang EJ, Hanson A, Gillman MS, Bumpstead S, et al. A multi-factorial analysis of response to warfarin in a UK prospective cohort. Genome Med 2016;8(1):2. doi:10.1186/s13073-015-0255-y
2. Sconce EA, Khan TI, Wynne HA, Avery P, Monkhouse L, King BP, et al. The impact of CYP2C9 and VKORC1 genetic polymorphism and patient characteristics upon warfarin dose requirements: proposal for a new dosing regimen. Blood 2005;106(7):2329-33. doi:10.1182/blood-2005-03-1108
3. Scott SA, Khasawneh R, Peter I, Kornreich R, Desnick RJ. Combined CYP2C9, VKORC1 and CYP4F2 frequencies among racial and ethnic groups. Pharmacogenomics 2010;11(6):781-91. doi:10.2217/pgs.10.49
4. Moyer TPP, O'Kane DJ, Baudhuin LM, Wiley CL, Fortini AM, Fisher PK, et al. Warfarin Sensitivity Genotyping: A Review of the Literature and Summary of Patient Experience. Mayo Clin Proc 2009;84:1079-94. doi:10.4065/mcp.2009.0278
5. Kalman LV, Agundez J, Appell ML, Black JL, Bell GC, Boukouvala S, et al. Pharmacogenetic allele nomenclature: International workgroup recommendations for test result reporting. Clin Pharmacol Ther 2016;99:172-85. doi:10.1002/cpt.280
6. Westervelt P, Cho K, Bright DR, Kisor DF. Drug-gene interactions: inherent variability in drug maintenance dose requirements. P T 2014;39(9):630-7
7. Niinuma Y, Saito T, Takahashi M, Tsukada C, Ito M, Hirasawa N, et al. Functional characterization of 32 CYP2C9 allelic variants. Pharmacogenomics J 2014;14(2):107-14. doi:10.1038/tpj.2013.22
8. Gulseth MP, Grice GR, Dager WE. Pharmacogenomics of warfarin: uncovering a piece of the warfarin mystery. Am J Health Syst Pharm 2009;66(2):123-33. doi:10.2146/ajhp080127
9. Kamali F, Wynne H. Pharmacogenetics of warfarin. Annu Rev Med 2010;61:63-75. doi:10.1146/annurev.med.070808.170037
10. Lane S, Al-Zubiedi S, Hatch E, Matthews I, Jorgensen AL, Deloukas P, et al. The population pharmacokinetics of R- and S- warfarin: effect of genetic and clinical factors. Br J Clin Pharmacol 2012;73:66-76. doi:10.1111/j.1365-2125.2011.04051.x
11. Wigle P, Hein B, Bloomfield HE, Tubb M, Doherty M. Updated guidelines on outpatient anticoagulation. Am Fam Physician 2013;87(8):556-66
12. Homme MB, Reynolds KK, Valdes R, Linder MW. Dynamic pharmacogenetic models in anticoagulation therapy. Clin Lab Med 2008;28(4):539-52. doi:10.1016/j.cll.2008.10.002
13. Wen MS, Lee MTM. Warfarin pharmacogenetics: new life for an old drug. Acta Cardiol Sin 2013;29(3):235-42
14. Borgiani P, Ciccacci C, Forte V, Sirianni E, Novelli L, Bramanti P, et al. CYP4F2 genetic variant (rs2108622) significantly contributes to warfarin dosing variability in the Italian population. Pharmacogenomics 2009;10(2):261-6. doi:10.2217/14622416.10.2.261
15. Cen HJ, Zeng WT, Leng XY, Huang M, Chen X, Li JL, et al. CYP4F2 rs2108622: a minor significant genetic factor of warfarin dose in Han Chinese patients with mechanical heart valve replacement. Br J Clin Pharmacol 2010;70(2):234-40. doi:10.1111/j.1365-2125.2010.03698.x
16. Tatarunas V, Lesauskaite V, Veikutiene A, Grybauskas P, Jakuska P, Jankauskiene L, et al. The effect of CYP2C9, VKORC1 and CYP4F2 polymorphism and of clinical factors on warfarin dosage during initiation and long-term treatment after heart valve surgery. J Thromb Thrombolysis 2014;37(2):177-85. doi:10.1007/s11239-013-0940-x
17. Cavallari LH, Perera MA. The future of warfarin pharmacogenetics in under-represented minority groups. Future Cardiol 2012;8(4):563-76. doi:10.2217/fca.12.31
18. McDonald MG, Rieder MJ, Nakano M, Hsia CK, Rettie AE. CYP4F2 is a vitamin K1 oxidase: an explanation for altered warfarin dose in carriers of the V433M variant. Mol Pharmacol 2009;75(6):1337-46. doi:10.1124/mol.109.054833

19. Zhang JE, Klein K, Jorgensen AL, Francis B, Alfirevic A, Bourgeois S, et al. Effect of genetic variability in the CYP4F2, CYP4F11, and CYP4F12 genes on liver mRNA levels and warfarin response. Front Pharmacol 2017;8:323. doi:10.3389/fphar.2017.00323
20. 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;10(1):11613. doi:10.1038/s41598-020-68519-9
21. 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
22. Mirghani RA, Chowdhary G, Elghazali G. Distribution of the major cytochrome P450 (CYP) 2C9 genetic variants in a Saudi population. Basic Clin Pharmacol Toxicol 2011;109(2):111-4. doi:10.1111/j.1742-7843.2011.00692.x
23. Mizzi C, Dalabira E, Kumuthini J, Dzimiri N, Balogh I, Başak N, et al. A European spectrum of pharmacogenomic biomarkers: implications for clinical pharmacogenomics. PLoS One 2016;11(9):e0162866. doi:10.1371/journal.pone.0162866
24. Saour JN, Shereen AW, Saour BJ, Mammo LA. CYP2C9 polymorphism studies in the Saudi population. Saudi Med J 2011;32(4):347-52.
25. Apostolakis S, Sullivan RM, Olshansky B, Lip GYH. Factors affecting quality of anticoagulation control among patients with atrial fibrillation on warfarin: the SAMe-TT_2R_2 score. Chest 2013;144(5):1555-63. doi:10.1378/chest.13-0054
26. Caldwell MD, Awad T, Johnson JA, Gage BF, Falkowski M, Gardina P, et al. CYP4F2 genetic variant alters required warfarin dose. Blood 2008;111(8):4106-12. doi:10.1182/blood-2007-11-122010
27. Danese E, Raimondi S, Montagnana M, Tagetti A, Langaee T, Borgiani P, et al. Effect of CYP4F2, VKORC1, and CYP2C9 in influencing coumarin dose: a single-patient data meta-analysis in more than 15,000 individuals. Clin Pharmacol Ther 2019;105(6):1477-91. doi:10.1002/cpt.1323
28. Al Ammari M, AlThiab K, AlJohani M, Sultana K, Maklhafi N, AlOnazi H, et al. Tele-pharmacy Anticoagulation clinic during COVID-19 pandemic: patient outcomes. Front Pharmacol 2021;12:652482. doi:10.3389/fphar.2021.652482
29. Al-Saikhan F, Abd-Elaziz MAR, Hamdy Asho R, Langaee T. Impact of cytochrome P450 2C9 polymorphism on warfarin therapy in Saudi population. Int J Pharmacol 2018;14:566-71. doi:10.3923/ijp.2018.566.571
30. Al-Saikhan F, Abd-Elaziz M, Ashour R. Influence of vitamin K epoxide reductase complex 1 polymorphism on warfarin therapy in a cohort study of Saudi patients. Int J Pharmacol 2018;14:414-20. doi:10.3923/ijp.2018.415.420
31. Kamali F, Khan TI, King BP, Frearson R, Kesteven P, Wood P, et al. Contribution of age, body size, and CYP2C9 genotype to anticoagulant response to warfarin. Clin Pharmacol Ther 2004;75(3):204-12. doi:10.1016/j.clpt.2003.10.001
32. Jorgensen AL, FitzGerald RJ, Oyee J, Pirmohamed M, Williamson PR. Influence of CYP2C9 and VKORC1 on patient response to warfarin: a systematic review and meta-analysis. PLoS One 2012;7(8):e44064. doi:10.1371/journal.pone.0044064
33. Loebstein R, Yonath H, Peleg D, Almog S, Rotenberg M, Lubetsky A, et al. Interindividual variability in sensitivity to warfarin--Nature or nurture? Clin Pharmacol Ther 2001;70(2):159-64. doi:10.1067/mcp.2001.117444
34. Rieder MJ, Reiner AP, Gage BF, Nickerson DA, Eby CS, Mcleod HL, et al. Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose. N Engl J Med 2005;352:2285-93. doi:10.1056/NEJMoa044503
35. Wadelius M, Chen LY, Downes K, Ghori J, Hunt S, Eriksson N, et al. Common VKORC1 and GGCX polymorphisms associated with warfarin dose. Pharmacogenomics J 2005;5:262-70. doi:10.1038/sj.tpj.6500313
36. Gage BF, Eby C, Johnson JA, Deych E, Rieder MJ, Ridker PM, et al. Use of pharmacogenetic and clinical factors to predict the therapeutic dose of warfarin. Clin Pharmacol Ther 2008;84(3):326-31. doi:10.1038/clpt.2008.10