

PREVALENCE OF CYP2C9 AND VKORC1 POLYMORPHISMS INFLUENCING WARFARIN METABOLISM IN AN ADULT POPULATION OF PESHAWAR, PAKISTAN

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Abstract

Warfarin is a common oral anticoagulant that has a low therapeutic index, in which the inter-individual variation in dose is strongly dependent on genetic polymorphisms in the CYP2C9 and VKORC1 genes. This research was aimed at determining the estimates of CYP2C9 (at least the alleles of the 2) and VKORC1 (-1639G>A) polymorphisms among the adult population in Peshawar, Pakistan. In the present, cross-sectional, observational study, genomic DNA was produced in 200 unrelated healthy participants (mostly of Pashtun heritage) and genotyped under Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The statistical analysis performed using SPSS v26.0 showed that the CYP2C9 2 allele was 9.0%, and the genotype frequencies were 84.0%, 14.0 and 2.0 respectively. In the case of VKORC1 (-1639G>A), the mutant A-allele frequency was 23.5, where genotype GG, GA, and AA frequencies stood at 59.0, 35.0, and 6.0, respectively. The two SNPs were discovered to be in Hardy-Weinberg Equilibrium ($p > 0.05$). The results indicate that there are considerable levels of reduced-function genotype within the Peshawar population, and thus pharmacogenetic screening and individualized dosing regimen are crucial in improving the safety and efficacy of the warfarin regimen within local clinical environments.

1. Introduction

1.1 Warfarin Clinical Use

One of the most popular oral anticoagulants that continues to be used to prevent thromboembolic events, including deep vein thrombosis, pulmonary embolism, and stroke, in patients with atrial fibrillation or mechanical heart valves, is warfarin [1]. Although effective, warfarin has a limited therapeutic index when the treatment efficacy is sensitive to small changes in dose (undertherapy resulting in the risk of clotting) or excessive anticoagulation (bleeding) [2]. Monitoring therapy is based on the International

Normalized Ratio (INR), which aims at a range of 2.0-3.0, although stabilization of the INR in most cases takes weeks of dose changes because of more than 10-fold interindividual variation [3]. Initial doses (usually 2 to 5 mg/day) are adjusted according to INR responses in clinical practice, yet serious adverse events (such as major hemorrhage) happen in 1-3% of patients every year, which highlights the importance of individual dosing schedules.

1.2 Genetic Factors

The variation in warfarin dosage is attributed to genetic polymorphism in the enzymes that metabolize drugs and the target proteins [4]. The drug warfarin acts on cytochrome P450 2C9 (CYP2C9), and the first drug substrate is vitamin K epoxide reductase complex subunit 1 (VKORC1), which uses vitamin K to produce the clotting factors [5].

CYP2C92 (rs1799853, c.430C> T,p Arg144Cys) reduces the activity of the enzyme by approximately 30-50, which increases the plasma warfarin concentration and decreases the necessary doses (Fung et al., 2013). Likewise, CYP2C93 (rs1057910, but it is not in the center of the present paper) also disrupts functionality [6]. In the case of VKORC1, the hypersensitivity to warfarin and a reduction of up to 30 percent warfarin dose in AA homozygotes are caused by a promoter region polymorphism -1639G>A (rs9923231, c.-1639G>A) which lowers gene expression (Yin et al., 2007).

These effects are confirmed in the meta-analyses of the populations: CYP2C9 A2B/3 carriers need to be given 20-40% less dose, whereas VKORC1 A-allele carriers need to be given 25-50% less dose [4]. These genotypes are included in algorithms, enhancing the initial dosing error by 1020% [5]. VKORC1 GG (54%) was the most frequent among the Saudi population, but higher doses were necessary, and CYP2C91/ 1 (71) exerted more than variants (1/2 14, and 1/3 11) required, but there were no differences in INR (Al Hamad, 2025). The same views were also expressed by Brazilian literature, where CYP2C92/CYP2C93 VKORC1 1639G>A was associated with dose variability upon maintenance (Santos et al., 2013). In Chinese cohorts, CYP2C9 rs10579100 and VKORC1

rs9923231 were found to predict the risk of bleeding [7].

1.3 Pakistan/Peshawar Gap

Although there are global and regional data (e.g., Saudi: VKORC1 AA 16; Chinese: high bleeding in CYP2C9 variants, there is a paucity of studies of pharmacogenetics in Pakistan, subject to frequent urban centres such as Lahore or Karachi [8]. Peshawar, Khyber Pakhtunkhwa, is an example of a particular ethnic mix (majorly Pashtun), where no specific population-level data of CYP2C9/VKORC1 is provided. Pakistani reports have already CYP2C9*2 of +1015% and VKORC1 of A +4050%, but regional differences (e.g., VKORC1 is more sensitive in South Asians) are uncharacterized. This disparity prevents the application of genotype-guided warfarin therapy in the local clinics where warfarin is a frequent therapy, even though there are difficulties in the monitoring of INR [8].

1.4 Objectives

General objective:

To estimate the prevalence of CYP2C9 (particularly *2 allele) and VKORC1 (-1639G>A) polymorphisms in an adult population of Peshawar, Pakistan.

Specific objectives:

- To determine genotype and allele frequencies of CYP2C9*2 and VKORC1 -1639G>A among adult participants.
- To analyze whether genotype distribution is in Hardy-Weinberg equilibrium using SPSS.
- To explore simple associations between these genotypes and demographic variables (sex and major ethnic group).

Table 1: Global CYP2C9*2 and VKORC1 -1639A Allele Frequencies

Population	CYP2C9*2 MAF (%)	VKORC1 A MAF (%)	Reference
Saudi (n=100)	14-15	31	[3]
Brazilian (n=206)	12	40	[6]
Chinese (n=896)	5-8	85	[7]
European	11-13	37-42	[2]
South Asian	10-15	40-50	[4]

2. Materials and Methods

2.1 Study Design and Population

It is a cross-sectional, observational study of 200 healthy adult volunteers aged 18-65 years old, who are students of the University of Peshawar and neighboring communities of Peshawar (Khyber Pakhtunkhwa Pakistani state) between August and December 2025. Participants were local Pakistani citizens who were not relatives of each other (they were mostly Pashtun). Inclusion criteria: Ages 18-65 years, voluntary participation, and no acute disease. Exclusion criteria: pregnancy, known hereditary coagulopathy, taking warfarin/anticoagulant therapy, severe liver/kidney disease, and age below 18/>65 years. The use of convenience sampling through university announcements and community outreach guaranteed the possibility of implementation on undergraduate level [3].

2.2 Ethical Approval

The Institutional Review Board/Ethics Committee of the University of Peshawar approved the protocol. Informed consent was provided in written form in Urdu/English after procedures and risks (minimal phlebotomy) had been explained, and the rights of voluntary withdrawal were provided. The anonymity of data was ensured through ID coding (Declaration of Helsinki compliant).

2.3 Sample Collection & DNA Extraction

Non-invasiveness was desired in the use of buccal swabs; 3 mL of venous blood in EDTA tubes (BD Vacutainer) was also used. Samples were stored at -20°C. The genomic DNA was isolated according to the instructions of the manufacturer: lysis (56 °C, 10 min), binding to a silica column, washes (AW1/AW2), and elution (50 µL AE buffer). Yield/quality evaluated through NanoDrop (A260/A280 1.82.0, concentration over

50ng/liter). This is an easy kit-based approach that is appropriate in low-resource undergraduate laboratories.

2.4 Genotyping

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique, based on adopted protocols, was used to identify the variants of CYP2C9*2 (rs1799853; C>T) and VKORC1 -1639G>A (rs9923231). The amplification of genomic DNA (50 -100 ng) was conducted using a 25 µL reaction with a mixture of 0.2 mM primers, 200 mM dNTPs, 1.5 mM 0MgCl₂, and 1 U Taq polymerase [9]. Bio-Rad T100 thermal cycling conditions included an initial denaturation of 95 °C during 5 minutes and 35 cycles of 95 °C during 30 seconds, annealing of 58 °C during 30 seconds in case of CYP2C9 and annealing of 55 °C during 30 seconds in case of VKORC1, and final extension of 72 °C during 5 minutes.

After amplification, 10 µL of PCR product was subjected to a restriction digestion reaction in 15 µL of a reaction consisting of specific enzymes at 37 °C over the period of 3-16 hours. The *Ava*I enzyme was used to identify the CYP2C9*2 variant, and the *Msp*I was used to identify VKORC1. The DNA fragments that were obtained were separated using gel electrophoresis on 2-3% agarose gels stained with Ethidium Bromide and analyzed under UV light.

Genotypes were assigned based on distinct band patterns: CYP2C9*2 wild-type (CC) produced 209 and 91 bp fragments, whereas VKORC1 wild-type (GG) remained at 140 bp. Stringent quality control measures were maintained, including the use of no-template blanks, known reference DNA controls, and a 10% random re-genotyping process, which yielded 100% concordance.

Table 2. PCR-RFLP Primers and Enzymes

SNP Target	PCR Product	Enzyme	Genotype Fragment Sizes (bp)
CYP2C9*2	~ 300 bp	<i>AvaII</i>	CC: 209 + 91 CT: 209, 130, 91 TT: 130 + 91
VKORC1	~ 140 bp	<i>MspI</i>	GG: 140 GA: 140, 110, 30 AA: 110 + 30

2.5 Statistical Analysis

Information was stored in Microsoft Excel and processed using IBM SPSS Statistics version 26.0. Descriptive frequencies/percentages (genotypes/alleles), means+SD (demographics). Allele frequency: $(2 \times \text{homozygote variant} + \text{heterozygote}) / 2N$. Hardy-Weinberg equilibrium (HWE): $2 p^2 / 2 p q$ vs. $p^2 / 2$. Associations (genotype vs. sex/ethnicity): Pearson-square or Fisher (expected less than 5). Significance: $p < 0.05$ (two-tailed). Calculation of power: $n=200$ with a difference of 10% MAF (80% power, $5.05=0.05$).

3. Results

This part includes the genetic character and demographic pattern of the study cohort ($n=200$). All statistical tests were conducted in order to give a baseline of the prevalence of CYP2C9*2 and VKORC1 variants in the target population of Peshawar, Pakistan.

3.1. Participant Characteristics

The sample size used in the study was 200 participants, and the mean age of the sample was 42.5 ± 12.3 years. The sample was mainly male (62.5) and not female (37.5). Regarding ethnicity, most of them were Pashtun (82.0%), with the rest constituting other regional ethnicities (18.0%). Table 3 provides a summary of the detailed demographic information.

Table 3: Demographic Characteristics of the Study Population

	Characteristic	Frequency (n)	Percentage (%)
Age (Years)	18–30	44	22.0%
	31–45	78	39.0%
	46–60	56	28.0%
	> 60	22	11.0%
Sex	Male	125	62.5%
	Female	75	37.5%
Ethnicity	Pashtun	164	82.0%
	Other	36	18.0%
	Total	200	100%

3.2. Genotype and Allele Frequencies

Genotype distribution of the CYP2C9*2 and VKORC1 polymorphisms in the absence of using the RFLP method was established through the PCR-RFLP analysis. The observed frequencies are

in line with the intermediate frequency patterns reported in South Asians and recent literature on the region (Al Hamad, 2025; Qayyum et al., 2018).

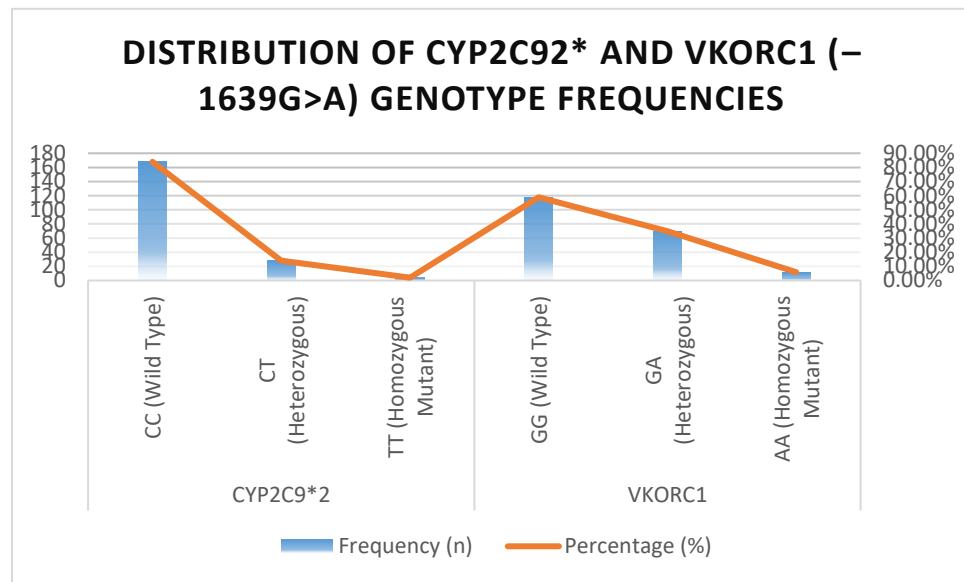


Figure 2. Distribution of CYP2C9*2 and VKORC1 (-1639G>A) Genotype Frequencies

Figure 2 shows the proportion of CYP2C9*2 and VKORC1 genotype frequencies of 200 participants, with wild-type alleles being the most

common (84 and 59 percent mutant and heterozygous alleles, respectively).

Table 4: CYP2C9*2 (rs1799853) Genotype and Allele Frequencies

Genotype/Allele	Frequency (n)	Percentage (%)	Allele Frequency
CC (Wild Type)	168	84.0%	C (Wild): 0.91
CT (Heterozygous)	28	14.0%	
TT (Homozygous Mutant)	4	2.0%	T (*2): 0.09

Table 4 represents how the CYP2C9*2 (rs1799853) polymorphism was common among 200 participants. The prevailing type of CC genotype is wild with 84.0%, and heterozygous and homozygous genotypes of CT and TT with

frequencies of 14.0 and 2.0, respectively. In line with this, the wild-type C allele frequency stands at 0.91, and the variant T (*2) allele frequency stands at 0.09.

Table 5: VKORC1 (-1639G>A) Genotype and Allele Frequencies

Genotype/Allele	Frequency (n)	Percentage (%)	Allele Frequency
GG (Wild Type)	118	59.0%	G (Wild): 0.765
GA (Heterozygous)	70	35.0%	
AA (Homozygous Mutant)	12	6.0%	A (Mutant): 0.235

Table 5 represents the distribution of polymorphisms of VKORC1 (A -1639G). The most prevalent genotype is wild-type GG (59.0%), heterozygous GA and mutant AA genotypes (35.0 and 6.0, respectively). The frequency of the A allele mutant is 0.235.

3.3. Hardy-Weinberg Equilibrium (HWE)

Frequencies of the genotypes were compared with expected frequencies to test the HWE by using the chi-square goodness of fit. The SNPs were both HWE ($p > 0.05$), which is an indication of

no significant selection bias or non-random

mating in the sample (Santos et al., 2013).

Table 6: Hardy-Weinberg Equilibrium Statistics

SNP	Observed (WT/Het/Mut)	Expected (WT/Het/Mut)	χ^2	p-value
CYP2C9*2	168 / 28 / 4	165.6 / 32.8 / 1.6	1.14	0.285
VKORC1	118 / 70 / 12	117.1 / 71.8 / 11.1	0.38	0.537

3.4. Associations

The Pearson Chi-square test was used in the determination of the statistical relationship between the genotypes and the demographic

variables (sex and ethnicity). In line with other multi-ethnic groups, no meaningful relationships were observed.

Table 7: Association of Genotypes with Demographic Variables

Variable	CYP2C9*2 (p-value)	VKORC1 (p-value)
Sex (Male vs. Female)	0.42	0.78
Ethnicity (Pashtun vs. Other)	0.61	0.35

4. Discussion

Warfarin is noted to be a difficult drug to manage clinically because of its narrow therapeutic index and inter-individual differences in dosage requirements. This paper determined the frequency of two major genetic risk factors of warfarin response, namely CYP2C9*2 and VKORC1 (-1639 G A), in a sample of adults in Peshawar, Pakistan. Our results present an essential pharmacogenetic foundational base to an area that has been previously underrepresented in genomic studies.

4.1. Key Findings and Clinical Phenotypes

The findings show that both genes have wild-type alleles that are the most dominant among the Peshawar cohort. In CYP2C9*2 (rs1799853), the minor allele frequency (MAF) was 9.0%, and 16.0% of the population had at least one allele of the dominant mutation (14.0% was heterozygous, and 2.0% was homozygous). These people have been categorized as intermediate or poor metabolizers of S-warfarin, and in most instances, they require much lower doses to prevent supratherapeutic anticoagulation and possible bleeding. In the case of VKORC1(G -1639G A), the A-allele was detected with a frequency of 23.5%. Weaknesses: About 41 percent (35.0 percent GA and 6.0 percent AA) of the participants have a genetic predisposition to warfarin hypersensitivity because of low levels of

vitamin K epoxide reductase enzyme. Both SNPs are observed to be in Hardy-Weinberg equilibrium, implying that the frequencies may reflect the equilibrium genetic structure of the Peshawar population [10].

4.2. Comparison with Literature

Analysis of our data with the regional and global works will give different ethnic trends (Table 7). CYP2C9*2 MAF in our analysis (9.0) is a bit less than the past Pakistani reports of Lahore and Karachi, which reported between 10 and 15. It is also significantly below European (1113), though it is slightly above East Asian cohorts (58%), where the variant with 1 opposite is frequently rare. In the case of VKORC1, the frequency of the A-allele seen in our study (23.5) is significantly less than the 40-50% of these meta-analyses and the past Pakistani research. It is also in stark contrast to the Chinese population, where the A-allele may reach up to 85 percent, resulting in extremely low mean warfarin dosage. The matches of our findings with the Saudi Arabian population (31%), however, make it possible to postulate that there might be a genetic gradient across West and South Asia. These differences emphasise the risks of direct application of dosing algorithms created with the Western or East Asian population on a Pashtun-majority population of Peshawar.

4.3. Clinical Implications

The discovery of a considerable portion of the population (16% in the case of CYP2C9 and 41% in the case of VKORC1) having sensitivity-related variants has direct effects on the local clinical practice. These patients have an increased risk of initial over-anticoagulation in Peshawar clinics, where warfarin is usually initiated with a standard dose that fits all patients [11]. To these users, we suggest that local healthcare providers should consider pharmacogenetic screening, even in simple forms of PCR-RFLP, before the therapy commences. When genotyping is not available, the carriers of such low-dose genetic signatures should be monitored more closely during the first two weeks of treatment to avoid hemorrhagic complications.

4.4. Strengths and Limitations

The research is the first focused pharmacogenetic survey of the Peshawar population that will offer the necessary information to the primarily Pashtun ethnic group. PCR-RFLP is a very economical and strict system of genotyping in resource-constrained environments. The drawbacks, however, are the convenience sample used, which can create selection bias. Moreover, clinical outcomes, i.e., actual maintenance doses or INR stability, were not documented in this study. We also selected two SNPs only, omitting other pertinent variants such as CYP2C9, CYP4F2, etc., that also cause dose variation.

5. Conclusion

This paper has been able to define the prevalence of CYP2C9*2 (MAF: 9.0) and VKORC1 -1639A (MAF: 23.5) in an adult population of Peshawar, Pakistan. We have found that a large fraction of the local population has genotypes that are related to slow warfarin metabolism and high sensitivity to drugs, with about 16 and 41 percent of the individuals having at least one allele variant of the two respective genes. These findings reveal the genetic peculiarity of the Peshawar cohort, which is mainly a Pashtun ethnic mixture, and which is not at all similar to the East Asian and European allele frequency distributions. These results are well argued in the

context of incorporating pharmacogenetic screening into the local clinical practice that excludes the use of standardized dosing in favor of a more personalized approach to warfarin therapy and reduces the likelihood of life-threatening hemorrhagic events. This study can provide a framework through which local clinicians can introduce more stringent INR oversight on identified high-risk genotypes by supplying the initially regional benchmarks of such SNPs. The improvement of future research must be conducted on prospective clinical trials in Peshawar hospitals to directly proportionate these determined genotype frequencies with longitudinal maintenance stability of INR and real maintenance dose in Pakistani patients.

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