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Original Article

Association between genetic polymorphisms of *CYP2C9* and *VKORC1* and safety and efficacy of warfarin: Results of a 5 years audit

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ABSTRACT

Objective: Genetic polymorphisms of *CYP2C9* and *VKORC1* play major role in pharmacokinetics and pharmacodynamics of warfarin, respectively. Purpose of our study was to assess the utility of pretesting patients for the above mutations in predicting tendency for bleeding and achieving target INR.
Methods: This was an audit of data collected between July 2011 and December 2016. For safety and efficacy, patients were divided into two subgroups: those with or without bleeding and those who achieved target INR or not. Chi square test was applied to compare the between group differences and crude Odds Ratio (cOR) calculated.
Results: Among 521 patients evaluated, most common indication for warfarin therapy was valvular heart disease (210/521 = 40%); 36% (187/521) had at least one bleeding episode; 56% (269/479) had below target INR. 26% (136/521) had polymorphic alleles of *CYP2C9* and 69% (358/521) had the GG haplotype of *VKORC1*. Polymorphic alleles of *CYP2C9* or AG/AA haplotype had twice the odds of bleeding (cOR = 2.14 and 2.44 respectively) relative to those with wild *CYP2C9* allele or GG haplotype. Combined *CYP2C9* mutant alleles and/or AG/AA haplotypes had thrice the odds of bleeding (cOR = 3.12) relative to those with wild *CYP2C9* alleles and GG haplotype. Those with GG haplotype had twice the odds (cOR = 1.81) and those with GG haplotype along with wild *CYP2C9* allele had four times the odds (cOR = 4.27) of not achieving the target INR relative to those with other haplotype/alleles. All these associations were statistically significant ($p < 0.05$).
Conclusions: Pretesting patients for genetic polymorphisms could aid in individualizing warfarin therapy.

1. Introduction

Warfarin is commonly prescribed to prevent venous thromboembolism in patients with prosthetic heart valve replacement, Budd-Chiari syndrome, myocardial infarction or chronic atrial fibrillation and to treat acute deep vein thrombosis or pulmonary thromboembolism.[1] Choosing the correct dose of warfarin is often challenging due to its unpredictable pharmacokinetics-pharmacodynamics, narrow therapeutic index and inter-individual variability of response to standard dose, which can lead to rapid rise in International Normalized Ratio (INR), resulting in various complications, mainly bleeding in the form of ecchymosis, gastrointestinal or intracranial hemorrhage. Few patients may also experience uncommon adverse effects such as skin necrosis, alopecia, purple toe syndrome etc.[1–3] Hence, though efficacious and inexpensive, warfarin is not optimally utilized in many individuals due to high rate of adverse events, lack of precise dosing parameters and the need of regular monitoring of response.[4] Overdose increases the risk of unexpected bleeding, while insufficient dose results in not

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achieving the desired target range of INR, thus leading to failure of therapy.[5]

Most of the inter-individual variability of warfarin pharmacokinetics or pharmacodynamics can be accounted for by polymorphisms in its metabolizing enzymes.[6,7] Warfarin is a racemic mixture containing S- and R-warfarin, metabolized by liver enzymes *CYP2C9, CYP3A4, CYP1A2* and *CYP2C19*. Of these, *CYP2C9* is the most significant contributor to Warfarin metabolism as it degrades S-Warfarin which is 5–6 times more biologically active than R-Warfarin.[8] The *CYP2C9**1/*2 (c.C430T; p.Arg144Cys, rs1799853) and *1/*3 (c.A1075C; p.Ile359Leu, rs1057910) polymorphisms have been found to have only 5–12% of the activity of its wild counterpart (*CYP2C9**1/*1), leading to a slower metabolism of warfarin. Patients with these polymorphisms are therefore likely to require a dose reduction or else they become more prone to experience bleeding as adverse event.[9]

Another important gene that contributes to inter-individual variability in warfarin response is the *VKORC1*, which codes for a sub-unit of the Vitamin K epoxide reductase (VKOR) enzyme.[10] Normally, Warfarin binds to VKOR and decreases the amount of Vitamin K available to produce coagulation factors. Altered genotypes caused by some single nucleotide polymorphisms (SNPs) in *VKORC1* such as *VKORC1* –1639G>A (rs9923231) are known to increase this effect of Warfarin leading to increased bleeding tendencies in these subsets of population.[6,9]

Studies have suggested that allelic and genotypic characteristics of *CYP2C9* and *VKORC1* variants in Indian population are quite different from those in other ethnic groups across the globe.[11] *CYP2C9**1/*2, *CYP2C9**1/*3 genotypes and *VKORC1* –1639G>A (rs9923231) haplotype are commonly encountered polymorphisms in the Indian population.[11,12] This audit was performed with the objectives of providing a Western Indian perspective of the distribution of *CYP2C9* and *VKORC1* polymorphisms and to assess the utility of pretesting patients on warfarin therapy for the polymorphisms in predicting the tendency for bleeding and achieving target INR.

2. Materials and methods

2.1. Ethics

Along with Institutional Ethics Committee approval (EC/OA-100/2014, amended on December 2016), consent waiver was sought and obtained as the study was based on reviewing past records of data collected from patients and no interventions or personal interactions with patients were performed. The study was conducted according to the Indian Council of Medical Research Ethical Guidelines for Biomedical Research on Human Participants, 2006.

2.2. Study site

Department of Clinical Pharmacology, Seth GS Medical College and KEM Hospital, Mumbai, Maharashtra, India.

2.3. Study design

Single center, cross-sectional audit of past records.

2.4. Selection criteria

Records of genotyping data from the Warfarin Clinic of our department over more than five years (July 2011–December 2016) were collated and analyzed. Inclusions were patients of any age, either gender, on warfarin therapy (regardless of duration and dose), referred to us for genetic testing, and whose results of the same were available. Exclusions were those on other anticoagulant therapy.

2.5. Genotyping method

Genotyping was carried out by using the method described by Gu et al.[13] Briefly, 5 ml of venous blood was collected in 100 μl of 10% disodium EDTA and genotyping was done using the Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR RFLP) method. DNA was extracted from whole blood using the phenol chloroform method and PCR was carried out for *CYP2C9*2 (rs1799853)* allele, *CYP2C9 *3 (rs1057910)* allele and VKORC1-1639 G>A *(rs9923231)* gene. All the digested samples were run on 3% agarose gel electrophoresis and visualized under UV detector using ethidium bromide. Allele frequencies were measured. The details of the primers and enzymes used for PCR-RFLP are given in Table 1.

2.6. Key variables recorded

Age, gender, indication for, dose and duration of warfarin therapy, results of *CYP2C9* genotype and *VKORC1* haplotype, history of bleeding episode with nature of the bleed and plasma INR value at the time of genotyping. All these data were transcribed into a pre-designed case record form.

2.7. Outcome measures

Demographics, proportions of each genotype and of *CYP2C9* and haplotypes of *VKORC1*; association between polymorphisms (either alone or combined) and occurrence of bleeding; association between polymorphisms (either alone or combined) and whether patients were in target INR for that indication.

Table 1
Primers used for amplification and sequencing of polymorphisms of the *CYP2C9*2, CYP2C9*3* alleles and *VKORC1* gene.

Genotype	Primer sequence	PCR product before digestion	Restriction enzyme used for digestion	Amplicons after treatment with digestion enzyme
*CYP2C9**2	F 5′-AATTTTGGGATGGGGAAGAG-3′ R5′-CCGCTTCACATGAGCTAACA-3′	213bp	*Sau96 I*	183bp-WT 213bp-MUT
*CYP2C9**3	F5′-AGGAAGAGATTGAACGTGTGA-3′ R(5′GGCAGGCTGGTGGGGAGAAGGCCAA-3′	130bp	*Sty I*	130bp-WT 104bp-MUT
VKORC1 –1639 G>A	F: 5′-GCCAGCAGGAGAGGGAAATA-3′; R: 5′-AGTTTGGACTACAGGTGCCT-3′	290bp	*Msp I*	290bp-AA HAP 122bp +168bp-GG HAP 122bp+168bp +290bp-AG HAP

WT = Wild type, MUT = Mutant type, HAP = Haplotype, bp = basepair, F = Forward, R = Reverse.

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2.8. Statistical analysis

Quantitative data were presented using measures of central tendency (median [range]). Categorical data were expressed as proportions. Based on genotyping results participants were divided into those with and without polymorphisms and those achieving target INR or not. Between group analysis was done using the Chi square test with Yates continuity correction and the strength of association between variables was expressed in terms of crude Odds Ratio (cOR) with 95% Confidence Interval (CI). P value <0.05 was considered significant. All the statistical analysis was performed using SPSS software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: USA.), at 5% significance level.

3. Results

3.1. Demographics

Of the 555 patients referred to our Warfarin Clinic from various departments, over the study duration, a total of 521 patients were included in the analysis. Of the 34 excluded, 28 were on concomitant or other anticoagulants (acenocoumarol) and six could not be genotyped as the sample volume was inadequate (needed 5 ml but obtained only 2 ml). INR value at the time of genotyping was available and recorded for 479 patients.

There were a total of 230 (44%) men and 291 (56%) women. The median (range) age (years) was 37 (7–89); the weekly warfarin dose (mg) was 35 (7–105); duration of therapy (months) was 25 (1–80); and the INR value was 1.65 (0.89–18.6).

3.2. Indications for warfarin therapy

The most common indication for warfarin therapy was valvular heart disease (210/521 = 40%), followed by Budd Chiari syndrome (80/521 = 15%), cortical venous thrombosis (74/521 = 14%) and deep vein thrombosis (62/521 = 12%). The remaining 95/521 (19%) fell into the miscellaneous category (including atrial fibrillation, pulmonary thromboembolism, scleroderma, aortic aneurysm, vasculitis and other inflammatory or autoimmune conditions, portal hypertension, portal venous thrombosis, other chronic liver diseases like cirrhosis and cases where a definite diagnosis could not be reached).

3.3. Bleeding episodes

As many as 36% patients (187/521) reported at least one episode of bleeding. Half of the cases had ecchymosis (96/187 = 51%), followed by gum bleeding (25/187 = 13%), hematuria (15/187 = 8%), hemoptysis (14/187 = 7.5%), bleeding per rectum (13/187 = 7%), epistaxis (11/187 = 6%), menorrhagia (8/187), subdural hemorrhage (2/187), hemarthrosis (2/187) and cardiac tamponade (1/187).

3.4. Genotype distribution

3.4.1. CYP2C9

Approximately 74% (385/521) of the study population had the wild type of *CYP2C9* genotype (*1/*1). The remaining patients had mutant variants with *1/*3 being the most prevalent genotype (85/521, 16%) followed by *1/*2 (41/521, 8%), *2/*3 (8/521, 1.5%) and *3/*3 (2 of 521, 0.5%).

3.4.2. VKORC1

Majority of the *VKORC1* haplotype was GG (358/521; 69%), followed by AG (142/521; 27%) and AA (21/521; 4%).

Fifty patients had both *CYP2C9* polymorphism and AG/AA haplotype of *VKORC1*.

All these genotype/haplotype frequency distributions were in accordance with Hardy-Weinberg equilibrium.

This distribution is summarized in Table 2.

3.5. Association of polymorphisms with history of bleeding

A significant association was found between the *CYP2C9* genotype and occurrence of bleeding. The presence of any polymorphism had twice the odds of bleeding relative to those with wild type alleles (cOR 2.14; 95% CI [1.44, 3.20], $p = 0.0002$). Similarly, a significant association was found between the AG and AA haplotypes and increasing risk of bleeding with a cOR of 2.44; 95% CI [1.66, 3.57], $p < 0.0001$.

The presence of *either* a *CYP2C9* mutant *or* the *VKORC1* AG/AA haplotype were associated with thrice the odds for bleeding relative to those with the wild type *CYP2C9* alleles or GG haplotype (cOR 3.12; 95% CI [2.15, 4.54], $p < 0.0001$).

Patients with *both* a mutant of *CYP2C9* and the *VKORC1* AG/AA haplotype, had approximately four times the odds of bleeding relative to patients with a wild *CYP2C9* and *VKORC1* GG haplotype (cOR 3.82; 95% CI [2.05, 7.11], $p < 0.0001$).

The results are summarized in Table 3.

3.6. Subgroup analysis for major indications

A subgroup analysis of the four main indications *viz*. valvular heart disease, Budd Chiari syndrome, cortical venous thrombosis and deep vein thrombosis was done to find the association between genotypes of *CYP2C9* or haplotypes of *VKORC1*, either alone or combined, and history of bleeding. A strong association (cOR > 1) was found in all the subgroups. However, only in valvular heart disease subgroup, this association was statistically significant ($p < 0.05$) across all combinations, whereas in Budd Chiari syndrome subgroup, none of the associations were statistically significant across any combination ($p > 0.05$).

This has been summarized in Tables 4A, 4B, 4C, 4D.

3.7. Association of polymorphism with achievement of target INR

Among the 521 enrolled, INR value was available for 479 patients. More than half of the referred patients (270/479; 56%) had below target INR (<2). Of the rest 209 patients (44%) who achieved target INR, 123 (26%) had plasma INR of more than 3.5 at the time of presentation to our department.

A significant association was found between *CYP2C9* polymorphisms or *VKORC1* haplotypes and proportion of patients achieving target plasma INR levels. Those with wild variant of *CYP2C9* had two and half times the odds (cOR 2.42; 95% CI [1.59, 3.68], $p < 0.0001$), those with GG haplotype had twice the odds (cOR 1.81; 95% CI [1.23, 2.67], $p = 0.0025$) and those with GG haplotype along with wild type *CYP2C9* had four times the odds (cOR 4.27; 95% CI [2.20, 8.27], $p < 0.0001$) of not achieving the target INR relative to those with other haplotype/alleles.

This association is described in Table 5.

Table 2
Distribution of *CYP2C9* & *VKORC1* polymorphisms (n = 521).

Genotype/Haplotype			Numbers (Percentage)
CYP2C9 (n = 521)	Wild	*1/*1	385 (74)
	Mutant	*1/*2	41 (8)
		*1/*3	85 (16)
		*2/*3	8 (1.5)
		*3/*3	2 (0.5)
VKORC1 (n = 521)		GG	358 (69)
		AG	142 (27)
		AA	21 (4)

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Table 3
Association between genotypes of *CYP2C9* and haplotypes of *VKORC1*, either alone or combined and history of bleeding.

Types of alleles	Bleeders	Non Bleeders	P value	cOR [95% CI]
Mutant alleles (*1/*2, *1/*3, *2/*3, *3/*3)	67	69	0.0002[#]	2.14 [1.44–3.20]
Wild (*1/*1)	120	265		
Total	187	334		
AG and AA haplotypes	82	81	<0.0001[#]	2.44 [1.66–3.57]
GG haplotype	105	253		
Total	187	334		
Mutant for *CYP2C9* or *VKORC1* (AG/AA)	122	127	<0.0001[#]	3.12 [2.15–4.54]
Wild for *CYP2C9* and *VKORC1* (GG)	64	208		
Total	186	335		
Mutant for *CYP2C9* and *VKORC1* (AG/AA)	27	23	<0.0001[#]	3.82 [2.05–7.11]
Wild for *CYP2C9* and *VKORC1* (GG)	64	208		
Total	91	231		

Chi square test with Yates continuity correction.
[#] $P < 0.05$ = statistically significant.

Table 4A
Association between genotypes of *CYP2C9* and haplotypes of *VKORC1*, either alone or combined and history of bleeding, subgroup: Valvular Heart Disease Patients (n = 210).

Types of alleles	Bleeders	Non Bleeders	P value	cOR [95% CI]
Mutant *CYP2C9* alleles (*1/*2, *1/*3, *2/*3, *3/*3)	30	18	0.001[@]	3.07 [1.57–5.98]
Wild *CYP2C9* allele (*1/*1)	57	105		
Total	87	123		
VKORC1: AG and AA haplotypes	42	32	0.001[@]	2.65 [1.48–4.75]
VKORC1: GG haplotype	45	91		
Total	87	123		
Mutant for *CYP2C9* and/or *VKORC1*: AG/AA	59	42	<0.0001[@]	4.06 [2.27–7.29]
Wild for *CYP2C9* and *VKORC1*: GG	28	81		
Total	87	123		

Chi square test with Yates continuity correction.
[@] $P < 0.05$ = statistically significant.

Table 4B
Association between genotypes of *CYP2C9* and haplotypes of *VKORC1*, either alone or combined and history of bleeding, subgroup: Budd Chiari Syndrome Patients (n = 80).

Types of alleles	Bleeders	Non Bleeders	P value	cOR [95% CI]
Mutant *CYP2C9* alleles (*1/*2, *1/*3, *2/*3, *3/*3)	6	16	0.421	1.60 [0.51–5.04]
Wild *CYP2C9* allele (*1/*1)	11	47		
Total	17	63		
VKORC1: AG and AA haplotypes	5	17	0.846	1.13 [0.35–3.68]
VKORC1: GG haplotype	12	46		
Total	17	63		
Mutant for *CYP2C9* and/or *VKORC1*: AG/AA	11	28	0.144	2.29 [0.75–6.97]
Wild for *CYP2C9* and *VKORC1*: GG	6	35		
Total	17	63		

Table 4C
Association between genotypes of *CYP2C9* and haplotypes of *VKORC1*, either alone or combined and history of bleeding, subgroup: Cortical Venous Thrombosis Patients (n = 74).

Types of alleles	Bleeders	Non Bleeders	P value	cOR [95% CI]
Mutant *CYP2C9* alleles (*1/*2, *1/*3, *2/*3, *3/*3)	10	9	0.095	2.48 [0.85–7.22]
Wild *CYP2C9* allele (*1/*1)	17	38		
Total	27	47		
VKORC1: AG and AA haplotypes	11	10	0.078	2.54 [0.90–7.18]
VKORC1: GG haplotype	16	37		
Total	27	47		
Mutant for *CYP2C9* and/or *VKORC1*: AG/AA	17	17	0.028[@]	3.00 [1.12–8.01]
Wild for *CYP2C9* and *VKORC1*: GG	10	30		
Total	27	47		

Chi square test with Yates continuity correction.
[@] $P < 0.05$ = statistically significant.

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Table 4D
Association between genotypes of *CYP2C9* and haplotypes of *VKORC1*, either alone or combined and history of bleeding, subgroup: Deep Vein Thrombosis Patients (n = 62).

Types of alleles	Bleeders	Non Bleeders	P value	cOR [95% CI]
Mutant *CYP2C9* alleles (*1/*2, *1/*3, *2/*3, *3/*3)	7	6	0.166	2.41 [0.69–8.34]
Wild *CYP2C9* allele (*1/*1)	16	33		
Total	23	39		
VKORC1: AG and AA haplotypes	10	4	0.005[@]	6.73 [1.79–25.3]
VKORC1: GG haplotype	13	35		
Total	23	39		
Mutant for *CYP2C9* and/or *VKORC1*: AG/AA	20	9	<0.0001[@]	22.2 [5.35–92.3]
Wild for *CYP2C9* and *VKORC1*: GG	3	30		
Total	23	39		

Chi square test with Yates continuity correction.
[@] P<0.05 = statistically significant.

Table 5
Association between genotypes of *CYP2C9* and haplotypes of *VKORC1*, either alone or combined and plasma INR.

Types of alleles	INR <2	INR >2	P value	cOR [95% CI]
CYP2C9 wild type	221	136	<0.0001[$]	2.42 [1.59–3.68]
CYP2C9 mutants	49	73		
VKORC1 haplotype GG	197	125	0.0025[$]	1.81 [1.23–2.67]
VKORC1 haplotype AG/AA	73	84		
CYP2C9 wild+*VKORC1*- GG	162	86	<0.0001[$]	4.27 [2.20–8.27]
CYP2C9 mutants+*VKORC1*- AG/AA	15	34		

Chi square test with Yates continuity correction.
[$] P < 0.05 = statistically significant.

4. Discussion

The present study is a large series of 555 patients and it documents the prevalence of polymorphisms of genes coding for *CYP2C9* and *VKORC1* enzymes, and their association with bleeding as side effect or non-achievement of target INR as non-efficacy in patients receiving warfarin for various indications. A total of 521 patients' data was analyzed. Of the 34 excluded, 28 were on concomitant or other anticoagulants (acenocoumarol). It was found in our study that a quarter of patients referred to us for genotyping had mutant alleles of *CYP2C9* with 16% having the *1/*3 polymorphism. The majority (69%) of the *VKORC1* haplotype was found to be GG. A significant association was found between the presence of any *CYP2C9* mutant allele or *VKORC1* AG/AA haplotypes (either alone or combined) and increased risk of bleeding. A significant association was also found between the presence of wild type allele of *CYP2C9* *1/*1 or GG haplotype of *VKORC1* (either alone or combined) and proportion of patients not achieving the target plasma INR.

The distribution of *CYP2C9* polymorphisms, namely *1/*2 & *1/*3 in our study (8% & 16% respectively, n = 521) was similar to that seen in the south Indian population in several studies by Krishna Kumar et al.[14] (7% & 14% respectively), Jose et al.[15] (5% & 15% respectively) and Madhan et al.[16] (7.6% & 11.8% respectively). Though, another study in the south Indian population by Nahar et al.[17] found much less frequency of *1/*2 and similar frequency of *1/*3 (1.2% & 11% respectively). A smaller sample size (n = 82) might have caused such under-detection of mutant variants. However, the frequency distribution of *1/*3 genotype was different in our study than that found in north Indian population by Kaur et al.[18] (7.2% for *1/*2 & 3.6% for *1/*3), Nahar et al.[17] (9.6% for *1/*2 & 20% for *1/*3), Rathore et al.[19] (6% for *1/*2 & 8% for *1/*3) and Chaudhary et al.[20] (8% for *1/*2 & 19% for *1/*3, in pediatric age group). Much less frequency of *1/*2 & *1/*3 genetic polymorphisms has been reported in literature amongst Chinese population (0% & 2–4% respectively).[21–24] Studies in Caucasians by Sconce et al.[9] show a similar frequency of *1/*3 as in our study (14%) but higher with *1/*2 genotype (22%). Similar observations were found in literature when distributions of *1/*2 & *1/*3 genotypes in Caucasians were compared with Chinese or Indian data.[23–25]

Frequency of the *VKORC1* −1639G>A polymorphism in the present study was 31%. Studies in south Indian population showed this frequency to be varied (20% by Madhan et al.,[16] 26% by Krishna Kumar et al.[14] and 28% by Nahar et al.[17]). Similar variation has been reported in the north Indian population as well (11.7% by Kaur et al.[18], 26.5% by Rathore et al.[19] and 36% by Nahar et al.[17]). While the Caucasian (65% and 75%)[9,25] and Chinese (99%)[21] population expressed consistently much higher proportion of *VKORC1* −1639G>A polymorphism compared to our study.

The marked inter-ethnic difference in the distribution of *CYP2C9*/*VKORC1* alleles and genotypes/haplotypes underscores the importance for studying population distribution of polymorphisms and haplotypes. This variability could be explained by true genetic differences across the population studied as also the difference in the sample sizes of the studies conducted in different set ups.[24] Even the variation in distribution of polymorphisms between north and south Indian population can be attributed to the fact that the people of northern part of India are descendants of Aryans while the people of southern part of India are considered to be of Dravidian origin.[26]

Patients having *CYP2C9* polymorphisms were found to have twice the odds for experiencing a bleeding episode as compared to those with a wild allele (*1/*1). Similarly, patients with AG and AA haplotypes had two and half odds of bleeding relative to those having the GG haplotype. The odds of bleeding increased to three times for patients having polymorphism of either *CYP2C9* or *VKORC1* AG/AA haplotypes than the other variants; with the chance of bleeding further increasing to almost four times in patients having both polymorphisms. A case control study by Sridharan et al.[27] involving 100 controls (those who did not bleed) and 38 cases (those who bled within three months of warfarin initiation) also found a significant association between *CYP2C9* mutant genotypes and *VKORC1* −1639G>A haplotype status with increased bleeding tendency due to warfarin (cOR of 7.8 and 2.7

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respectively for *CYP2C9* and *VKORC1*). This difference of cOR can be attributed to the study design used (prospective case controlled study for Sridharan et al.[27] and cross sectional audit in ours without follow-up).

Similar associations were found in three subgroups (valvular heart disease, cortical venous thrombosis, deep vein thrombosis) in the present study, except in patients with Budd-Chiari syndrome, where cOR was low and not statistically significant. However, a prospective study with the same set of patients of Budd-Chiari syndrome with a one-year follow-up at our liver clinic, found strong association between bleeding episode and presence of polymorphisms (cOR 2.65, p = 0.06 for *CYP2C9*; cOR 1.46, p = 0.48 for *VKORC1*; cOR 3.13, p = 0.04 for *CYP2C9* and/or *VKORC1*). This could be attributed to a longer follow up period as few patients experienced bleeding at a delayed time point. This data is being reported separately.

In the present study, we did not evaluate dosage change subsequent to genotyping as our study was a one-time point audit. Studies with genotyping that have involved a prospective design have documented a significant change in warfarin dosing resulting from information obtained from the genetic tests. Krishna Kumar et al.[14] found that in patients with *CYP2C9**1/*2, *1/*3 and *2/*3 genotypes required a 51%, 60.9% and 62.2% lower daily maintenance dose of warfarin, respectively, than those patients with *CYP2C9**1/*1 wild-type genotype ($p < 0.001$). Sconce et al.[9] showed mean warfarin daily dose requirement was highest in *CYP2C9* wild-type (*1/*1), compared with those having *2 and *3 mutant alleles ($p < 0.001$); and highest in patients with the *VKORC1* GG haplotype compared with those with the AG or AA ($p < 0.001$).

Those with GG haplotype (relatively resistant to warfarin) had twice the odds and those with GG haplotype along with wild type *CYP2C9* (*1/*1) had four times the odds of not achieving the target INR, relative to those with other haplotype/alleles. The target INR ranges from 2 to 3.5 depending on the indication for use of warfarin. A randomized trial by Pirmohamed et al.[28] found that 82% patients in the genotype-guided group reached a stable dose by 3 months, as compared with 70% patients in the non genotype guided group. Also the median time to reach the therapeutic INR was less and the mean percentage of time in the therapeutic range was higher in the genotype-guided group as compared to the control group with the difference being statistically significant in both cases ($p < 0.001$).

A major limitation of our study was a retrospective analysis of data and no patient follow up data. Additionally, we have included only patients that were referred to us and this could have led to a selection bias. Information on INR trends and dose adjustments also have not been done in our study, again limited by its cross-sectional nature. In addition, other rare but possible polymorphisms of ABCB1, CYP4F2, GGCX which could impact the outcome, were not studied. We also did not measure plasma vitamin K or S-warfarin [the active moiety] concentration. There are many known SNPs of the (for e.g. rs1799853, rs1057910, rs2108622, rs9934438, rs7294, rs9923231 etc.) in the three genes related to warfarin dosages and they vary across Indian populations. We could check and provide the results for only two of them (*2, rs1799853 and *3, rs1057910). This is justified because these two alleles have got the highest prevalence in Indian population, amongst different allelic frequencies.[24] We used the PCR-RFLP method for analysis, which has got its own drawbacks (real time PCR or micro array methods are better choices in this regard, but more expensive). The duration of treatment with warfarin ranged from 1 to 80 months, which also could be a potential confounder in terms of ascertaining bleeding episodes.

None of the variables except plasma INR level could predict the occurrence of bleeding, both in univariate and multivariate analysis. This could be explained by the presence of confounders and the study design being a retrospective audit without any scope for follow-up. On the other hand, a controlled prospective study by Gaikwad et al.[29] in western Indian patients on warfarin demonstrated that five variables (*VKORC1*–1639 G>A, *CYP2C9* *2, *CYP2C9* *3, age, and diet) were significantly associated with warfarin response in an univariate analysis. After stepwise multiple regression analysis with these five variables, the developed prediction model was found to be explaining approximately 67% of warfarin dose variability ($R^2 = 0.67$). The maximum dose variability was explained by CYP2C9 (*2 and *3) and VKORC1 genotypes, followed by age and diet.

Thus our study showed that individuals with polymorphism of *CYP2C9* and *VKORC1*, either alone or combined, are more susceptible to experience bleeding episodes as adverse effect in comparison to individuals having no polymorphism. Similarly, patients having wild (*1/*1) *CYP2C9* or *VKORC1* GG haplotype may need higher dose of warfarin to maintain INR in therapeutic range. Both sets of patients need more frequent INR monitoring to optimise the individual dosing. In 2007, the US FDA modified the warfarin label mentioning that *CYP2C9* and *VKORC1* genotyping may be useful in determining the optimal initial dose. Subsequently, the label was further updated in 2010 and included a table recommending different initial dosing ranges for patients with different combinations of *CYP2C9* and *VKORC1* genotypes.[30]

The expected turnaround time for genetic testing at our setup is 4–5 days. At the time the data was collected and analysed we were in the period when genetics was not standard of care for patient management. Patients were referred to us post-facto (after the bleeding episode or if the target INR was not achieved). Based on this audit, we have now started the Pharmacogenetics OPD for testing polymorphisms and subsequent tailoring of dose, according to the genetic results.

In literature, the impact of genotyping has been shown to be equivocal in prospective controlled studies. Researchers of COAG (Clarification of Optimal Anticoagulation through Genetics) trial concluded that genotype-guided dosing of warfarin did not improve anticoagulation control during the first four weeks of therapy.[31] They however stated that the maximum benefit is obtained in the first few days.[32] Another study showed that both *CYP2C9* genotype and *VKORC1* haplotype had a significant influence on the required warfarin dose after the first two weeks of therapy but initial variability in the INR response to warfarin was more strongly associated with *VKORC1* than with *CYP2C9*.[6] Studies from western India recommended that inclusion of genetic testing data along with clinical parameters would help in better prediction of safe and effective warfarin dosing in Indian patients.[12,33] A recently published meta-analysis concluded that genotype-guided dosing compared with standard dosing algorithms did not decrease a composite of death, thromboembolism and major bleeding, but it did result in improved TTR (Time in Therapeutic Range).[34]

In tertiary care public hospitals such as ours, it is difficult to check INR frequently to adjust individual dosing of warfarin as patients from remote parts of the country do not follow up once they are discharged. This is something that does not happen in developed nations. Thus, one time pre-prescription genotyping could present a potential solution in the absence of frequent INR monitoring. However, this must be proven through robust well designed prospective Randomized Controlled Trials comparing genotyping guided warfarin dosing versus non genotype guided dosing, in populations that cannot be easily followed up or cannot undergo frequent INR monitoring, along with pharmacoeconomic assessments, to guide evidence based clinical practice, for individualization of therapy and to determine the cost effectiveness.

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Conflict of interest

None.

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