EFFECT OF *CYP2C9* AND *VKORC1* GENETIC POLYMORPHISMS ON WARFARIN DOSE REQUIREMENT IN SOUTH INDIAN POPULATION

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Abstract : Cytochrome P450 2C9 *(CYP2C9)* and vitamin K epoxide reductase complex subunit 1 (*VKORC1*) genetic polymorphisms were strongly associated with warfarin dose requirement in Caucasians, African Americans and other populations. Our aim was to evaluate the effect of *CYP2C9* and *VKORC1* genetic polymorphisms on warfarin dose requirement in south Indian population. A total of 150 patients on warfarin with stable INR (2-3.5) for the past 3 months were recruited. The genotypes of *CYP2C9*2* and **3* and *VKORC1 -1639G>A* were compared with mean daily warfarin dose (MDWD). The variant allele frequency of *VKORC1 -1639G>A* was found to be 10.4% and *CYP2C9*2* and *CYP2C9*3* were found to be 4.5% and 6.6%, respectively. Our study showed that the mean daily warfarin dose is higher in patients with wild type genotypes of *CYP2C9* and *VKORC1* compared to those with variant genotypes. Multivariate regression analysis revealed that age, body mass index (BMI), duration of therapy and genetic polymorphisms of *CYP2C9* and *VKORC1* together contribute to 36.1% variability in MDWD in south Indian population.

Key words : CYP2C9   VKORC1   genetic polymorphism
          warfarin   south Indian

INTRODUCTION

Warfarin, an oral anticoagulant, is a racemic mixture of R and S- warfarin. S-warfarin is more potent than R-warfarin and it contributes to 70% of the anticoagulant effect of warfarin (1). It has been in clinical use for more than 60 years. It is indicated for both the prophylaxis and treatment of thromboembolic conditions like deep vein thrombosis, pulmonary embolism, atrial fibrillation, prosthetic heart valves and also

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for the reduction of death risk in myocardial infarction (2, 3). Warfarin is a drug with narrow therapeutic index, small change in dose can lead to bleeding risk or lack of therapeutic effect and it requires careful monitoring. The anticoagulation profile is monitored by International Normalized Ratio (INR) which is an index of its therapeutic effect and safety (4). Besides this limitation of being a narrow therapeutic index drug, large interindividual variability exists attributable to both genetic and non-genetic factors (5, 6, 7).

The cytochrome P450 enzymes are involved in the metabolism of warfarin. S-warfarin is metabolised predominantly by CYP2C9 and R-warfarin is metabolised by multiple enzymes such as CYP3A4, CYP1A2 and CYP1A1 (8). Since the greater proportion of anticoagulant effect is contributed by S-warfarin, abnormal function of CYP2C9 enzyme leads to altered dose-response to warfarin (9). CYP2C9 enzyme is encoded by the gene *CYP2C9* which is polymorphically expressed in different ethnic populations (10, 11, 12, 13). Genetic polymorphisms in *CYP2C9* affect the drugs metabolised by CYP2C9 like phenytoin, warfarin, NSAIDs etc. (14, 15, 16). *CYP2C9*2* and *CYP2C9*3* are found to be important variants in warfarin metabolism (17). The individuals with these variants have reduced capacity to metabolise warfarin which is clinically implicated in the requirement of lower dose and increased risk of bleeding (5, 18). The frequency of these genetic polymorphisms differs between various ethnic populations (10, 11, 12, 13). Studies have shown the variation in dose and risk of over anticoagulation correlating with the variant allele in different population (9, 19, 20, 21, 22, 23).

On the other hand, vitamin K epoxide reductase complex subunit 1 (VKORC1) is the target site for warfarin, which it inhibits for its anticoagulation effect. This enzyme is encoded by the gene *VKORC1* (24). Number of single nucleotide polymorphisms in *VKORC1* has been identified. Of these promoter region polymorphism (*VKORC1 – 1639G>A*) appears to be the most important. Polymorphism at this region leads to abnormal expression of the enzyme which correlates well with the dose required by those individuals with this variant (25, 26). Several studies have shown that the gene *VKORC1* as the single biggest predictor of warfarin dose requirement (6, 27, 28, 29). The *VKORC1 -1639 GG* (wild) genotype requires high dose and *VKORC1 -1639 AA* (homomutant) genotype lower while the *VKORC1 -1639 GA* (heteromutant) genotype requires intermediate dose (25, 26). The frequency of these genotypes varies in different ethnic groups (25, 27, 30, 31, 32, 33). Studies have shown that the Caucasians require higher dose compared to Asians which correlates well with their higher *VKORC1 -1639 GG* and *VKORC1 -1639 AA* genotype frequency respectively in these populations (20, 21, 22, 26, 33).

The frequency of *CYP2C9* polymorphism has been established in south Indian population (34, 35). Limited information is available on *VKORC1* polymorphism in south Indian population and the influence of these polymorphisms on warfarin dose requirements (36). The present study was designed to find out the effect of *CYP2C9* and *VKORC1* genetic polymorphisms on warfarin dose requirement in south Indian population.

MATERIALS AND METHODS

Study settings

The study was done in the Department of Pharmacology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry in collaboration with the Department of Cardiology and Department of Medicine, JIPMER. This study was conducted in patients on warfarin therapy visiting Cardiology and Medicine out-patients department and also in-patients of these departments during the period from March 2010 to July 2011.The study protocol was approved by the Institute Ethics Committee (IEC) prior to commencement of the study.

Sample size

The sample size calculations were undertaken before the study. The minimum important difference (difference in mean dose) between the groups was taken to be 0.8% with a standard deviation of ± 1.2 with ratio of subjects between wild normal and variant allele groups 4:1. Using the Power and Sample Size (PS) Calculations software version 3.0 (Vanderbilt University, Nashville, Tennessee, USA) for a power of 80% and a significance level of 5%, the sample size was calculated to be a total of 110 for the study.

Study subjects

Patients receiving warfarin maintenance therapy for cardiovascular disorders, cerebrovascular disorders and deep vein thrombosis with an INR within the range of 2.0 to 3.5 for the past 3 months, age ranged 18-65 years, either gender and of south Indian origin were included. The nativity was assessed by family history of three generations living in Tamil Nadu, Pondicherry, Kerala, Karnataka, Andhra Pradesh, and speaking any of the south Indian languages as their native language. Patients with liver or renal dysfunction, taking CYP2C9 inducers and inhibitors (eg. barbiturates, carbamazepine, rifampicin, amiodarone, ketoconazole etc.), pregnant and lactating women, smokers and alcoholics were excluded. Written informed consent was obtained from patients participated in this study. Mean daily warfarin maintenance dose (MDWD) [the mean of two recent doses over a period when two consecutive stable INR values achieved 2.0 to 3.5] was calculated.

Genotyping for *CYP2C9* and *VKORC1 -1639G>A*

Five millilitre of venous blood was collected from the study participants for genotyping. DNA was extracted by using phenol-chloroform extraction method (37). Genotyping for *CYP2C9*2* and *CYP2C9*3* polymorphisms and *VKORC1 -1639G>A* were done by Real Time Thermocycler (ABI Prism 7300, Foster City). TaqMan drug metabolism genotyping assays were obtained from Applied Biosystems, Foster City for *CYP2C9* alleles [*CYP2C9*2 (rs1057910)* (Assay by design), *CYP2C9*3* (Assay ID: C_27104892_10)] and *VKORC1 -1639G>A* (C_30996661_30). The qRT-PCR reaction was carried out using a final volume of 15 µl (7.5 µl of Taqman Universal master mix (2X), 0.375 µl of 20X working stock of genotyping assay, 3.375 µl of deionized water and 3.750 µl of genomic DNA (50 ng/µl) diluted in DNAase free water). The allelic discrimination was performed using 7300 SDS software (version 1.3.1).

Data analysis

Statistical analysis was performed using SPSS version 16.0 and GraphPad Instat version 3.06 software packages. Unpaired t-test, Chi-square and Fischer's exact test were used appropriately to compare between the baseline parameters. Comparison of MDWD in *CYP2C9* and *VKORC1* genotype was done by using Mann-Whitney U test and in combined *CYP2C9* and *VKORC1* genotypes was done by Kruskal-Wallis test. Multiple linear regression analysis was done to study the association of independent variables known to influence MDWD. P value less than 0.05 was considered statistically significant.

RESULTS

Study participants

A total of 150 patients were recruited

depending on the inclusion and exclusion criteria. Among them 6 patients were excluded for the data analysis due to the poor quality or missing of the DNA samples for the analysis. The patient characteristics are given in Table I. The baseline characteristics in patients with wild and variant genotype for *CYP2C9* and *VKORC1 -1639G>A* were compared. The baseline characteristics were not significantly different in *CYP2C9* genotype and in *VKORC1* genotype groups. The baseline characteristics across combined genotype of *CYP2C9* and *VKORC1 -1639G>A* were found to be statistically non-significant.

CYP2C9, VKORC1 -1639G>A allele and genotype frequencies of study participants

The allele and genotype frequencies of *CYP2C9* and *VKORC1* are given in the Table II. The genotype frequencies of *CYP2C9* and

TABLE I : Subject characteristics.

Parameters	Overall (N=144)	VKORC1		CYP2C9	
		GG (N=115)	Non GG (N=29)	*1*1 (N=114)	Non *1*1 (N=30)
Age (years)#	43.4±11.6	42.5±11.2	46.7±12.5	43.1±11.9	44.5±10.5
Height (cm)#	156.6±6.9	156.8±6.9	155.8±6.9	156.4±7.1	157.7±6.2
Weight (kg)#	56.11±8.2	56.3±8.5	55.5±6.7	56.6±8.1	54.4±8.1
Body mass index (kg/m²)#	22.8±2.9	22.8±2.9	22.9±2.6	23.1±2.8	21.9±3.1
Duration (months)#	10.8±7.8	10.7±7.8	10.4±7.7	11.6±8.2	8.8±5.4
Hypertensive (N)(%)	17(11.8)	12(10.4)	5(17.2)	12(10.5)	5(16.7)
Diabetic (N)(%)	9(6.2)	8(7)	1(3.4)	8(7)	1(3.3)
Indications (N)(%)					
Valvular heart disease	104 (72.2)	80(73)	20(68.9)	80(70.2)	24(80)
Prosthetic valves	3(2.1)	2(1.7)	1(3.4)	2(1.8)	1(3.3)
Atrial fibrillation	11(13.7)	10(8.7)	1(3.4)	9(7.9)	2(6.7)
Deep vein thrombosis	8(5.6)	6(5.2)	2(6.9)	7(6.1)	1(3.3)
Others	18(12.5)	15(13)	3(10)	16(14)	2(6.7)
Concomitant drugs (N)(%)					
Phenoxymethylpenicillin	73(62.9)	59(51.3)	14(48.3)	57(50)	16(53.3)
Furosemide	89(76.7)	71(61.7)	18(62.1)	73(64)	16(53.3)
Digoxin	54(46.6)	40(34.8)	14(48.3)	42(36.8)	12(40)
Atenolol	41(54)	32(27.8)	9(31)	33(28.9)	8(26.7)

(#-*Values in mean±SD; N=Number of subjects*).

TABLE II : Genotype and allele frequency of *CYP2C9* and *VKORC1 -1639G>A* (n=number of subjects).

Gene	Nucleotide position	n	Genotype frequency (%)				n	Allele frequency (%)		
CYP2C9	CYP2C9*2 (430 C>T) CYP2C9*3 (1075 A>C)	144	*1*1 114(79.2)	*1*2 11(7.6)	*1*3 17(11.8)	*2*3 2(1.4)	288	*1 256(88.9)	*2 13(4.5)	*3 19(6.6)
VKORC1	-1639G>A	144	GG 115(79.9)	GA 28(19.4)	AA 1(0.7)		288	G 258(89.6)	A 30(10.4)	

VKORC1 -1639G>A in the present study was found to be in Hardy–Weinberg equilibrium. *CYP2C9* homozygous variants *(*2/*2 or *3/ *3)* were not reported in the patients.

Comparison of MDWD in *CYP2C9* and *VKORC1 -1639G>A* genotype

MDWD was compared between wild and variant genotypes of *CYP2C9* and *VKORC1 - 1639 G>A* and also the effect of combined genotypes of both the genes were studied (Table III). There was significant difference observed when the MDWD was compared between the *CYP2C9* genotype wild and *CYP2C9* variant genotype (4.8±1.6 mg vs. 2.9±1.1 mg, P<0.0001). Similarly significant difference was observed between *VKORC1*

TABLE III : Comparison of MDWD in *CYP2C9*, *VKORC1 -1639 G>A* and combined *CYP2C9* and *VKORC1 -1639 G>A* genotypes.

CYP2C9		N=144 (%)	MDWD (mg) (95% CI)	P value
*1/*1		114(79.17)	4.8±1.6 (4.47–5.07)	P<0.0001#
non*1/*1		30(20.83)	2.9±1.1 (2.46–3.35)	
VKORC1 -1639G>A		**N=144 (%)**	**MDWD (mg)**	**P value**
G G		115(79.9)	4.6±1.6 (4.32–4.97)	P<0.001#
nonGG		29(20.1)	3.4±1.1 (2.96–3.84)	
VKORC1 -1639G>A	**CYP2C9**	**N=144(%)**	**MDWD(mg)**	**P value**
G G	*1/*1	95(66)	4.97±1.5 (4.64–5.30)	P<0.0001$
nonGG	non*1/*1	10(7)	2.6±0.4 (2.29–2.93)	
nonGG	*1/*1	19(13.2)	3.8±1.1 (3.23–4.37)	
G G	non*1/*1	20(13.8)	3.0±1.3 (2.39–3.72)	

Values in mean±SD; P<0.05 is significant; N=Number of subjects.
\# - Mann-Whitney U test was used.
$ - Kruskal-Wallis test was used.

TABLE IV : Multiple linear stepwise regression analysis for factors determining MDWD.

S. No.	Independent variable	Unstandardized regression coefficient	Standard error	Standardized regression coefficient	P value
1.	Constant	3.05			
2.	Age	−0.036	0.010	−0.255	<0.0001
3.	BMI	0.129	0.041	0.226	<0.05
4.	Duration of therapy	0.038	0.015	0.178	<0.05
5.	CYP2C9 Genotype	−0.691	0.159	−0.308	<0.0001
6.	VKORC1 Genotype	−0.859	0.276	−0.221	<0.05

Predictors in the Model: (Constant), Age, BMI, duration of therapy, *CYP2C9* genotypes, *VKORC1* genotypes, R=0.621; R^2=0.386; Adjusted R^2=0 .361; P=<0.0001.

Independent variables: Age, BMI, duration of therapy, *VKORC1 -1639G>A* genotype and *CYP2C9* genotype were considered.

wild and variant genotype (4.6±1.6 mg vs. 3.4±1.1 mg, P<0.001). When comparing combined wild genotypes with combined variant genotypes a strong significant difference were observed.

Multiple linear regression for factors determining MDWD

Multiple linear regression analysis was carried out for the factors which can determine the MDWD. Age, body mass index (BMI), duration of therapy, *CYP2C9* and *VKORC1 -1639G>A* genotypes were the independent variable considered. Age, BMI, duration of therapy and genotypes of *CYP2C9* and *VKORC1 -1639G>A* were found to be significantly associated with the MDWD in this study (Table IV). All these factors together contributed to 36.1% variability in MDWD (adjusted R^2 = 0.361).

DISCUSSION

The frequency of *CYP2C9* in the current study did not differ from the previously established frequency in our population (Table II) (38). The frequency of *VKORC1* genotypes was found to be similar to few studies done in Indian population and was quite different from other world population particularly the Chinese and Japanese population (Table II) (22, 25, 30, 32, 39, 40). The frequency distribution of variant alleles differs in various ethnic groups and so the mean dose required. Studies have shown that frequency of AA genotype is higher in Asians when compared to the Caucasians and thus Asians require lower maintenance dose to maintain a stable INR (25, 27, 30, 31, 32, 33). In our study the patients carrying the wild genotype (*CYP2C9*1/*1*) required higher MDWD (4.8±1.6 mg) when compared to the variant genotypes (*CYP2C9 non*1/*1*) (2.9±1.1 mg). These findings are in concordance with findings obtained in the studies conducted previously in various population (Table III) (19, 22, 23, 41,42, 43, 44).

In the present study, an association between the *VKORC1* genotype and MDWD was observed. MDWD was significantly higher in the wild genotype compared the variant genotype (4.6±1.6 mg vs. 3.4±1.1 mg, P<0.001) (Table III). A study done in 104 Chinese patients showed that AA genotype required lower dose when compared to

combined GA and GG genotype (2.61±1.10 mg/ day vs 3.81±1.24 mg/day, P<0.0001). This study also assessed the enzymatic activity in wild and variant allele which showed that *VKORC1* promoter with the G allele had a 44% increase in luciferase activity when compared with the A allele. This increase in enzymatic activity could result in increase in dose requirement in those carrying G allele (25).

In our study we studied the promoter region polymorphism alone in *VKORC1*. Studies have demonstrated that this polymorphism is in strong linkage disequilibrium with six other SNPs (rs7196161, rs2884737, rs9934438, rs8050894, rs2359612 and rs7294) and this polymorphism alone to some extent explains the pharmacologic variation to oral anticoagulants (45). Studies have demonstrated that the promoter region polymorphism *VKORC1 -1639G>A,* is the most important predictor of warfarin initiation dose (6, 27, 28, 46). The current study findings are in agreement with the studies done elsewhere and also few studies done in Indian population (7, 22, 27, 30, 31, 32, 33, 41, 47). MDWD was significantly higher in the combined wild genotype of *CYP2C9* and *VKORC1* when compared with the combined variant genotypes (Table III). This finding is similar to the previous study done in Caucasian population (39). The major finding of this study was that MDWD was lower in patients carrying variant combined genotypes *CYP2C9* and *VKORC1* when compared to the wild genotypes. Both these polymorphisms along with age, BMI and duration of therapy contributed to 36.1% variability in MDWD (Table IV). In a study done in Andhra Pradesh population genetic and clinical factors contributed up to 61%

variability in warfarin dose requirement. They have explained that age, gender, BMI, vitamin K intake, *CYP2C9 (*2,*3* and *8)* and *VKORC1 (*3, *4* and *-1639 G>A), CYP4F2 V433M, GGCX G8016A* and thyroid status contributed the variability (36). Previous studies have shown the effect of age, weight and height on warfarin maintenance dose requirements (6, 39, 48, 49). In our study we found that age and BMI significantly influenced the MDWD (Table IV).

Many of the drugs which can potentially interact with warfarin were excluded as part of the exclusion criteria (eg. barbiturates, rifampicin, carbamazepine, fluconazole, cimetidine etc.). Other concurrent medications which were essential as a part of the treatment required by the patients were not excluded and they were not found to be contributing to the MDWD variation since they were equally distributed among different genotype groups.

Diet, particularly the amount of vitamin K intake can also affect anticoagulant response to warfarin (50). In this study although we did not consider dietary consumption of vitamin K, we assumed that all patients had relatively stable vitamin K consumption, given that they had stable INR. Patient compliance is another factor which may affect the anticoagulation response to warfarin (51). The patients had relatively good compliance as their INR was stable in our study. They may be the patients who were counseled well when starting the anticoagulation therapy by the clinicians. In addition to *CYP2C9* and *VKORC1*, other genetic polymorphisms in gamma glutamyl carboxylase (*GGCX*), *CYP4F2*, microsomal epoxide hydrolase 1(*EPHX1*), calumenin

(*CALU*) and apolipoprotein E (*APOE*) are minor contributors for warfarin dose requirement (52, 53, 54, 55, 56). In the present study we have not focussed on these genes. The strengths of our study were that we followed strict exclusion criteria to exclude the co-morbid conditions, smoking status and other concomitant medications that may potentially interact with warfarin. This implies that the effect of *CYP2C9* and *VKORC1* polymorphism on warfarin dose requirement found in our study is more reliable. The major limitation of our study was that the contribution by other genetic factors like gamma glutamyl carboxylase, epoxide hydrolase and *CYP4F2* polymorphisms and also other polymorphisms in *VKORC1* were not addressed.

CONCLUSION

MDWD is higher in patients with wild genotypes of *CYP2C9* and *VKORC1* compared to those with variant genotypes and along with age, BMI and duration of therapy they contribute to 36.1% variability in MDWD. In future replicate study with large sample size with additional genetic factors taken into consideration needs to be carried out to develop an algorithm for determining warfarin dose before introduction into routine clinical practice. The present study provides the basic information for larger studies in our population and contributes to establish the pharmacogenetic based warfarin therapy in south Indian population.

REFERENCES

1. Choonara IA, Haynes BP, Cholerton S, Breckenridge AM, Park BK. Enantiomers of warfarin and vitamin K1 metabolism. *Br J Clin Pharmacol* 1986; 22: 729–732.

2. Hirsh J, Dalen J, Anderson DR, et al. Oral anticoagulants: mechanism of action, clinical effectiveness, and optimal therapeutic range. *Chest* 2001; 119: 8S–21S.

3. Tadros R, Shakib S. Warfarin—indications, risks and drug interactions. *Aust Fam Physician* 2010; 39: 476–479.

4. Ng VL. Anticoagulation monitoring. *Clin Lab Med* 2009; 29: 283–304.

5. Lindh JD, Holm L, Andersson ML, Rane A. Influence of *CYP2C9* genotype on warfarin dose requirements—a systematic review and meta-analysis. *Eur J Clin Pharmacol* 2009; 65: 365–375.

6. Gage BF, Eby C, Johnson JA, et al. Use of pharmacogenetic and clinical factors to predict the therapeutic dose of warfarin. *Clin Pharmacol Ther* 2008; 84: 326–331.

7. Yang L, Ge W, Yu F, Zhu H. Impact of *VKORC1* gene polymorphism on interindividual and interethnic warfarin dosage requirement—a systematic review and meta analysis. *Thromb Res* 2010; 125: e159–166.

8. Porter WR. Warfarin: history, tautomerism and activity. *J Comput Aided Mol Des* 2010; 24: 553–573.

9. Kealey C, Chen Z, Christie J, et al. Warfarin and cytochrome P450 2C9 genotype: possible ethnic variation in warfarin sensitivity. Pharmacogenomics 2007; 8: 217–225.

10. Hamdy SI, Hiratsuka M, Narahara K, et al. Allele and genotype frequencies of polymorphic cytochromes P450 (CYP2C9, CYP2C19, CYP2E1) and dihydropyrimidine dehydrogenase (DPYD) in the Egyptian population. *Br J Clin Pharmacol* 2002; 53: 596–603.

11. LLerena A, Dorado P, O'Kirwan F, Jepson R, Licinio J, Wong M-L. Lower frequency of CYP2C9*2 in Mexican-Americans compared to Spaniards. *Pharmacogenomics J* 2004; 4: 403–406.

12. Nasu K, Kubota T, Ishizaki T. Genetic analysis of CYP2C9 polymorphism in a Japanese population. *Pharmacogenetics* 1997; 7: 405–409.

13. Dorado P, Berecz R, Norberto M-J, Yasar U, Dahl M-L, LLerena A. CYP2C9 genotypes and diclofenac metabolism in Spanish healthy volunteers. *Eur J Clin Pharmacol* 2003; 59: 221–225.

14. Hung C-C, Huang H-C, Gao Y-H, et al. Effects of polymorphisms in six candidate genes on phenytoin maintenance therapy in Han Chinese patients. *Pharmacogenomics* 2012; 13: 1339–1349.

15. Carbonell N, Verstuyft C, Massard J, et al. CYP2C9*3 Loss-of-Function Allele Is Associated With Acute Upper Gastrointestinal Bleeding Related to the Use of NSAIDs Other Than Aspirin. *Clin Pharmacol Ther* 2010; 87: 693–698.

16. López-Rodríguez R, Novalbos J, Gallego-Sandín S, et al. Influence of CYP2C8 and CYP2C9 polymorphisms on pharmacokinetic and pharmacodynamic parameters of racemic and enantiomeric forms of ibuprofen in healthy volunteers. *Pharmacol Res* 2008; 58: 77–84.

17. Miners JO, Birkett DJ. Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. *Br J Clin Pharmacol* 1998; 45: 525–538.

18. Sanderson S, Emery J, Higgins J. CYP2C9 gene variants, drug dose, and bleeding risk in warfarin-treated patients: a HuGEnet systematic review and meta-analysis. *Genet Med* 2005; 7: 97–104.

19. Aithal GP, Day CP, Kesteven PJ, Daly AK. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. *Lancet* 1999; 353: 717–719.

20. Carlquist JF, Horne BD, Muhlestein JB, et al. Genotypes of the cytochrome p450 isoform, CYP2C9, and the vitamin K epoxide reductase complex subunit 1 conjointly determine stable warfarin dose: a prospective study. *J Thromb Thrombolysis* 2006; 22: 191–197.

21. Cavallari LH, Langaee TY, Momary KM, et al. Genetic and clinical predictors of warfarin dose requirements in African Americans. *Clin Pharmacol Ther* 2010; 87: 459–464.

22. Gan GG, Phipps ME, Lee MMT, et al. Contribution of VKORC1 and CYP2C9 polymorphisms in the interethnic variability of warfarin dose in Malaysian populations. *Ann Hematol* 2011; 90: 635–641.

23. Higashi MK, Veenstra DL, Kondo LM, et al. Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. *JAMA* 2002; 287: 1690–1698.

24. Rost S, Fregin A, Ivaskevicius V, et al. Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2. *Nature* 2004; 427: 537–541.

25. Yuan H-Y, Chen J-J, Lee MTM, et al. A novel functional VKORC1 promoter polymorphism is associated with inter-individual and inter-ethnic differences in warfarin sensitivity. *Hum Mol Genet* 2005; 14: 1745–1751.

26. Rieder MJ, Reiner AP, Gage BF, et al. Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose. *N Engl J Med* 2005; 352: 2285–2293.

27. Wadelius M, Chen LY, Lindh JD, et al. The largest prospective warfarin-treated cohort supports genetic forecasting. *Blood* 2009; 113: 784–792.

28. Huang S-W, Chen H-S, Wang X-Q, et al. Validation of VKORC1 and CYP2C9 genotypes on interindividual warfarin maintenance dose: a prospective study in Chinese patients. *Pharmacogenet Genomics* 2009; 19: 226–234.

29. Klein TE, Altman RB, Eriksson N, et al. Estimation of the warfarin dose with clinical and pharmacogenetic data. *N Engl J Med* 2009; 360: 753–764.

30. Yoshizawa M, Hayashi H, Tashiro Y, et al. Effect of VKORC1-1639 G>A polymorphism, body weight, age, and serum albumin alterations on warfarin response in Japanese patients. *Thromb Res* 2009; 124: 161–166.

31. Oner Ozgon G, Langaee TY, Feng H, et al. VKORC1 and CYP2C9 polymorphisms are associated with warfarin dose requirements in Turkish patients. *Eur J Clin Pharmacol* 2008; 64: 889–894.

32. Schelleman H, Chen Z, Kealey C, et al. Warfarin response and vitamin K epoxide reductase complex 1 in African Americans and Caucasians. *Clin Pharmacol Ther* 2007; 81: 742–747.

33. Aquilante CL, Langaee TY, Lopez LM, et al. Influence of coagulation factor, vitamin K epoxide reductase complex subunit 1, and cytochrome P450 2C9 gene polymorphisms on warfarin dose requirements. *Clin Pharmacol Ther* 2006; 79: 291–302.

34. Adithan C, Gerard N, Vasu S, Balakrishnan R, Shashindran CH, Krishnamoorthy R. Allele and genotype frequency of CYP2C9 in Tamilnadu population. *Eur J Clin Pharmacol* 2003; 59: 707–709.

35. Rosemary J, Surendiran A, Rajan S, Shashindran CH, Adithan C. Influence of the CYP2C9 and CYP2C19 polymorphisms on phenytoin hydroxylation in healthy individuals from south India. *Indian J Med Res* 2006; 123: 665–670.

36. Pavani A, Naushad SM, Mishra RC, et al. Retrospective evidence for clinical validity of expanded genetic model in warfarin dose optimization in a South Indian population. *Pharmacogenomics* 2012; 13: 869–878.

37. Green MR, Sambrook J. Molecular Cloning: A Laboratory Manual (Fourth Edition): Three-volume set 4th ed Cold Spring Harbor Laboratory Press 2012.

38. Jose R, Chandrasekaran A, Sam SS, et al. CYP2C9 and CYP2C19 genetic polymorphisms: frequencies in the south Indian population. *Fundam Clin Pharmacol* 2005; 19: 101–105.

39. Sconce EA, Khan TI, Wynne HA, 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: 2329–2333.

40. Rathore SS, Agarwal SK, Pande S, Mittal T, Mittal B. Frequencies of VKORC1 -1639 G>A, CYP2C9*2 and CYP2C9*3 genetic variants in the Northern Indian population. *Biosci Trends* 2010; 4: 333–337.

41. Takahashi H, Wilkinson GR, Nutescu EA, et al. Different contributions of polymorphisms in VKORC1 and CYP2C9 to intra- and inter-population differences in maintenance dose of warfarin in Japanese, Caucasians and African-Americans. *Pharmacogenet Genomics* 2006; 16: 101–110.

42. Takahashi H, Kashima T, Nomizo Y, et al. Metabolism of warfarin enantiomers in Japanese patients with heart disease having different CYP2C9 and CYP2C19 genotypes. *Clin Pharmacol Ther* 1998; 63: 519–528.

43. Miao L, Yang J, Huang C, Shen Z. Contribution of age, body weight, and CYP2C9 and VKORC1 genotype to the anticoagulant response to warfarin: proposal for a new dosing regimen in Chinese patients. *Eur J Clin Pharmacol* 2007; 63: 1135–1141.

44. Halkin H, Lubetsky A. Warfarin dose requirement and CYP2C9 polymorphisms. *Lancet* 1999; 353: 1972–1973.

45. Kumar DK, Shewade DG, Surendiran A, Adithan C. Genetic variation and haplotype structure of the gene Vitamin K epoxide reductase complex, subunit 1 in the Tamilian population. *J Pharmacol Pharmacother* 2013; 4: 53–58.

46. Obayashi K, Nakamura K, Kawana J, et al. VKORC1 gene variations are the major contributors of variation in warfarin dose in Japanese patients. *Clin Pharmacol Ther* 2006; 80: 169–178.

47. Lee S-C, Ng S-S, Oldenburg J, et al. Interethnic variability of warfarin maintenance requirement is explained by VKORC1 genotype in an Asian population. *Clin Pharmacol Ther* 2006; 79: 197–205.

48. Gurwitz JH, Avorn J, Ross-Degnan D, Choodnovskiy I, Ansell J. Aging and the anticoagulant response to warfarin therapy. *Ann Intern Med* 1992; 116: 901–904.

49. Tham L-S, Goh B-C, Nafziger A, et al. A warfarin-dosing model in Asians that uses single-nucleotide polymorphisms in vitamin K epoxide reductase complex and cytochrome P450 2C9. *Clin Pharmacol Ther* 2006; 80: 346–355.

50. Custódio das Dôres SM, Booth SL, Martini LA, et al. Relationship between diet and anticoagulant response to warfarin: a factor analysis. *Eur J Nutr* 2007; 46: 147–154.

51. Kimmel SE, Chen Z, Price M, et al. The influence of patient adherence on anticoagulation control with warfarin: results from the International Normalized Ratio Adherence and Genetics (IN-RANGE) Study. *Arch Intern Med* 2007; 167: 229–235.

52. Rieder MJ, Reiner AP, Rettie AE. Gamma-glutamyl carboxylase (GGCX) tagSNPs have limited utility for predicting warfarin maintenance dose. *J Thromb Haemost* 2007; 5: 2227–2234.

53. Caldwell MD, Awad T, Johnson JA, et al. CYP4F2 genetic variant alters required warfarin dose. *Blood* 2008; 111: 4106–4112.

54. Pautas E, Moreau C, Gouin-Thibault I, et al. Genetic factors (VKORC1, CYP2C9, EPHX1, and CYP4F2) are predictor variables for warfarin response in very elderly, frail inpatients. *Clin Pharmacol Ther* 2010; 87: 57–64.

55. Voora D, Koboldt DC, King CR, et al. A polymorphism in the VKORC1 regulator calumenin predicts higher warfarin dose requirements in African Americans. *Clin Pharmacol Ther* 2010; 87: 445–451.

56. Kimmel SE, Christie J, Kealey C, et al. Apolipoprotein E genotype and warfarin dosing among Caucasians and African Americans. *Pharmacogenomics J* 2008; 8: 53–60.