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Original Article

Value of *VKORC1* (−1639G>A) rs9923231 genotyping in predicting warfarin dose: A replication study in South Indian population

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ABSTRACT

Objective: Warfarin is the most commonly prescribed oral anticoagulant, although having a narrow therapeutic index and wide interindividual variability. The aim of this study was to replicate the utility of *VKORC1* (−1639G>A) rs9923231 genotyping in predicting the mean daily dose and to evaluate its ability to categorize warfarin-treated patients to high-, intermediate-, or low-dose categories in the South Indian population.

Materials and methods: A cohort of 222 warfarin-treated patients was genotyped using restriction fragment length polymorphism method. The influence of the rs9923231 polymorphism on the variations in the mean daily dose was compared using one-way analysis of variance and linear regression analysis. Discriminatory ability of the rs9923231 polymorphism to group the patients into ordered dose categories was assessed by estimating the proportional odds ratios using the ordered logit regression analysis.

Results: The frequency of AA genotype and A allele in the study sample was found to be 1.8% and 9.23%, respectively, which was similar to reports from other South Indian populations. The mean daily dose required to achieve the optimum international normalized ratio was significantly lower in AA homozygous genotype carriers (3.99 ± 1.67 mg/day) and GA heterozygous (4.26 ± 1.57 mg/day) compared to the GG genotype carriers (5.51 ± 2.13 mg/day), $p = 0.003$. The A allele carriers (GA+AA genotypes) had a 3.23 higher odds of being grouped as a low-dose requiring category compared to non-carriers (95% CI 1.49–6.98, $p = 0.003$).

Conclusions: These preliminary results strongly support the use of *VKORC1* (−1639G>A) rs9923231 polymorphism for genetically guided initial warfarin dosing in South Indian patients with heart valve replacements.

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1. Introduction

Genotypic variations in the *VKORC1* gene contribute significantly to warfarin sensitivity among patients.[1,2] Inherent differences in allele frequencies have revealed varying dose requirements for warfarin across populations.[3] Various studies have reported significant geographic differentiation in the observed allele frequencies for the *VKORC1* and *CYP2C9* gene polymorphisms from Asian populations, and distinct Indian subpopulations have also shown a great degree of pharmacogenetic variations in the *VKORC1, CYP2C9,* and *CYP4F2* genes.[4] In this context, subpopulation-specific replication studies designed to capture the genotypic distribution of pharmacogenetic variants are essential to assist clinicians in optimizing dose regimes. Moreover, incorporating the ethnic background of the patient as an additional variable has been shown to significantly improve the predictability of anticoagulant dosing algorithms.[5]

The polymorphisms in the *VKORC1* gene explain up to 25–50% of the variance in anticoagulant dose.[6–8] The strong association between haplotypes of the *VKORC1* gene polymorphisms and warfarin dose phenotypes are distinguished by the tag-single nucleotide polymorphism (SNP) rs9923231 present in the promoter of the gene. At the transcriptional level, the A allele of the

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rs9923231 SNP has been found to be associated with a 70% reduction of *VKORC1* mRNA levels compared to the G allele, by abolishing an E-box consensus sequence in the promoter region.[9] The Clinical and Pharmacogenetic Consortium guidelines provided varying levels of evidence linking the *VKORC1* gene polymorphisms to warfarin phenotypes.[10] A high level of evidence shows that *VKORC1* is the protein target of warfarin, where the *VKORC1*-16339G>A variant and SNPs in high linkage disequilibrium with it are associated with reduced warfarin maintenance dose. They report clinical studies that show that with empirical dosing, *VKORC1*-16339G>A carriers require shorter time to achieving first international normalized ratio (INR) in therapeutic range but have no difference in time to stable dose. Other evidences include that *VKORC1*-16339G>A carriers have less time in therapeutic range early in the course of therapy and are associated with increased risk of over-anticoagulation (INR>4) in Caucasians and Asians but not in African Americans. Johnson et al, also provided moderate level evidence to suggest that with empiric warfarin dosing and INR monitoring, individuals with *VKORC1* –1639G>A are not at increased risk for major or minor bleeding event.[10]

A few studies from South Indian states have published data regarding the *VKORC1* (–1639G>A) rs9923231 polymorphism and its effect on daily warfarin dose requirement.[11–16] Meanwhile, other studies on South Indian populations have also reported allele and genotypic frequencies without accessing association with dosage requirements.[17–20] Comparative studies on North Indian populations have reported conflicting allele and genotype frequencies possibly due to varied sample sizes.[19,21,22] At present, there are no reports on the *VKORC1* (–1639G>A) rs9923231 polymorphism and its association with warfarin dose requirements exclusively from Kerala, the southernmost state of India with a predominant Dravidian population. Therefore, the aims of this study were to determine the frequency of the *VKORC1* (–1639G>A) rs9923231 polymorphism in the South Indian population of Kerala and to assess variation of mean daily dose among carriers of the three genotypes. The incremental information sought in this study was to test the ability of the *VKORC1* (–1639G>A) rs9923231 polymorphism to discriminate among the patients belonging to high-, intermediate-, and low-dose categories and to compute their genotype-based predicted doses.

2. Materials and methods

2.1. Patient sample

A total of 222 patients having a stable therapeutic INR were recruited from the INR clinic of the Cardiology Department, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Trivandrum, Kerala. Stable maintenance dose was conservatively defined as the average daily dosage that consistency yielded a minimum of three consecutive INR results within therapeutic range of 2.5–3.5 over previous 3 visits, over at least a 3 month period. Patients aged above 18 years who needed anticoagulation with warfarin after prosthetic mitral valve replacement with tilting disc valve for rheumatic heart disease and having normal prosthetic valve function were included in this study. Patients with renal dysfunction, hepatic dysfunction, and patients on other anticoagulants such as Dindevan (phenindione) were excluded from the study. At initiation of anticoagulant therapy, patients were advised to maintain a diet that kept vitamin K at a moderate yet constant level and were given food charts that excluded green leafy vegetables. Diet was not perceived as major risk factor that influenced dose in the Keralite population because more than 95% of the population are non-vegetarians, as reported in a recent cross-sectional study by Harikrishnan et al, 2018.[23] All participants belonged to the Malayalam-speaking South Indian population of Kerala. The study was approved by the Institutional Ethics Committee of the SCTIMST and conformed to the guidelines set by the Declaration of Helsinki.

2.2. DNA isolation and polymerase chain reaction–restriction fragment length polymorphism

Genomic DNA was isolated from three milliliters of whole blood samples taken from patients after obtaining written informed consent, using the modified rapid method.[24] A polymerase chain reaction (PCR)–restriction fragment length polymorphism method was designed to identify the *VKORC1* –1639G>A (rs9923231) SNP. A 15 μl PCR reaction mixture was prepared to amplify 100 ng gDNA mixed with 1 μl 10× Taq buffer, 200 μM dNTPs (Invitrogen), 250 μM $MgCl_2$, 0.5U Taq polymerase (NEB), and 10 μM each of forward and reverse primers. PCR cycling conditions consisted of an initial denaturation for 2 min at 95 °C, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 45 s, and extension at 72 °C for 45 s, with a 10 min final extension at 72 °C, done using Biorad MJ Mini™ thermal cycler. Human-specific primers were *VKORC1-F*, 5′-TTGCTGCCCACGCCATAAACTA, and *VKORC1-R*, 5′-ATCACAGACGCCAGAGGAAGAG, designed using the Primer Premier v.5 software.

The 496-bp PCR product was digested with *Nci*I restriction enzyme, according to manufacturer's protocol (NEB, Inc., USA). Products were separated on 2.5% agarose gel alongside a 100 bp DNA ladder and stained with ethidium bromide for visualization (Fig. 1). Scoring of the AA homozygous genotype was done by observing the presence of a single uncut band of size 496 base pairs (bp). The GG homozygous genotype was scored by two bands of 392 and 104 bp, and the GA heterozygous genotype by the presence of three bands of sizes 496, 392, and 104 bp. Approximately, 10% of the samples were reanalyzed to ascertain 100% concordance in genotyping. The genotypes inferred from the *Nci*I digestion patterns were further confirmed by direct sequencing using ABI PRISM 3730 Genetic Analyser (Applied Biosystems), as shown in Fig. 2. The sequence traces were queried for sequence similarity using the BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.3. Statistical analysis

Deviation of allelic frequencies from Hardy–Weinberg equilibrium was examined using the Pearson's chi-square test implemented in the FINETTI program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). The mean INR-based daily dosage of anticoagulant required by

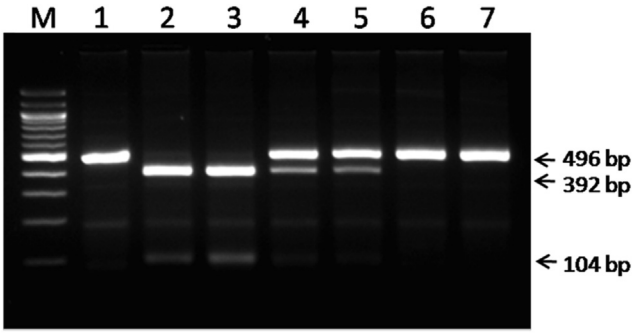

Fig. 1. *Nci*I RFLP analysis of the *VKORC1* (–1639G>A) polymorphism. Lane 1 is an undigested PCR product as control, lanes 2 and 3 show patient samples with GG homozygous genotype, lanes 4 and 5 show patient samples with GA heterozygous genotype, and lanes 6 and 7 show patient samples with AA homozygous genotype. M: 100 bp DNA ladder.

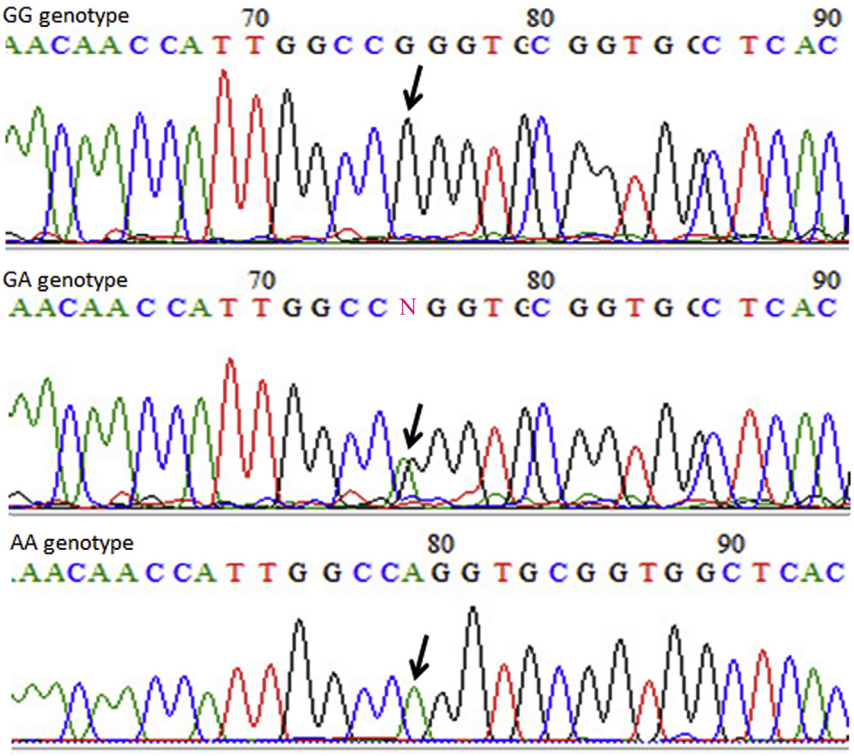

Fig. 2. Representative sequence traces showing GG homozygous, GA heterozygous and AA homozygous genotypes of the *VKORC1* (−1639G>A) polymorphism.

the genotypes of the *VKORC1* (−1639G>A) polymorphism was estimated using a linear regression analysis. Two models were analyzed; the first unadjusted model had only the genotype variables, and the second model was adjusted for age, sex, body mass index, hypothyroidism, and amiodarone. Negative regression coefficients corresponded to the reduced amount of warfarin dose required in the presence of the variant genotypes compared to the GG genotype used as the reference. The genotype-based predicted daily doses were predicted using the regression coefficient from the second regression model. Student *t* test was used to compare means of the prothrombin time (PT)-INR–based and genotype-based predicted daily doses. The variation in mean daily dose among patients carrying the three different genotypes GG, GA, and AA of the *VKORC1* (−1639G>A) rs9923231 polymorphism were compared using one-way analysis of variance test. To compare the standard deviations of the PT-INR–based and genotype-based predicted doses among the genotypes, a scale-free relative measure of variability, such as the coefficient of variation was calculated as standard deviation (±SD) divided by the mean.

Ordered logit regression analysis was used to evaluate the predictive value of *VKORC1* (−1639G>A) rs9923231 polymorphism in dose categorization of the patients. To measure the predictive ability of the genotypes to segregate the study cohort into any of the three dose categories, the therapeutic dose estimates were categorized as high-dose (≥49 mg per week), intermediate-dose (>21 and < 49 mg per week), and low-dose (≤21 mg per week) groups. This was in accordance with the International Warfarin Pharmacogenetics Consortium, 2009 and recoded as 1, 2, and 3, respectively.[1] Two models were used to calculate the unadjusted odds and the odds ratios adjusted for age, sex, body mass index, hypothyroidism, and amiodarone. All analyses were performed using the statistical software STATA, version 13.0, (Stata Corp, TX). The $p < 0.05$ was considered statistically significant.

3. Results

The study group comprised of 112 females (50.4%) with a mean age of 48.3 ± 10.8 years and 110 males (49.6%) with a mean age of 48.8 ± 12.6 years. Twenty-one patients had adverse events such as bleeding (9.5%) during the 1-year follow-up period, with no mortalities. Atrial fibrillation was present in 99 patients (44.6%). Other risk factors included smoking (8.1%), systemic hypertension (9.5%), dyslipidemia (7.2%), and diabetes mellitus (5.4%). Concomitant medications included digoxin (33.3%), aspirin (17.1%), clopidogrel (3.2%), and amiodarone (4.5%).

The frequency of the GG, GA, and AA genotypes of *VKORC1* (−1639G>A) rs9923231 polymorphism among the 222 patients were 83.33%, 14.86%, and 1.8%, respectively (Table 1). The allele frequency of the G allele was 90.77% and that of the A allele was 9.23%, with no significant deviation from Hardy–Weinberg equilibrium ($p = 0.10$). The association of the *VKORC1* (−1639G>A) genotypes with the required PT-INR–based daily dose is shown in Table 2. Analyses of both models showed that the GA genotype and the combined GA+AA genotypes required significantly lower dose compared to the GG genotype (adjusted model, 1.15 mg/day and 1.37 mg/day less, respectively, $p < 0.01$).

Among the three genotypes, the mean PT-INR–based and the genotype-based predicted daily dose were significantly different, $p = 0.0029$ and $p < 0.0001$, respectively (Table 3). The GG genotype

Table 1
Genotype and allele frequencies of *VKORC1* (−1639G>A) rs9923231 polymorphism in South Indian patients.

Genotypes, n = 222	Frequency (n)%	Allele	Frequency (n)%
GG	185 (83.33)	G	403 (90.77)
GA	33 (14.86)	A	41 (9.23)
AA	4 (1.80)		
Total	222		

Table 2
Association of *VKORC1* (−1639G>A) genotypes with the required INR-based daily dose in 222 patients.

VKORC1 −1639G>A, Genotypes	Model 1	95% CI	p-value	Model 2	95% CI	p-value
GG	Ref			Ref		
GA	−1.23	−2.01 to −0.49	**0.001**	−1.15	−1.93 to −0.37	**0.004**
AA	−1.52	−3.56 to 0.53	0.145	−1.33	−3.38 to 0.73	0.205
GA+AA	−1.28	−2.01 to −0.55	**0.001**	−1.17	−1.91 to −0.43	**0.002**

$p < 0.05$ is significant, indicated in bold.
Model 1: unadjusted for other independent variables.
Model 2: adjusted for age, BMI, sex, hypothyroidism, and amiodarone.

Table 3
Comparison of the mean PT-INR–based and genotype-based predicted daily dose among *VKORC1* (−1639G>A) genotypes.

Genotype	n = 222	PT-INR–based daily dose	CV[b]	Genotype-based predicted daily dose	CV[b]
	Frequency	Mean mg (±SD)		Mean mg (±SD)	
GG	185	5.51 ± 2.13	0.39	5.51 ± 0.33	0.06
GA	33	4.26 ± 1.57	0.37	4.24 ± 0.38	0.09
AA	4	3.99 ± 1.67	0.42	4.15 ± 0.31	0.07
[a]p-value		**0.0029**		**<0.0001**	

[a] One-way ANOVA test, $p < 0.05$ is significant, indicated in bold.
[b] Coefficient of variation (CV = SD/mean).

carriers required a PT-INR–based daily dose of 5.51 mg/day compared to the GA and AA carriers which required a daily dose of 4.26 and 3.99 mg/day, respectively. The overall mean of the PT-INR–based daily dose was similar to the predicted genotype-based predicted daily dose with 5.38 ± 2.34 mg/day and 5.38 ± 0.66 mg/day, respectively ($p = 0.50$). However, among all three genotypes, the PT-INR–based daily dose showed greater variation in standard deviations compared to the genotype-based predicted daily dose, as indicated by the higher coefficients of variation (Table 3).

Table 4 shows the proportional odds ratios and 95% confidence intervals of the ordered logit regression analyses. Each model shows the proportional odds ratio of a patient carrying a variant genotype, either GA or AA or grouped as GA+AA (dominant genetic model), of being in a low dose category versus the combined intermediate and high dose. For GA genotype carriers in model 2, the adjusted odds of being in the low warfarin dose category versus the intermediate- and high-dose categories were 2.89 times higher than for GG genotype carries ($p = 0.01$). Likewise, for the AA genotype carriers being in the low-dose category versus the intermediate and high warfarin dose categories were 8.06 times higher than for GG genotype carries ($p = 0.039$). When GA and AA genotypes are grouped as in a dominant genetic model, the adjusted odds of them being in the low versus the combined intermediate and high–dose category was 3.23 times higher compared to GG genotype carriers ($p = 0.003$).

Among the 21 patients who had bleeding, 15 carried the GG genotype associated with high dose, whereas only 4 and 2 patients had the GA and AA genotypes associated with low dose, respectively. Comparison of the prevalence of genotypes among patients with and without bleeding showed that patients who carried the GA or AA genotypes were not at an increased risk for a bleeding event ($p = 0.124$). None of these 21 patients were on amiodarone, and none had clinical hypothyroidism.

4. Discussion

The *VKORC1* gene is considered the most important individual predictor of warfarin dose.[25] Initially, D'Andrea et al had identified the *VKORC1* (1173C>T) rs9934438 polymorphism in intron 1 to correlate with higher warfarin dose requirement.[26] A subsequent study in Asian patients by Yuan et al found the *VKORC1* (−1639G>A) rs9923231 promoter polymorphism as a novel candidate, which was replicated simultaneously by other studies that included additional SNPs or by complete mapping of all SNPs in the *VKORC1* gene.[9,27] In a later study by Wadelius et al, 29 genes in the warfarin pharmacological pathway were tested for association using approximately 900 SNPs in 201 patients of North European descent.[7] They found the *VKORC1* as the single gene most strongly associated with warfarin dose. Further high-density mapping of the *VKORC1* region on chromosome 16 revealed that three SNPs (rs2359612, rs9934438, and rs9923231) were in strong linkage disequilibrium (LD) and accounted for approximately 30% of the warfarin dose variance.

The present study estimated the frequency of the high dose–associated G allele at 0.908 (90.8%), while the low dose–associated A allele frequency was 0.092 (9.2%) in a cohort of 222

Table 4
Proportional odds ratios of *VKORC1* (−1639G>A) A allele carriers of being in the low-dose category (≤21 mg per week) versus the combined categories of intermediate (>21 and < 49 mg per week) and high dose (≥49 mg per week).

VKORC1 −1639G>A, Genotypes	Model 1	95% CI	p-value	Model 2	95% CI	p-value
GG	Ref			Ref		
GA	3.03	1.37–6.69	**0.006**	2.89	1.29–6.45	**0.010**
AA	9.14	1.29–64.69	**0.027**	8.06	1.12–58.16	**0.039**
GA+AA	3.42	1.60–7.30	**0.001**	3.23	1.49–6.98	**0.003**

$p < 0.05$ is significant, indicated in bold.
Model 1: unadjusted for other independent variables;
Model 2: adjusted for age, BMI, sex, hypothyroidism, and amiodarone.

patients belonging to the South Indian population from Kerala. This was well within the allele frequency range reported from other South Indian studies. Other significant findings revealed a distinct variation of mean warfarin daily dose among the GG, GA, and AA genotype carriers, with the GA and AA genotype carriers requiring a lower dose relative to the GG genotype carriers. The presence of the low dose–associated A allele when present either as heterozygous or homozygous genotypes showed a statistically significant capacity to discriminate patients belonging to high-, intermediate-, and low-dose categories. This underscored the predictive value of *VKORC1* (−1639G>A) rs9923231 genotyping for clinical use. We also found that the mean genotype-based predicted daily dosage was similar and with lesser standard deviation compared to the PT-INR–based daily dosage. This could imply that the fluctuations in warfarin dosing in individual patients can be reduced if using a genetically guided dosing strategy. We also observed that patients with the *VKORC1*-1639G>A allele were not at an increased risk for a bleeding event, which was consistent with the evidences gathered in the Clinical and Pharmacogenetic Consortium guidelines by Johnson et al, 2017.[10]

Despite the unusual allele frequency distribution observed worldwide, the *VKORC1* gene along with *CYP2C9* has been found to consistently affect warfarin dose across populations. The extensive geographic differentiation of rs9923231 SNP is notable, in that the A allele is almost absent in African populations, whereas it shows extremely high frequencies in East Asian populations, indicative of positive selection among East Asians.[25] The high level of geographic differentiation along with its significant effect on warfarin drug dosing makes it an important gene to be studied in detail. In the Indian context, following the first study by Rathore et al, there are 25 reports that have measured the association of genetic polymorphisms with various different outcomes such as warfarin or acenocoumarol dose requirement, risk of adverse events, or reported the frequency of variant alleles (Supplementary Table 1).[4,11–22,28–39] Since the present work was designed as a pilot study, the effects of variants in the *CYP2C9* and *CYP4F2* genes were not studied and may be perceived as a limitation of the study. The impetus to genotype the *VKORC1* (−1639G>A) polymorphisms was due its direct correlation with warfarin dose requirement and relatively higher frequency reported in the South Indian population. The average frequency of the high dose–associated G allele ranged from 0.760 to 0.913, while the frequency of the low dose–associated A allele ranged from 0.087 to 0.240.[11–16,19,20,29] Though the presence of *CYP2C9* *2 and *3 alleles influences the metabolism of warfarin, genotyping these variants was not initiated in the present study, owing to their low frequency reported in nine studies from the South Indian population. Review of all the South Indian studies conducted to date showed that the *CYP2C9* *2 and *3 alleles are less polymorphic than the *VKORC1* (−1639G>A) polymorphism. The average minor allele frequency (MAF) of the *2 allele ranged from 0.006 to 0.088 and that of the *3 allele ranged from 0.028 to 0.094.[11–16,19,20,29] Subsequent studies that would include the *CYP2C9* and *CYP4F2* genes, and additional environmental risk factors, conducted with larger sample sizes could provide a more comprehensive model to predict individual dose requirement for the South Indian population.

It is also worth mentioning that interstudy differences in the association of genetic variants with the anticoagulant dose requirement have been observed. For instance, Rathore et al found distinct frequencies of *CYP4F2* 1347 G>A and *GGCX* 12970 C>G polymorphisms in 225 North Indian patients (MAF, 43.6% and 0.03%, respectively).[36,40] They reported that these polymorphisms did not have a significant influence on the maintenance dose of acenocoumarol oral anticoagulant in cardiac valve replacement patients. On the contrary, Krishna Kumar et al reported that the *CYP4F2* rs2108622 and *GGCX* rs11676382 polymorphisms were important predictive factors of warfarin for 240 patients of South Indian ethnicity (MAF, 34.2% and 1.0%, respectively).[14] This indicates that fundamental differences in allele and haplotype frequencies leading to distinct differences in linkage disequilibrium patterns in Indian subpopulations may explain variability in warfarin dose requirements. Kumar et al had exemplified this by studying the haplotype structure and pair-wise LD pattern of the eight *VKORC1* SNPs identified in the Tamil South Indian population.[17] A strong LD pattern (D'>0.8) was observed between seven out of eight SNPs which included *VKORC1* (−1639G>A) rs9923231 in the promoter region and *VKORC1* (1173C>T) rs9934438 in intron 1. Hence, identifying genetic polymorphisms that are predominant or exclusive to Indian subpopulations through resequencing, and construction of LD maps could significantly increase the proportion of variability that can be explained for warfarin dose requirements.

Analyzing genetic polymorphisms will help in predicting the average dose requirement in patients after a valve replacement. This will help in reducing the time to titration to the target dose and reduce the hospital stay and the number of INR checks required to attain the target dose. This reduces costs and is important in a developing country like India where accessibility to INR checking facilities and affordability is a major issue.

Conflicts of interest

All authors have none to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ihj.2018.07.006.

References

1. International Warfarin Pharmacogenetics Consortium, 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.
2. Limdi NA, Wadelius M, Cavallari L, et al. Warfarin pharmacogenetics: a single VKORC1 polymorphism is predictive of dose across 3 racial groups. *Blood*. 2010;115:3827–3834.
3. Tang W, Shi QP, Ding F, et al. Impact of VKORC1 gene polymorphisms on warfarin maintenance dosage: a novel systematic review and meta-analysis of 53 studies. *Int J Clin Pharmacol Therapeut*. 2017;55:304–321.
4. Giri AK, Khan NM, Grover S, et al. Genetic epidemiology of pharmacogenetic variations in CYP2C9, CYP4F2 and VKORC1 genes associated with warfarin dosage in the Indian population. *Pharmacogenomics*. 2014;15:1337–1354.
5. Hernandez W, Gamazon ER, Aquino-Michaels K, et al. Ethnicity-specific pharmacogenetics: the case of warfarin in African Americans. *Pharmacogenomics J*. 2014;14:223–228.
6. 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.
7. Wadelius M, Chen LY, Eriksson N, et al. Association of warfarin dose with genes involved in its action and metabolism. *Hum Genet*. 2006;121:23–34.
8. 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.
9. Yuan HY, Chen JJ, Lee MT, 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.
10. Johnson JA, Caudle KE, Gong L, et al. Clinical pharmacogenetics Implementation Consortium (CPIC) guideline for pharmacogenetics-guided warfarin dosing: 2017 Update. *Clin Pharmacol Ther*. 2017;102:397–404.
11. 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*. 2012a;13:869–878.

12. Pavani A, Naushad SM, Rupasree Y, et al. Optimization of warfarin dose by population-specific pharmacogenomic algorithm. *Pharmacogenomics J*. 2012b;12:306–311.
13. Madhan S, Kumar DK, Kumar DT, et al. Effect of CYP2C9 and VKORC1 genetic polymorphisms on warfarin dose requirement in South Indian population. *Indian J Physiol Pharmacol*. 2013;57:308–317.
14. Krishna Kumar D, Shewade DG, Loriot MA, et al. Effect of CYP2C9, VKORC1, CYP4F2 and GGCX genetic variants on warfarin maintenance dose and explicating a new pharmacogenetic algorithm in South Indian population. *Eur J Clin Pharmacol*. 2014;70:47–56.
15. Krishna Kumar D, Shewade DG, Loriot MA, et al. An acenocoumarol dosing algorithm exploiting clinical and genetic factors in South Indian (Dravidian) population. *Eur J Clin Pharmacol*. 2015;71:173–181.
16. Kalpana SR, Bharath G, Manjunath CN, et al. Influence of VKORC1 and CYP2C9 polymorphisms on daily acenocoumarol dose requirement in South indian patients with mechanical heart valves. *Clin Appl Thromb Hemost*. 2016;23:876–882.
17. Kumar DK, Shewade DG, Surendiran A, et al. Genetic variation and haplotype structure of the gene Vitamin K epoxide reductase complex, subunit 1 in the Tamilian population. *J Pharmacol Pharmacother*. 2013a;4, 53-58.0.
18. Kumar DK, Shewade DG, Manjunath S, et al. Inter and intra ethnic variation of vitamin K epoxide reductase complex and cytochrome P450 4F2 genetic polymorphisms and their prevalence in South Indian population. *Indian J Hum Genet*. 2013b;19:301–310.
19. Nahar R, Deb R, Saxena R, et al. Variability in CYP2C9 allele frequency: a pilot study of its predicted impact on warfarin response among healthy South and North Indians. *Pharmacol Rep*. 2013;65:187–194.
20. Mahadevan L, Yesudas A, Sajesh PK, et al. Prevalence of genetic variants associated with cardiovascular disease risk and drug response in the Southern Indian population of Kerala. *Indian J Hum Genet*. 2014;20:175–184.
21. Rathore SS, Agarwal SK, Pande S, et al. The impact of VKORC1-1639 G>A polymorphism on the maintenance dose of oral anticoagulants for thromboembolic prophylaxis in North India: a pilot study. *Indian J Hum Genet*. 2011;17(Suppl 1):S54–S57.
22. Kaur A, Khan F, Agrawal SS, et al. Cytochrome P450 (CYP2C9*2,*3) & vitamin-K epoxide reductase complex (VKORC1 -1639G<A) gene polymorphisms & their effect on acenocoumarol dose in patients with mechanical heart valve replacement. *Indian J Med Res*. 2013;137:203–209.
23. Harikrishnan S, Sarma S, Sanjay G, et al. Prevalence of metabolic syndrome and its risk factors in Kerala, South India: analysis of a community based cross-sectional study. *PLoS One*. 2018;27(13):e0192372.
24. Koshy L, Anju AL, Harikrishnan S, et al. Evaluating genomic DNA extraction methods from human whole blood using endpoint and real-time PCR assays. *Mol Biol Rep*. 2017;44:97–108.
25. Ross KA, Bigham AW, Edwards M, et al. Worldwide allele frequency distribution of four polymorphisms associated with warfarin dose requirements. *J Hum Genet*. 2010;55:582–589.
26. D'Andrea G, D'Ambrosio RL, Di PP, et al. A polymorphism in the VKORC1 gene is associated with an interindividual variability in the dose-anticoagulant effect of warfarin. *Blood*. 2005;105:645–649.
27. Geisen C, Watzka M, Sittinger K, et al. VKORC1 haplotypes and their impact on the inter-individual and inter-ethnical variability of oral anticoagulation. *Thromb Haemost*. 2005;94:773–779.
28. 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.
29. Arunkumar G, Vishnuprabu D, Nupur B, et al. Genotyping of CYP2C9 and VKORC1 polymorphisms predicts south Indian patients with deep vein thrombosis as fast metabolizers of warfarin/acenocoumarin. *Drug Discov Ther*. 2017;11:198–205.
30. Biswas M, Bendkhale S, Deshpande SP, et al. Association between genetic polymorphisms of CYP2C9 and VKORC1 and safety and efficacy of Warfarin: results of a 5 years Audit. *Indian Heart J*; 2018. https://doi.org/10.1016/j.ihj.2018.02.005.
31. Gaikwad T, Ghosh K, Kulkarni B, et al. Influence of CYP2C9 and VKORC1 gene polymorphisms on warfarin dosage, over anticoagulation and other adverse outcomes in Indian population. *Eur J Pharmacol*. 2013;710:80–84.
32. Gaikwad T, Ghosh K, Avery P, et al. Warfarin dose model for the prediction of stable maintenance dose in indian patients. *Clin Appl Thromb Hemost*. 2018;24:353–359.
33. Kumar S, Dubey AK, Kalita J, et al. The Role of clinical variables and VKORC1 polymorphism in efficacy and Stability of acenocoumarol in Neurological patients. *J Neurosci Rural Pract*. 2018;9:186–192.
34. Nahar R, Saxena R, Deb R, et al. CYP2C9, VKORC1, CYP4F2, ABCB1 and F5 variants: influence on quality of long-term anticoagulation. *Pharmacol Rep*. 2014;66:243–249.
35. Natarajan S, Ponde CK, Rajani RM, et al. Effect of CYP2C9 and VKORC1 genetic variations on warfarin dose requirements in Indian patients. *Pharmacol Rep*. 2013;65:1375–1382.
36. Rathore SS, Agarwal SK, Pande S, et al. Therapeutic dosing of acenocoumarol: proposal of a population specific pharmacogenetic dosing algorithm and its validation in north Indians. *PLoS One*. 2012;7:e37844.
37. Sehgal T, Hira JK, Ahluwalia J, et al. High prevalence of VKORC1*3 (G9041A) genetic polymorphism in North Indians: a study on patients with cardiac disorders on acenocoumarol. *Drug Discov Ther*. 2015;9:404–410.
38. Shalia KK, Doshi SM, Parikh S, et al. Prevalence of VKORC1 and CYP2C9 gene polymorphisms in Indian population and its effect on warfarin response. *J Assoc Physicians India*. 2012;60:34–38.
39. Sridharan K, Modi T, Bendkhale S, et al. Association of genetic polymorphisms of CYP2C9 and VKORC1 with bleeding following warfarin: a case-control study. *Curr Clin Pharmacol*. 2016;11:62–68.
40. Rathore SS, Agarwal SK, Pande S, et al. CYP4F2 1347 G>A & GGCX 12970 C>G polymorphisms: frequency in North Indians & their effect on dosing of acenocoumarol oral anticoagulant. *Indian J Med Res*. 2014;139:572–578.