

Inter and intra ethnic variation of vitamin K epoxide reductase complex and cytochrome P450 4F2 genetic polymorphisms and their prevalence in South Indian population

Dhakchinamoorthi Krishna Kumar, Deepak Gopal Shewade, Sajjanavar Manjunath¹, Prayaga Ushakiran², Gangadharan Reneega³, Chandrasekaran Adithan

Department of Pharmacology, Indian Council of Medical Research Centre for Advance Research in Pharmacogenomics, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry, ¹Department of Pharmacology, M.R Medical College, Gulbarga, Karnataka, ²Department of Pharmacology, Rangaraya Medical College, Kakinada, Andhra Pradesh, ³Department of Pharmacology, Thiruvananthapuram Medical College, Thiruvananthapuram, Kerala, India

BACKGROUND: Genetic variation in the vitamin K epoxide reductase complex (*VKORC1*) and cytochrome P450 4F2 (*CYP4F2*) genes were found to be strongly associated with the oral anticoagulant (OA) dose requirement. The distribution of genetic variation in these two genes was found to show large inter- and intra-ethnic difference.

MATERIALS AND METHODS: A total of 470 unrelated, healthy volunteers of South Indians of either sex (age: 18-60 years) were enrolled for the study. A 5 ml of venous blood was collected and the genomic deoxyribonucleic acid (DNA) was extracted by using phenol-chloroform extraction method. Real-time quantitative polymerase chain reaction (RT-PCR) method was used for genotyping.

RESULTS: The variant allele frequencies of *VKORC1* *rs2359612* (T), *rs8050894* (C), *rs9934438* (T) and *rs9923231* (A) were found to be 11.0%, 11.8%, 11.7% and 12.0%, respectively. The variant allele *VKORC1* *rs7294* was (80.1%) more frequent and the variant allele *CYP4F2* * 3 was found to be 41.8% in South Indians. The allele, genotype and haplotype frequencies of *VKORC1* and *CYP4F2* gene were distinct from other compared HapMap populations ($P < 0.0001$).

CONCLUSION: The findings of our study provide the basic genetic information for further pharmacogenetic based investigation of OA therapy in the population.

Key words: Cytochrome P450 4F2, haplotypes, oral anticoagulants, South Indians, vitamin K epoxide reductase complex

Introduction

Vitamin K epoxide reductase (VKOR) enzyme converts vitamin K (VK) 2, 3-epoxide to VK hydroquinone, a required cofactor for the post-translational gamma-carboxylation of several blood coagulation factors (factors II, VII, IX, X, Protein C and S) and other VK-dependent proteins, such as osteocalcin bone Gla protein and matrix Gla protein.^[1-3] The gamma- glutamyl carboxylase enzyme requires reduced VK as a cofactor in the clotting pathway. Oral anticoagulants (OAs) (warfarin, acenocoumarol and phenprocoumon) inhibit the regeneration of reduced VK by targeting the enzyme VKOR and there by blocks the clotting mechanism.^[1,4] The inter-individual variability in OA dose is influenced by the genes of two enzymes, cytochrome P450 (CYP) 2C9, the enzyme that metabolizes the OAs and vitamin K epoxide reductase complex (*VKORC1*), the pharmacological target enzyme of those drugs. The genetic variation in the *CYP2C9* gene (in particular, the common *CYP2C9**2 and *CYP2C9**3 alleles) was a result in decreased

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Address for correspondence: Dr. Dhakchinamoorthi Krishna Kumar, Department of Pharmacology, Indian Council of Medical Research Centre for Advance Research in Pharmacogenomics, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry - 605 006, India. E-mail: krishnakumarx@hotmail.com

enzyme activity and affects the pharmacokinetics of OA and its dose requirement. The variants in the VKORC1 gene influence the pharmacodynamics response to OAs.^[5-7] Polymorphisms in the *VKORC1* gene encoding the VKOR enzyme leads to inter individual variability in susceptibility to develop bleeding and other adverse reactions to oral anti-coagulants.^[8,9] The impact of *VKORC1* genetic variation on warfarin dose requirement confirmed that the genetic polymorphisms of VKORC1 are significant determinants of inter- individual dose requirement. It has been found that genetic variations in the *CYP2C9* and *VKORC1* genes together account for 35-50% variability in the dose requirement for initiation and maintenance of OAs.^[5,10,11] There is an evidence that specific haplotypes can help to determine the dose of OAs. Single nucleotide polymorphisms (SNPs) found in haplotype A (3730G > A, 2255C > T, 1542G > C, 1173 C > T and – 1639 G > A) versus wild type alleles in haplotype B can be used to determine whether the person require low, intermediate or high dose of OAs.^[12]

CYP4F2 genetic polymorphism (rs2108622) was found to be associated with reduced hepatic CYP4F2 enzyme activity and higher levels of VK. The presence of this variant allele is associated with a higher warfarin dose requirement.^[13-15] *CYP4F2* functions as a VK mono-oxidase, probably generating the ω -hydroxy derivative of the substrate. Carriers of the *CYP4F2* 433M allele have a reduced capacity to metabolize VK, secondary to a *CYP4F2* dependent decrease in the steady state hepatic concentrations of the enzyme. Therefore, patients with the *CYP4F2**3 polymorphism are likely to have an elevated hepatic level of VK and thus are likely to require a higher warfarin dose to prompt the expected anticoagulant response.^[16]

South Indians constitute about 21.7% of the total population of India and residing at four states (Tamil Nadu, Andhra Pradesh, Kerala and Karnataka) (<http://www.census.india.govt.in/2011census>, accessed on August, 2011). This population shares a common ancestry (Dravidians), but the present day population distinguishes themselves from one another in terms of language, culture and dietary habits with limited admixture. Reich et al.^[17] found that the ancestral South Indians were distinct from the ancestral North Indians and East Indians.

In our previous studies, we have found that the polymorphic profile of the genes encoding some of the Phase I and Phase II drug metabolizing enzymes (CYP2E1, CYP2A6, CYP3A5, CYP2C9, CYP2C19, CYP2D6, thiopurine methyl transferase and uridine diphosphate-glucoronyl transferases) as well as drug transporters (multidrug resistance 1 and organic cation transporters 1) were significantly different between South Indians and other population groups.^[18-23] These findings raise the possibility that the frequency of variants of other genes including the OA dose determining *VKORC1* and *CYP4F2* genes may be different in our population. This is the first study to establish the haplotype structure of the *VKORC1* and genotype/allele frequencies of the *CYP4F2* in India, in particular for South Indians. In this study, we have also compared our data with those of other population groups included in the HapMap project (<http://hapmap.ncbi.nlm.nih.gov/>).

Materials and Methods

Study subjects

The present study included 470 unrelated healthy volunteers from South Indian states (Tamil Nadu, Andhra Pradesh, Kerala and Karnataka) of both genders, aged between 18 and 60 years with a family history of three generations in South India and speaking any one of the South Indian languages. Blood samples were collected from Tamilians ($n = 110$), Andhrites ($n = 120$), Kannadigas ($n = 120$) and Keralites ($n = 120$). The Institute ethics committee approved the study and was conducted according to declaration of Helsinki. Written informed consent was obtained from all volunteers.

Genotyping procedure

A volume of 5 ml of venous blood was collected using sodium ethylene diamine tetra acetic acid as anticoagulant. Deoxyribonucleic acid (DNA) was extracted by using a standard phenol: chloroform protocol. Genotyping of *VKORC1* and *CYP4F2* was carried out by real-time thermo cycler (7300 Applied Biosystems; Life Technologies Corporation, Carlsbad, CA, USA) using TaqMan SNP genotyping assays (Assay ID for *VKORC1* C__7473918_10, rs7294, C__26291751_10, rs2359612, C__2847860_10, rs8050894, C__30204875_10,

rs9934438, C__30996661_30, rs9923231 and assay ID for *CYP4F2* C_16179493_40, rs2108622). The polymerase chain reaction (PCR) was carried out in duplicate in a 20- μ L final volume that contained 10 μ L of TaqMan universal PCR master mix (2x), 0.5 μ L of 20x working stock of SNP genotyping assay and 4.5 μ L of genomic DNA diluted in DNAase free water and 5 μ L of Milli-Q water (Millipore Corporate Headquarters, Billerica, MA, USA). The thermocycler conditions included one cycle at 50°C for 2 min; one cycle at 95°C for 10 min to activate the AmpliTaq Gold polymerase followed by 40 cycles of denaturation at 92°C for 15 s and annealing/extension at 60°C for 1 min. The allelic discrimination was performed using 7300 SDS software version 1.4 (Applied Biosystems; Life Technologies Corporation, Carlsbad, CA, USA).

Statistical analysis

Statistical analysis was performed using the GraphPad InStat 3 software (GraphPad Software Inc., San Diego, CA, USA). Hardy – Weinberg equilibrium was tested by Chi-square test to compare the observed genotype frequencies of South Indian population with the expected genotype frequencies calculated from the observed allele frequencies. Chi-square test was used for comparisons of two different ethnic groups. $P < 0.05$ was considered as statistically significant. Pairwise linkage disequilibrium (LD) pattern and haplotype frequencies were estimated using HAPLOVIEW 4.1^[24] (Daly Lab, Broad Institute, Cambridge, MA02141, USA). All SNPs with minor allele frequencies of 0.01% were excluded and minimum haplotype frequency was set as 1%. Haplotype blocks were defined by using four gametes rule incorporated by analysis in HAPLOVIEW software. Haplotypes were estimated by accelerated expectation-maximization algorithm in HAPLOVIEW. The confidence interval range for LD was set between 0.7 and 0.98. D' values from 0.7 to 1.0 indicates strong LD between the pair of SNPs. Whereas, D' value < 0.7 indicates moderate LD and D' value of < 0.2 indicates no LD.

Results

Among the 470 (245 men, 225 women; age $[\pm SD]$ 27.0 \pm 7.9 years) healthy volunteers, 445 samples genotyping was successful and included in further

analysis. The frequency distribution of *VKORC1* and *CYP4F2* genotypes and allele in four South Indian populations was found to be in Hardy – Weinberg equilibrium (4×3 contingency table; rs7294- $\chi^2 = 11.52$, $P = 0.0734$, rs2359612- $\chi^2 = 12.34$, $P = 0.0547$, rs8050894- $\chi^2 = 9.85$, $P = 0.1308$, rs9934438- $\chi^2 = 7.48$, $P = 0.2781$, rs9923231- $\chi^2 = 8.12$, $P = 0.2295$, rs2108622, $\chi^2 = 5.845$, $P = 0.4408$).

The overall allele and genotype frequency [Tables 1a, b and 2] was also calculated. The genotype and allele frequency of *VKORC1* and *CYP4F2* in South Indians was compared with HapMap populations (African ancestry in Southwest USA, Utah residents with Northern and Western European, Han Chinese in Beijing [CHB], Chinese in Metropolitan Denver, Gujarati Indians in Houston [GIH], Texas, Japanese in Tokyo [JPT], Japan, Luhya in Webuye, Kenya, Mexican ancestry in Los Angeles, California, Maasai in Kinyawa, Kenya, Tuscans in Italy, Yoruba in Ibadan [YRI], Nigeria).

The haplotype frequencies of *VKORC1* in South Indians were compared with major HapMap populations (namely African American, Caucasians, CHB and GIH). Data was obtained from PharmGKB database [Table 3]. The Haplotype structure and LD pattern of *VKORC1* in South Indian was compared with the HapMap population [Figure 1].

In South Indian population, a strong LD pattern ($D' > 0.8$) was observed between all the SNPs except the LD pattern between rs8050894 and rs9934438 only moderate LD ($D' < 0.8$) was observed. In Africans (YRI), a very strong LD pattern ($D' = 1$) was observed between rs7294 and rs2359612, rs2359612 and rs9934438, rs2359612 and rs9923231, rs7294 and rs9923231 rs9934438 and rs9923231, rs7294 and rs9934438. Only low LD ($D' < 0.5$) was observed between rs2359612 and rs8050894. In Caucasians, all the SNPs were in strong LD ($D' > 0.8$). In CHB and JPT strong LD pattern ($D' = 1$) was observed between all the SNPs. In GIH for rs9923231 genotype data was not available for many samples, but strong LD pattern ($D' > 0.8$) was observed between other SNPs. In the four South Indian populations, the LD pattern was observed. In Tamilians only moderate LD pattern ($D' < 0.8$ to > 0.5) was observed between rs7294 and rs9934438, rs8050894 and rs9934438, rs9934438 and

Table 1a: Comparison of genotype and allele frequencies of VKORC1 gene in SI and other HapMap population. Comparison of rs7294 and rs2359612

SNP	Population (number of subjects)	Genotype frequency % (95% CI)			Allele frequency % (95% CI)	
		GG	GA	AA	G	A
VKORC1 rs7294	Tamilians (105)	1.9 (0.5-6.6)	24.8 (17.4-33.8)	73.3 (64.2-80.9)	13.3 (8.7-17.9)	85.7 (81.0-90.4)
	Andhrites (113)	4.4 (1.9-9.9)	33.6 (25.6-42.8)	62.0 (52.7-70.4)	21.2 (15.9-26.6)	78.8 (73.4-84.1)
	Kannadigas (113)	8.8 (4.9-15.5) ^a	35.4 (27.2-44.6) ^a	55.8 (46.6-64.6) ^a	26.5 (20.8-32.3)	73.5 (73.4-84.0)
	Keralites (144)	3.5 (1.4-8.7)	35.1 (26.9-44.2)	61.4 (52.2-69.8)	21.1 (15.8-26.3)	78.9 (73.2-83.7)
	SI (445)	4.7 (3.1-7.1)	30.3 (26.2-34.8)	64.9 (60.4-69.2)	20.0 (17.4-22.6)	80.1 (77.4-82.6)
	CEU (226)	39.8 (33.7-46.3)	47.8 (41.4-54.3)	12.4 (8.7-17.3)	63.7 (59.2-68.0)	36.3 (31.2-0.8)
	HCB (86)	88.4 (79.9-93.6)	11.6 (6.4-20.1)	-	94.2 (89.6-96.8)	5.8 (3.2-10.4)
	JPT (170)	80.0 (73.4-85.3)	20.0 (14.7-26.7)	-	90.0 (86.4-92.8)	10.0 (7.2-13.7)
	YRI (226)	23.9 (18.8-29.9)	45.1 (38.8-51.7)	31.0 (25.3-37.3)	46.5 (41.9-1.0)	53.5 (48.9-58.0)
	ASW (98)	22.4 (15.3-31.7)	49.0 (39.3-58.7)	28.6 (20.6-38.2)	46.9 (40.1-3.9)	53.1 (46.1-59.9)
	CHD (168)	85.7 (79.6-90.2)	14.3 (9.8-20.4)	-	92.9 (89.6-5.2)	7.1 (4.8-10.4)
	GIH (176)	3.4 (1.6-7.2)	55.7 (48.3-62.8)	40.9 (33.9-48.3)	31.2 (26.6-6.2)	68.8 (63.7-73.3)
	LWK (180)	28.9 (22.8-35.9)	55.3 (48.2-62.6)	17.8 (12.8-24.0)	55.6 (50.4-0.6)	44.4 (39.4-49.6)
	MEX (100)	44.0 (34.7-53.8)	46.0 (36.6-55.7)	10.0 (5.5-17.4)	67.0 (60.2-73.1)	33.0 (26.9-39.8)
	MKK (284)	27.5 (22.6-32.9)	43.0 (37.3-48.8)	29.6 (24.6-35.1)	48.9 (44.9-3.0)	51.1 (46.9-55.1)
	TSI (176)	40.9 (33.9-48.3)	51.1 (43.8-58.4)	8.0 (4.8-12.9)	66.5 (61.4-1.2)	33.5 (28.8-38.6)
VKORC1 rs2359612		CC	CT	TT	C	T
	Tamilians (105)	85.7 (77.8-91.2)	20.0 (13.8-28.7)	-	92.9 (88.5-96.0)	7.1 (4.4-11.5)
	Andhrites (113)	79.7 (71.3-86.0)	23.1 (16.2-31.5)	-	89.8 (85.9-93.8)	10.2 (6.8-14.8)
	Kannadigas (113)	71.7(62.8-79.2) ^b	28.3 (20.8-37.2) ^b	-	85.8 (81.3-90.4)	14.2 (10.2-19.3)
	Keralites (144)	76.3 (67.7-83.2)	21.1 (14.6-29.4)	2.6 (0.9-7.5)	87.7 (83.5-92.0)	12.3 (8.6-17.2)
	SI (445)	78.4 (74.3-82.0)	21.1 (17.6-25.2)	0.5 (0.1-0.6)	89.0 (86.8-90.9)	11.0 (9.1-13.2)
	CEU (226)	19.5 (14.8-25.1)	40.7 (34.5-47.2)	39.8 (33.7-46.3)	39.8 (35.4-44.2)	60.2 (55.6-64.6)
	HCB (86)	88.4 (79.9-93.6)	11.6 (6.4-20.1)	-	94.2 (89.6-96.8)	5.8 (3.1-10.4)
	JPT (172)	80.2 (73.7-85.5)	19.8 (14.5-26.4)	-	90.1 (86.5-92.8)	9.9 (7.2-13.5)
	YRI (226)	0.9 (0.2-3.2)	28.3 (22.9-34.5)	70.8 (64.6-76.3)	15.0 (12.0-18.6)	85.0 (81.3-87.9)
	ASW (98)	6.1 (2.8-12.7)	24.5 (17.0-33.9)	69.4 (59.7-77.6)	18.4 (13.6-24.4)	81.6 (75.6-86.4)
	CHD (170)	85.9 (79.8-90.3)	14.1(9.7-20.1)	-	92.9 (89.7-95.2)	7.1 (4.8-10.3)
	GIH (176)	1.1 (0.-4.0)	36.4 (29.6-43.7)	62.5 (55.1-69.3)	19.3 (15.5-23.8)	80.7 (76.2-84.4)
	LWK (180)	3.3 (1.5-7.0)	30.0 (23.8-37.1)	66.7 (59.5-73.1)	18.3 (14.7-22.7)	81.7 (77.3-85.3)
	MEX (100)	22.0 (15.0-31.0)	48.0 (38.5-57.7)	30.0 (21.9-39.6)	46.0 (39.2-52.9)	54.0 (47.0-60.7)
	MKK (286)	7.0 (4.5-10.5)	31.5 (26.4-37.1)	61.5 (55.8-67.0)	22.7 (19.4-26.3)	77.3 (73.7-80.5)
	TSI (176)	22.7 (17.1-29.4)	50.0 (42.7-57.3)	27.3 (21.2-34.2)	47.7 (42.6-52.9)	52.3 (47.0-57.4)

* $P < 0.001$ Kannadigas versus Tamilians, ^a $P < 0.05$ Kannadigas versus Tamilians, No significant difference ($P > 0.05$) SI versus JPT (rs2359612, rs9934438), SI versus MKK (rs9923231), $P < 0.0001$ SI versus HapMap populations. VKORC1: Vitamin K epoxide reductase complex, SNP: Single nucleotide polymorphisms, CI: Confidence interval, SI: South Indian, CEU: Utah residents with Northern and Western European, HCB: Han Chinese in Beijing, JPT: Japanese in Tokyo, YRI: Yoruba in Ibadan, ASW: African ancestry in Southwest USA, CHD: Chinese in Metropolitan Denver, GIH: Gujarati Indians in Houston, LWK: Luhya in Webuye, Kenya, MEX: Mexican ancestry in Los Angeles, MKK: Maasai in Kinyawa, Kenya, TSI: Tuscans in Italy

rs9923231. In Andhrites, a moderate LD pattern ($D' < 0.8$) was observed between rs2359612 and rs8050894, rs8050894 and rs9934438, rs8050894 and rs9923231. Strong LD pattern was observed between other SNPs. In Kannadigas and Keralites, a strong LD pattern was observed between all the SNPs. Allele frequencies of most studied SNPs (*CYP2C9**2 [rs1799852], *CYP2C9**3 [rs1057910], VKORC1 [rs9923231, rs7294, rs9934438] and *CYP4F2* [rs2108622]) in South Indians and other HapMap populations were compared [Figure 2].

Discussion

The frequencies of genetic variations may vary between populations as highlighted by several reports from different populations and from the HapMap

projects.^[25,26] Hence, it is useful to have the population distribution profile of the genetic polymorphisms and LD pattern in the study population prior to genetic association studies. The present study is the first to report the genotype, allele and haplotype distribution and LD pattern of VKORC1 and CYP4F2 genes in South Indian population with a large sample size. A significant difference in genotype, allele and Haplotype distribution was observed between the present study groups and other populations.

It is well-documented that the two alleles *CYP2C9**2 (rs1799852) and *CYP2C9**3 (rs1057910) were significantly associated with decreased warfarin dose requirements (warfarin sensitivity) and higher susceptibility to overdose.^[27] A direct association of *CYP2C9* genotype and anticoagulation status

Table 1b: Comparison of genotype and allele frequencies of VKORC1 gene in SI and other HapMap population. Comparison of rs8050894, rs9934438 and rs9923231

SNP	Population (number of subjects)	Genotype frequency % (95% CI)			Allele frequency % (95% CI)	
		GG	GC	CC	G	C
<i>VKORC1</i> rs8050894	Tamilian (105)	80 (71.4-86.5)	20 (13.8-28.7)	-	90.0 (85.1-93.7)	10.0 (6.6-14.8)
	Andhrites (113)	76.9 (68.4-83.7)	23.1 (16.2-31.5)	-	87.2 (82.8-91.5)	12.8 (9.1-17.8)
	Kannadigas (113)	74.3 (65.6-81.5)	25.7 (18.5-34.4)	-	88.5 (84.3-92.7)	11.5 (7.9-16.3)
	Keralites (144)	76.3 (67.7-83.2)	21.1 (14.6-29.4)	2.6 (0.9-7.5)	86.8 (82.5-91.2)	13.2 (9.4-18.2)
	SI (445)	77.0 (72.9-80.7)	22.3 (18.6-26.3)	0.7 (0.2-2.0)	88.2 (85.9-90.2)	11.8 (9.8-14.1)
	CEU (120)	45.0 (36.4-53.9)	35.0 (27.1-43.9)	20.0 (13.8-28.0)	62.5 (56.2-68.4)	37.5 (31.6-43.8)
	HCB (90)	-	11.1 (6.2-19.3)	88.9 (80.7-93.8)	5.6 (3.0-9.9)	94.4 (90.0-96.9)
	JPT (88)	-	20.5 (13.3-30.0)	79.5 (69.9-86.6)	10.2 (6.5-15.5)	89.8 (84.4-93.4)
		CC	CT	TT	C	T
<i>VKORC1</i> rs9934438	Tamilian (105)	82.9 (74.5-88.9)	17.1 (11.1-25.5)	-	91.4 (86.8-94.8)	8.6 (5.5-13.1)
	Andhrites (113)	77.9 (69.4-84.5)	22.1 (15.5-30.6)	-	88.9 (84.8-93.0)	11.1 (7.6-15.8)
	Kannadigas (113)	72.5 (63.8-79.9)	24.8 (17.7-33.5)	2.6 (0.9-7.5)	85.0 (80.3-89.6)	15.0 (10.9-20.3)
	Keralites (144)	77.2 (68.7-83.9)	21.1 (14.6-29.4)	1.8 (0.5-6.2)	87.7 (83.5-92.0)	12.3 (8.6-17.2)
	SI (445)	77.8 (73.7-81.4)	21.1 (17.6-25.2)	1.1 (0.5-2.6)	88.3 (86.0-90.1)	11.7 (9.7-14.0)
	CEU (226)	19.5 (14.8-25.1)	40.7 (34.5-47.2)	39.8 (33.6-46.3)	39.8 (35.4-44.4)	60.2 (55.6-64.6)
	HCB (86)	88.4 (79.9-93.5)	11.6 (6.4-20.1)	-	94.2 (89.6-96.8)	5.8 (3.2-10.4)
	JPT (172)	80.2 (73.6-85.5)	19.8 (14.5-26.4)	-	90.1 (85.0-91.7)	9.9 (7.0-13.3)
	YRI (226)	-	4.4 (2.4-7.9)	95.6 (92.0-97.6)	2.2 (1.2-4.0)	97.8 (95.9-98.7)
	ASW (98)	6.1 (2.8-12.7)	8.2 (4.2-15.3)	85.7 (77.4-91.3)	10.2 (6.7-15.2)	89.8 (84.7-93.3)
	CHD (170)	86.9 (81.2-91.3)	13.1 (8.7-18.8)	-	93.5 (90.4-95.7)	6.5 (4.3-9.6)
	GIH (176)	1.1 (0.3-4.0)	36.4 (29.6-43.7)	62.5 (55.1-69.3)	19.3 (15.5-23.7)	80.7 (76.2-84.4)
	LWK (180)	-	12.2 (8.2-17.8)	87.8 (82.2-91.8)	6.1 (4.0-9.0)	93.9 (90.9-95.9)
	MEX (100)	22.0 (15.0-31.0)	48.0 (38.4-57.6)	30.0 (21.9-39.6)	46.0 (39.2-52.9)	54.0 (47.0-60.7)
	MKK (286)	2.1 (0.9-4.5)	28.0 (23.0-33.4)	69.9 (64.4-74.9)	16.1 (13.3-19.3)	83.9 (80.7-86.7)
	TSI (176)	22.7 (17.1-29.5)	50.0 (42.7-57.3)	27.3 (21.2-34.3)	47.7 (42.6-52.9)	52.3 (47.0-57.4)
		GG	GA	AA	G	A
<i>VKORC1</i> rs9923231	Tamilian (105)	83.8 (75.6-89.6)	16.1 (10.4-24.4)	-	91.9 (87.4-97.2)	8.1 (5.1-12.6)
	Andhrites (113)	76.9 (68.4-83.8)	23.1 (16.2-31.6)	-	88.5 (84.3-92.7)	11.5 (7.9-16.3)
	Kannadigas (113)	73.4 (64.6-80.7)	23.9 (16.9-32.5)	2.7 (0.9-7.5)	85.4 (80.8-90.0)	14.6 (10.5-19.8)
	Keralites (144)	73.7 (64.9-80.9)	23.7 (16.8-32.3)	1.8 (0.5-6.2)	86.4 (82.0-90.9)	13.6 (9.8-18.6)
	SI (445)	77.1 (72.9-80.7)	21.8 (18.2-25.9)	1.1 (0.5-2.6)	88.0 (85.7-90.0)	12.0 (10.1-14.3)
	CEU (226)	39.8 (33.6-46.3)	40.7 (34.5-47.2)	19.5 (14.8-25.1)	60.2 (55.6-64.5)	39.8 (35.4-44.4)
	HCB (86)	-	11.6 (6.4-20.1)	88.4 (79.9-93.5)	5.8 (3.1-10.4)	94.2 (89.6-96.8)
	JPT (172)	-	19.8 (14.5-26.4)	80.2 (73.7-85.5)	9.9 (7.1-13.5)	90.1 (86.5-92.8)
	YRI (226)	95.6 (92.0-97.5)	4.4 (2.4-7.9)	-	97.8 (95.9-98.8)	2.2 (1.2-4.0)
	ASW (98)	85.7 (72.4-91.3)	8.2 (4.1-15.3)	6.1 (2.8-12.7)	89.8 (84.7-93.3)	10.2 (6.7-15.2)
	CHD (170)	-	14.1 (9.6-20.1)	85.9 (79.9-90.3)	7.1 (4.8-10.3)	92.9 (89.7-95.2)
	GIH (176)	62.5 (55.1-69.3)	36.4 (29.6-43.7)	1.1 (0.3-4.0)	80.7 (76.2-84.5)	19.3 (15.5-23.8)
	LWK (180)	87.8 (82.1-91.8)	12.2 (8.2-17.8)	-	93.9 (90.9-95.9)	6.1 (4.0-9.0)
	MEX (100)	30.0 (21.9-39.6)	48.0 (38.4-57.7)	22.0 (15.0-31.0)	54.0 (47.0-60.7)	46.0 (39.2-52.9)
	MKK (286)	69.9 (64.4-75.0)	28.0 (23.1-33.4)	2.1 (0.9-4.5)	83.9 (80.6-86.7)	16.1 (13.3-19.3)
	TSI (176)	27.3 (21.2-34.3)	50.0 (42.7-57.3)	22.7 (17.1-29.5)	52.3 (47.0-57.4)	47.7 (42.6-52.9)

* $P < 0.001$ Kannadigas versus Tamilians, * $P < 0.05$ Kannadigas versus Tamilians, No significant difference ($P > 0.05$) SI versus JPT (*rs2359612*, *rs9934438*), SI versus MKK (*rs9923231*), $P < 0.0001$ SI versus HapMap populations. *VKORC1*: Vitamin K epoxide reductase complex, SNP: Single nucleotide polymorphisms, CI: Confidence interval, SI: South Indian, CEU: Utah residents with Northern and Western European, HCB: Han Chinese in Beijing, JPT: Japanese in Tokyo, YRI: Yoruba in Ibadan, ASW: African ancestry in Southwest USA, CHD: Chinese in Metropolitan Denver, GIH: Gujarati Indians in Houston, LWK: Luhya in Webuye, Kenya, MEX: Mexican ancestry in Los Angeles, MKK: Maasai in Kinyawa, Kenya, TSI: Tuscans in Italy

and bleeding was first reported by Higashi *et al.*^[28] Furthermore, the allele frequencies of *CYP2C9**2 and *CYP2C9**3 diverge considerably among different ethnic groups.^[29,30] In our previous study, we have established the genotype and allele frequency of *CYP2C9**2 and *CYP2C9**3 in South Indian population.^[19]

In the present study, we have focused on the *VKORC1* and *CYP4F2* genes. Apart from SNPs in *CYP2C9* gene and SNPs in the *VKORC1* has been also correlated with warfarin dose requirement.^[12,31] The rs9934438 and rs9923231 polymorphisms are most commonly

studied and associated with a lower warfarin dose requirement.^[32-34] Another common SNP, the 3730 G > A (rs7294), located in the 3' untranslated region of the gene, is associated with increased warfarin dose requirements (warfarin resistance).^[12,35] In our study, we found that the rs7294 variant was highly frequent (80.1%).

Rieder *et al.*^[12] conducted the haplotype analysis and established a significant contribution of *VKORC1* to inter individual variability in warfarin dose. The ten most common SNPs (at positions 381, 861, 2653, 3673, 5808, 6009, 6484, 6853, 7566 and 9041 of the

Table 2: Comparison of genotype and allele frequencies of CYP4F2 gene in SI and other HapMap population

SNP	Population (number of subjects)	Genotype frequency % (95% CI)			Allele frequency % (95% CI)	
		GG	GA	AA	G	A
CYP4F2 rs2108622	Tamilian (105)	25.2 (17.9-34.2)	51.4 (42.1-60.6)	21.5 (14.8-30.2)	51.9 (45.1-58.7)	48.1 (41.4-54.8)
	Andhriles (113)	34.5 (26.4-43.6)	50.4 (41.4-59.5)	15.1 (9.6-22.8)	59.7 (53.3-66.1)	40.3 (34.1-46.8)
	Kannadigas (113)	38.9 (30.5-48.2)	44.3 (35.4-53.4)	16.8 (11.0-24.8)	62.1 (55.6-68.3)	38.9 (32.8-45.5)
	Keralites (144)	34.2 (26.1-43.3)	50.9 (41.8-59.9)	14.9 (9.5-22.5)	59.6 (53.3-66.0)	40.4 (34.2-46.8)
	SI (445)	33.5 (29.3-37.9)	49.4 (44.8-54.1)	17.1 (13.9-20.9)	58.2 (54.9-61.4)	41.8 (38.6-45.1)
	CEU (224)	60.7 (54.2-66.9)	32.1 (26.4-38.5)	7.1 (4.4-11.3)	76.8 (72.7-80.5)	23.2 (19.5-27.3)
	HCB (84)	54.8 (44.1-64.9)	40.5 (30.6-51.2)	4.8 (1.9-11.6)	75.0 (67.9-80.9)	25.0 (19.1-32.1)
	JPT (170)	63.5 (56.1-70.4)	25.9 (19.9-32.9)	10.5 (6.8-16.1)	76.4 (71.7-80.7)	23.5 (19.3-28.3)
	YRI (226)	89.4 (84.7-92.8)	9.7 (6.5-14.3)	0.8 (0.2-3.2)	94.3 (91.7-96.0)	5.8 (3.9-8.2)
	ASW (98)	81.6 (72.8-88.1)	18.4 (11.9-27.2)	-	90.8 (85.9-94.1)	9.2 (5.9-14.1)
	CHD (170)	60.0 (52.5-67.1)	36.5 (29.6-43.9)	3.5 (1.6-7.5)	78.2 (73.5-82.3)	21.8 (17.7-26.5)
	GIH (176)	32.9 (26.4-40.2)	47.7 (40.5-55.1)	19.3 (14.2-25.8)	56.8 (51.6-61.9)	43.2 (38.1-48.4)
	LWK (180)	81.8 (75.5-86.8)	19.3 (14.2-25.8)	1.1 (0.3-3.9)	89.4 (85.8-92.2)	10.6 (7.8-14.2)
	MEX (98)	55.1 (45.3-64.6)	44.9 (35.4-54.8)	-	77.6 (71.2-82.8)	22.5 (17.2-28.8)
	MKK (286)	61.5 (55.8-66.9)	35.6 (30.3-41.4)	2.8 (1.4-5.4)	79.4 (75.9-82.5)	20.6 (17.5-24.1)
	TSI (176)	45.5 (38.3-52.8)	39.8 (32.8-47.2)	14.8 (10.3-20.8)	65.3 (60.2-70.1)	34.7 (29.9-39.8)

No significant differences observed SI versus GIH (*rs2108622*), SI versus HapMap populations $P < 0.0001$. CYP4F2: Cytochrome P450 4F2, SNP: Single nucleotide polymorphisms, CI: Confidence interval, SI: South Indian, CEU: Utah residents with Northern and Western European, HCB: Han Chinese in Beijing, JPT: Japanese in Tokyo, YRI: Yoruba in Ibadan, ASW: African ancestry in Southwest USA, CHD: Chinese in Metropolitan Denver, GIH: Gujarati Indians in Houston, LWK: Luhya in Webuye, Kenya, MEX: Mexican ancestry in Los Angeles, MKK: Maasai in Kinyawa, Kenya, TSI: Tuscans in Italy

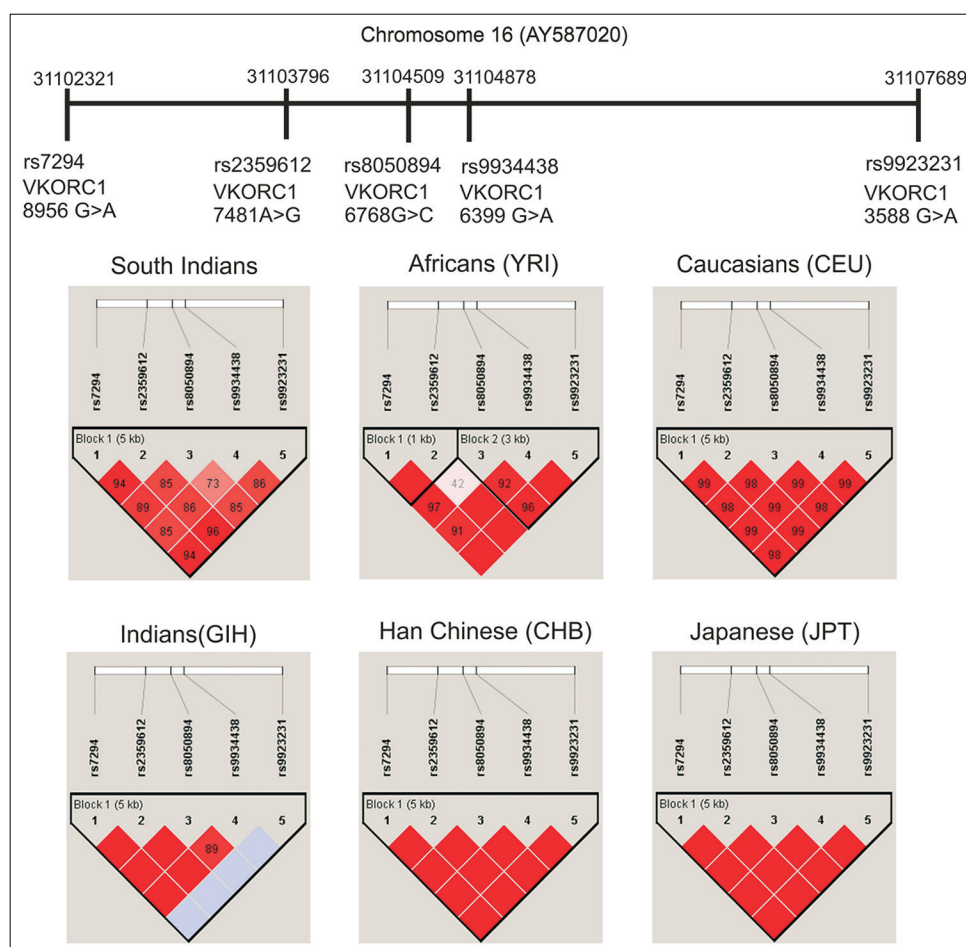


Figure 1: Linkage disequilibrium pattern of vitamin K epoxide reductase complex genetic variants in south Indians and other HapMap populations (The single nucleotide polymorphisms in Chromosome 16 were positioned according to the order and orientation. Each of the variants is given with its specific chromosomal position and the rsID. Red and pink colors represent a very strong LD pattern ($D' > 0.8$) and white color represents moderate to low LD ($D' < 0.8$ to > 0.5). The blue color in Indian (GIH) indicates missing genotype data)

Table 3: Haplotype frequencies (% [95% CI]) of VKORC1 gene in South Indians and other major HapMap population

Haplotype	rs7294	rs2359612	rs8050894	rs9934438	rs9923231	South Indians (n=445)	Africans (YRI) ^a (n=462)	Caucasian (CEU) ^a (n=3239)	Han Chinese (CHB) ^a (n=250)	Japanese (JPT) ^a (n=828)	Gujarati Indians in Houston ^b (n=43)
H1	A	C	G	C	G	75.6 (71.3-79.3)	43.7 (39.3-48.3)	38.3 (36.7-40.0)	11.5 (8.2-16.2)	8.7 (7.0-10.8)	80.9 (67.4-90.26)
H2	G	T	C	T	A	8.6 (6.3-11.5)	10.1 (7.7-13.3)	36.7 (35.1-38.4)	88.0 (83.4-91.5)	91.3 (89.2-93.0)	10.9 (5.1-24.5)
H3	G	C	G	C	G	8.0 (5.9-11.0)	16.8 (13.7-20.6)	23.3 (21.9-24.8)	0.5 (0-2.2)	-	2.3 (0.4-12.0)
H4	A	C	C	T	G	1.1 (0.4-2.6)	-	-	-	-	2.3 (0.4-12.0)
H5	G	C	G	C	G	0.6 (0.2-1.9)	19.0 (15.7-22.9)	0.9 (0.6-1.2)	-	-	-
H6	G	T	C	C	G	-	9.6 (7.2-12.5)	0.2 (0-0.4)	-	-	-
H7	A	T	C	T	A	0.1 (0.04-1.2)	0.2 (0.4-1.2)	0.2 (0-0.4)	-	-	-
H8	G	T	G	T	A	-	0.2 (0.4-1.2)	0.1 (0-0.2)	-	-	-
H9	G	C	G	C	A	0.6 (0.2-1.9)	-	0.1 (0-0.2)	-	-	-
H10	G	T	C	T	G	-	-	0.1 (0-0.2)	-	-	-
H11	G	C	C	T	A	0.4 (0.1-1.6)	-	-	-	-	-
H12	G	C	G	T	A	0.5 (0.2-1.9)	-	-	-	-	-
H13	G	C	C	C	A	0.3 (0.1-1.6)	-	0.2 (0-0.4)	-	-	-
H14	A	C	C	C	A	0.4 (0.1-1.6)	-	-	-	-	-
H15	G	C	G	C	A	0.9 (0.4-2.3)	-	-	-	-	-
H16	A	C	C	C	G	0.6 (0.2-1.9)	-	-	-	-	-
H17	A	C	C	T	A	0.4 (0.1-1.6)	-	-	-	-	-
H18	A	T	G	C	A	0.3 (0.1-1.6)	-	-	-	-	-
H19	A	C	G	T	A	0.1 (0.04-1.2)	-	-	-	-	-
H20	G	T	G	T	A	1.0 (0.4-2.3)	-	-	-	-	-

^aSignificantly differed from South Indians $P < 0.0001$, ^bNot significantly differed from South Indians ($P = 0.26$). VKORC1: Vitamin K epoxide reductase complex, CI: Confidence interval, YRI: Yoruba in Ibadan, Nigeria, CEU: Utah residents with Northern and Western European, CHB: Han Chinese in Beijing, JPT: Japanese in Tokyo

VKORC1 reference sequence [GenBank accession number AY587020]) were used to construct the five major haplotypes and the association of these haplotypes were observed in Caucasian patients. A low dose haplotype group A and high dose haplotype group B were identified. These haplotype frequencies also vary among the different ethnic populations. Haplotype A is more frequent in Asians (89%); whereas in Caucasians, haplotype B is more frequent (58%).^[12]

The previous pharmacogenetics studies have provided the indications that the South Indian population may have distinct polymorphic distribution features regarding the VKORC1 locus. In our study, we did not observe a significant difference in the genotyping frequencies of Tamilians, Kannadigas, Andhrites and Keralites. Only the genotype frequencies of *rs7294* and *rs2359612* in Kannadigas were significantly different from the Tamilians population. The pooled results of South Indians genotypes were compared with that of the HapMap population. We have observed a highly significant difference between the different ethnic population groups and South Indians. Only the Japanese population did not significantly differ for the SNPs *rs7294*, *rs2359612* and *rs9934438*.

The haplotype frequencies of the VKORC1 locus in South Indians were compared with those of the HapMap population (<http://hapmap.ncbi.nlm.nih.gov/>) by establishing the haplotype structure for Africans, Caucasians, Han Chinese, Japanese and Indian groups. The haplotype frequencies in South Indians were significantly different from Africans, Han Chinese and Japanese, but not from the GIH. The major Haplotype observed in our population is ACGCG (75.6%), but this haplotype was observed at different levels in Africans (43.7%), Caucasians (38.3%), Han Chinese (11.5%) and Japanese (8.7%), but close to that of GIH (80.9%). Thus, the present study reveals that the South Indians are anticipated to fall under the high dose requirement groups for the OAs.

A previous study has established the haplotype frequency (*rs7196161*, *rs17880887*, *rs9923231*, *rs2884737*, *rs9934438* and *rs17880624*) in the Malaysian Indians and found that the TCGTCA (H7) is more frequent in Indians as compared with Chinese and Malays. The

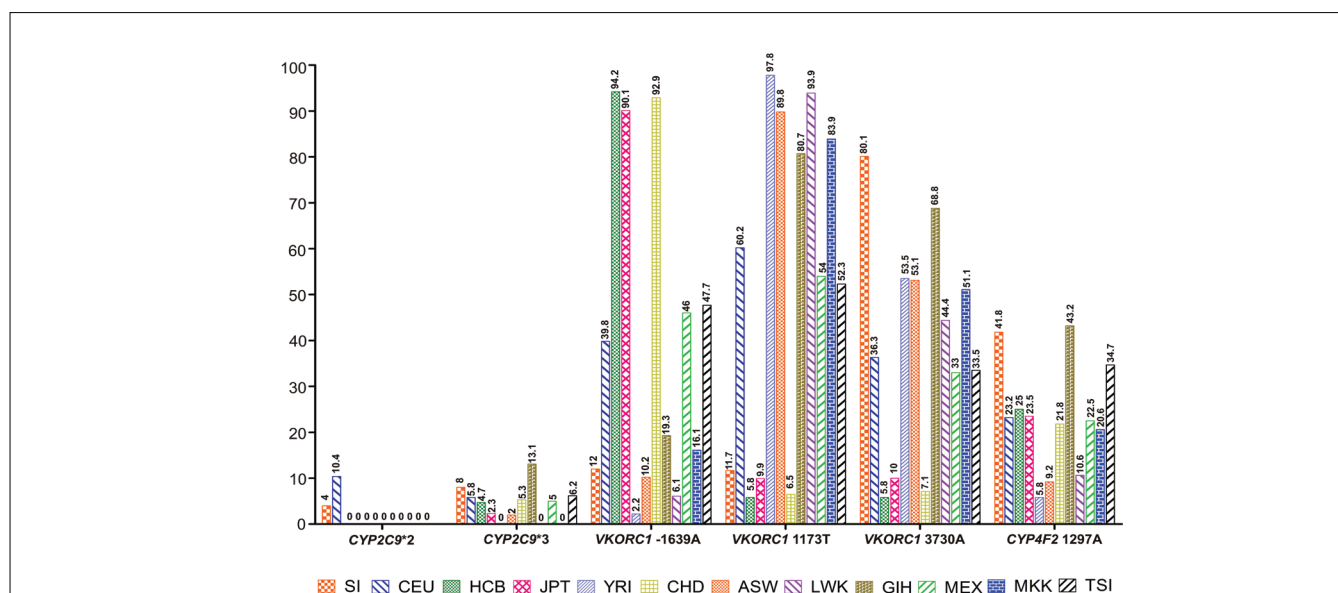


Figure 2: Allele frequencies of most studied single nucleotide polymorphisms (cytochrome P450 2C9 [CYP2C9]*2 [rs1799852], CYP2C9 * 3 [rs1057910], Vitamin K epoxide reductase complex [rs9923231, rs7294, rs9934438] and CYP4F2 [rs2108622]) genes in South Indians and other HapMap populations

H7 haplotype group is associated with the higher dose requirement.^[36] Our study on South Indians is in line with this observation.

In addition to the *CYP2C9* and *VKORC1* polymorphisms, a SNP in the *CYP4F2* gene has been shown to contribute to warfarin dose requirement, albeit to a lesser extent, through genome wide association studies.^[14] Another study has shown that patients with a variant allele namely *CYP4F2**3, require higher maintenance warfarin dose as compared with those with wild type allele in Han Chinese patients.^[33] In this study, we have established the frequency of *CYP4F2**3 allele and compared it with that of the HapMap populations. Both the allele and genotype frequencies significantly differed across these population groups with the exception of Gujarati Indians from Houston.

The United States Food and Drug Administration updated the label of warfarin twice: In 2007 advising physicians to consider the use of “genetic tests to improve their initial estimate” of the initial dosage, then in 2010 adding a new table with the range of expected therapeutic warfarin doses based on *CYP2C9* and *VKORC1* genotypes. Based on the evidences many studies have proposed the algorithms for calculating the maintenance dose and initial dose of OAs using the multivariate statistical techniques.^[11,37-44] However,

the proposed algorithms appear to be specific for each population group very likely due to differences in distribution of polymorphisms.

Thus, it is useful to establish the polymorphic profile of the study population/cohort before conducting pharmacogenetic studies on OAs. At this end, we have established the genotype, allele and haplotype frequencies of *VKORC1* and *CYP4F2* genotype and allele frequencies for the South Indian population.

Conclusion

The frequencies of the *VKORC1* and *CYP4F2* allele/genotype in the South Indian population were distinct from other world population groups. These results will not only contribute to the better understanding of the genetic basis of ethnic variation in OA dose requirement response, but also establishes the frame work for future algorithm based dose determination of OAs in Indians.

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