Original Article

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High prevalence of *VKORC1*3* (G9041A) genetic polymorphism in north Indians: A study on patients with cardiac disorders on acenocoumarol

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Summary **Coumarin derivatives such as warfarin and acenocoumarol are used in various disorders such as deep venous thrombosis, pulmonary embolism, atrial fibrillation and artificial heart valves. They have improved prognosis of patients with thromboembolic disease. An individual's response to coumarins depends on several factors. The non-genetic factors include age, gender, body mass index, diet and interacting drugs. Among the genetic factors, the cytochrome P450 system and vitamin K epoxide reductase complex subunit 1 play a key role in drug metabolism. This was a prospective hospital based study in which allele and genotypic frequencies of *CYP2C9* gene polymorphisms; 430C>T and 1075A>C and *VKORC1* gene polymorphisms; 1639G>A, 9041G>A and 6009C>T in 106 alleles of north Indian patients with valve replacement on acenocoumarol were determined and their effect on acenocoumarol dosing was studied. To the best of our knowledge, this is first report of *VKORC1* 9041G>A and 6009C>T gene polymorphisms and their effect on acenocoumarol dosing from north India. In 53 patients with valve replacement on acenocoumarol with stable INR, the allele frequency of *CYP2C9*2* and *CYP2C9*3* gene polymorphisms was 0.05 and 0.17 respectively and that of *VKORC1 *2,*3* and **4* gene polymorphisms was 0.15, 0.72 and 0.11 respectively. The presence of *CYP2C9*3* or *VKORC1*2* gene polymorphism were associated with decrease in acenocoumarol dose requirements (p values 0.03 and 0.02 respectively).This study confirmed the association of lower mean weekly dosages of acenocoumarol in patients with *CYP2C9*3* and *VKORC1*2* gene polymorphisms. An unusually high frequency of 9041A polymorphism in *VKORC1* was found in study population.**

Keywords: *CYP2C9*, *VKORC1*, acenocoumarol, dosage, INR, PCR-RFLP

1. Introduction

Both warfarin and acenocoumarol are used in various disorders such as deep venous thrombosis, pulmonary embolism, atrial fibrillation and artificial heart valves (*1,2*). They have improved the prognosis of patients with thromboembolic disease. An individual's response to coumarin derivatives depends on several factors. The non-genetic factors include age, gender, body mass index (BMI), diet and interacting drugs (*1*).

Among the genetic factors, the cytochrome P450 (CYP) system and vitamin K epoxide reductase complex subunit 1 (VKORC1) play a key role in the drug metabolism (*3*). The cytochrome P450s are a multigene family of enzymes found predominantly in the liver and are responsible for the metabolic elimination of most of the drugs. CYP2C9 is the second family of cytochrome P450 system. To date, 65 *CYP2C9* variant alleles have been reported (*4*). *CYP2C9*1* is the wild-type allele. There are two

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important single nucleotide polymorphisms (SNPs), the *CYP2C9*2* (C430T, exon 3) associated with a functionally important Arg144Cys substitution and the *CYP2C9*3* (A1075C, exon 7) associated with Ile359Leu substitution. Both variants are associated with a reduced enzymatic activity and hence a lower drug requirement (*5*). Acenocoumarol and warfarin are metabolized by CYP2C9 enzyme system. It has been shown that *CYP2C9* gene polymorphism contributes up to 15% variability in case of warfarin and about 5% in case of acenocoumarol (*2*).

VKORC1 is the target enzyme of oral anticoagulants. The inhibition of this enzyme reduces the regeneration of active form of vitamin K from vitamin K epoxide reductase (*6*). Many polymorphisms have been found both in the coding and the non-coding regions of the *VKORC1* gene. *VKORC1* −1639G>A is a polymorphism in the promoter region, *VKORC1* 9041G>A is a polymorphism in the 3'UTR region and *VKORC1* 6009 C>T variant is polymorphism in the intron- 1 region of *VKORC1* gene. The presence of these polymorphisms are known to contribute up to 30% in the dose requirements of warfarin and acenocoumarol (*3,6*).

There are a few studies from India about these polymorphisms and their effects on patients on long term oral anticoagulation (*7-11*). In this study, the allele and genotypic frequencies of two of the *CYP2C9* gene polymorphisms; 430C>T and 1075A>C and three of the *VKORC1* gene polymorphisms; 1639G>A, 9041G>A and 6009C>T in 106 alleles of north Indian patients with valve replacement on acenocoumarol were determined and their effect on acenocoumarol dosing was studied. To the best of our knowledge, this is the first report of *VKORC1* 9041G>A and 6009C>T gene polymorphisms and their effect on acenocoumarol dosing from north India.

2. Materials and Methods

2.1. *Study subjects*

This was a prospective hospital based study on fifty-three patients who attended the out-patient clinic of the Department of Cardiology over a period of 1 year from September 2013 to August 2014 and gave consent for the study and fulfilled the inclusion criteria. Institutional ethics committee approval was obtained prior to the study. Written informed consent was obtained from the patients participating in this study. The inclusion criteria comprised patients with prosthetic heart valves, between 18-65 years of age on anticoagulation treatment with acenocoumarol for prevention of thromboembolism. The mean daily maintenance dose (mg/day) of acenocoumarol was defined as "the dose of acenocoumarol for minimum of 3 months with two or more consecutive INR measurements done at least 7 days apart being within target range (2 to 3.5) to prevent thromboembolism" (*12*). Data on participants' age, height, weight, body mass index, medication history, INR values, and acenocoumarol dose was recorded. The exclusion criteria comprised concomitant therapy with drugs potentially interacting with acenocoumarol, liver or renal dysfunction, pregnant and lactating women, smokers, chronic alcoholics and patients on warfarin.

2.2. *INR testing*

INR testing was performed on peripheral blood on the fully automated STA-R Evolution coagulation analyzer on citrated blood a per the manufacturer's instruction. The reagent used to determine the PT had an ISI value of 0.9-1.1 (Diagnostica Stago (STA) Neoplastin R, Asnieres, France). The expected INR range was 2-3.5.

2.3. *PCR-RFLP*

DNA was isolated from two ml of EDTA venous blood using Midi-Kit method (QIAGEN amp DNA Midi Kit, California, USA) as per instruction provided by the manufacturer. PCR-RFLP was performed using primers and PCR conditions as described previously with modifications (given in supplementary material) (*13,14*).

2.4. *Statistical analysis*

Normality of quantitative data was checked by measures of Kolmogorov Smirnov tests of normality. The patient's data was normally distributed hence, discrete categorical data is presented as n (%) and continuous data is presented as mean ± standard deviation (SD) and the confidence intervals (CI) were calculated, as appropriate. The allele and genotype frequencies for *CYP2C9* and *VKORC1* gene polymorphisms were expressed as percentage (%) and CI. Categorical data for *e.g*. age and dose was compared by Chi-square or Fisher's exact test. All statistical tests were two-sided and performed at a significance level of $p = 0.05$. All analyses were performed using SPSS for Windows (version 17.0; SPSS Inc., Chicago, IL, USA). To find independent predictors of dose per week of acenocoumarol both multivariate regression analysis and bivariate logistic regression analysis were applied. Pearson's coefficient of regression was applied to analyze the relation of dose with different variables.

3. Results

A total of 53 patients from north India were enrolled in the study. All patients had undergone heart valve replacement surgery and were receiving regular oral anticoagulant therapy in the form of acenocoumarol. The primary indication for valve replacement was

rheumatic heart disease in 92% cases (49/53), the other indications were bicuspid aortic valve in 2 cases and degenerated aortic valve and dilated cardiomyopathy in 1 case each. Single heart valve replacement was seen in 81% cases (43/53) and double valve in 19% cases (10/53). The mean INR was 2.42 (range, 2-3) and the mean follow-up period was 3.35 years (range, 1-12 years). The mean dose of acenocoumarol was 17.5 mg/week. For assessing the association of drug dose in relation to the gene polymorphisms, the patients were classified based on mean dose of acenocoumarol into 2 arbitrary groups; low dose (≤ 17.5 mg/week) and high dose (> 17.5 mg/week) groups. The characteristics of patients with respect to dosage of acenocoumarol are given in Table1.

The allele and genotype frequencies of both *CYP2C9* and *VKORC1* gene polymorphisms were determined (Table 2). The allele frequencies were in Hardy-Weinberg equilibrium.

The mean weekly dose of acenocoumarol in mg/week was lower in a large proportion of the patients with the mutant allele for *CYP2C9*2* (C430T), *CYP2C9*3* (A1075C) and *VKORC1*2* (G1639A) gene polymorphisms when compared with those with wild type allele and the mean dose of acenocoumarol in mg/week was higher in patients with the mutant allele for *VKORC1*3* (G9041A) and *VKORC1*4* (C6009T) gene polymorphisms when compared with those with wild

Table 1. Characteristics of patients (*n* = 53) with respect to dosage of acenocoumarol

Parameters	Variables	Low dose of acenocoumarol (≤ 17.5 mg/week), *n* = 33	High dose of acenocoumarol (> 17.5mg/week), *n* = 20	*p* value
Age in years, mean ± SD	38.1 ± 13.3	38.3 ± 14	37.7 ± 12.2	0.88
Sex				
Males *n* (%)	29 (55)	18	9	0.9
Females *n* (%)	24 (45)	15	11	
Height in cm, mean ± SD	162.3 ± 9	161.9 ± 7.1	162.8 ± 11.7	0.7
Weight in kg, mean ± SD	60.2 ± 11.5	58.9 ± 11.0	62.30 ± 12.2	0.3
Body mass index in kg/m^2, mean ± SD	22.9 ± 4.2	22.5 ± 4.1	23.5 ± 4.3	0.6
Patients taking concomitant amiodarone, *n* (%)	8 (15.2)	7	1	*
Patients taking concomitant atorvastatin, *n* (%)	2 (3.8)	2	0	*

*small number of patients

Table 2. Allele and genotype frequencies of *CYP2C9* and *VKORC1* gene polymorphisms

CYP2C9 Gene Polymorphism	Alleles	Number of alleles (*n* = 106)	Allele frequency in %	95% Confidence Interval in %
*2 (C430T)	C	100	94.33	88-97
	T	06	05.67	2.6-12
*3 (A1075C)	A	88	83.01	75-89
	C	18	16.98	11-25
CYP2C9 Gene Polymorphism	Genotype	Number of subjects (*n* = 53)	Genotype frequency in %	95% Confidence Interval in %
*2 (C430T)#	CC	47	88.68	77-95
	CT	06	11.32	5.3-26
*3 (A1075C)	AA	36	67.92	55-79
	AC	16	30.18	19-44
	CC	01	01.88	3.3-9.9
VKORC1 Gene Polymorphism	Alleles	Number of alleles (*n* = 106)	Allele frequency in %	95% Confidence Interval in %
*2 (G1639A)	G	90	84.9	77-90
	A	16	15.1	9.5-23
*3 (G9041A)	G	30	28.3	21-38
	A	76	71.7	62-79
*4 (C6009T)	C	94	88.7	81-93
	T	12	11.3	6.6-19
VKORC1 Gene Polymorphism	Genotype	Number of subjects (*n* = 53)	Genotype frequency in %	95% Confidence Interval in %
*2 (G1639A)#	GG	37	69.81	56-80
	GA	16	30.19	19-44
*3 (G9041A)	GG	06	11.32	5.3-23
	GA	18	33.96	23-48
	AA	29	54.72	42-67
*4(C6009T)#	CC	41	77.36	64-86
	CT	12	22.64	13-36

#No mutant genotypes were found

Table 3. Association of *CYP2C9* and *VKORC1* gene polymorphisms with the likelihood of requiring a low (≤ 17.5mg/week) or a high dose (> 17.5mg/week) of acenocoumarol

CYP2C9 Gene Polymorphism	Genotype	Dose category in mg/week		*p* value
		Low dose, *n* = 33 (%)	High dose, *n* = 20 (%)	
*2 (C430T)	CC	28 (85)	19 (95)	0.28
	CT	05 (15)	01 (05)	
*3 (A1075C)	AA	20 (61)	16 (80)	0.19
	AC	12 (36)	04 (20)	
	CC	01 (03)	0	
VKORC1 Gene Polymorphism				
*2 (G1639A)	GG	20 (61)	17 (85)	0.07
	GA	13 (39)	03 (15)	
*3 (G9041A)	GG	06 (18)	0	0.09
	GA	09 (27)	09 (45)	
	AA	18 (55)	11 (55)	
*4 (C6009T)	CC	26 (79)	15 (75)	0.74
	CT	07 (21)	05 (25)	

type allele. However, the difference was not statistically significant (Table 3).

A haplotype analysis for each patient based on the individual's *CYP2C9* and *VKORC1* genotype was performed (Table 4). In the 106 alleles analysed for different *CYP2C9* polymorphisms, the most frequent haplotype observed was *1/*1 (56.6%) followed by *1/*3 (30.19%), *1/*2 (11.32%) and*3/*3 (1.88%). Homozygous mutant haplotype*3/*3 was found in one subject. The most frequent *VKORC1* gene haplotype was *3/*3 (54.7%) followed by *2/*3 (18.8%), *3/*4 (13.2%) and*2/*4 (9.4%). Haplotypes*1/*2 and *1/*3 were found in one subject each.

The most common combined haplotype for *CYP2C9* and *VKORC1* genes respectively was *1/*3 and*3/*3 respectively, seen in 23% (12/53) patients and the least common were haplotypes *1/*1 and *1/*3, *1/*2 and *1/*2, and *3/*3 and *3/*3 for *CYP2C9* and *VKORC1* genes respectively present in 1 patient each (Table 5).

Binary logistic regression analysis model revealed that both *CYP2C9*3*, and *VKORC1*2* (1639 G>A) gene polymorphisms contributed to the variability in low dose (≤ 17.5mg/week) of acenocoumarol, p value 0.038 and 0.025 respectively. Stepwise regression analysis model showed that *VKORC1*2* (1639 G>A) contributed to 7.6% to the mean dose variation of acenocoumarol (r^2 = 0.076, p = 0.025). Among the non-genetic factors, it was found that the dose requirements fell with increasing age however, the difference was not statistically significant. Significant dose differences were not seen with respect to the other non-genetic factors including age, gender and BMI.

Table 4. Distribution of various haplotypes of *CYP2C9* and *VKORC1* gene polymorphisms

Gene Polymorphism	Haplotype	*n* (%)
CYP2C9[#]	*1/*1	30 (56.6)
(C430T, A1075C)	*1/*2	6 (11.3)
	*1/*3	16 (30.2)
	*3/*3	1 (1.9)
VKORC1[##]	*1/*2	1 (1.9)
(G1639A,C6009T,G9041A)	*1/*3	1 (1.9)
	*2/*3	10 (18.9)
	*2/*4	5 (9.4)
	*3/*3	29 (54.7)
	*3/*4	7 (13.2)

[#]Haplotypes CYP2C9 *2/*2 and *2/*3 were not found in the study population. [##]Haplotypes VKORC1*1/*1, *1/*4, *2/*2 and *4/*4 were not found in the study population.

Table 5. Distribution of haplotype combinations of *CYP2C9* and *VKORC1* gene polymorphisms

CYP2C9	*VKORC1*	*n* = 53 (%)
*1/*1	*1/*3	01 (1.9)
*1/*1	*2/*3	08 (15)
*1/*1	*2/*4	03 (5.6)
*1/*1	*3/*3	11 (21)
*1/*1	*3/*4	07 (13)
*1/*2	*1/*2	01 (1.9)
*1/*2	*3/*3	05 (9.4)
*1/*3	*2/*3	02 (3.8)
*1/*3	*2/*4	02 (3.8)
*1/*3	*3/*3	12 (23)
*3/*3	*3/*3	1 (1.9)

p value 0.11 (ANOVA). Haplotypes *1/*1 and*1/*1, *1/*1 and *1/*2, *1/*1 and *1/*4, *1/*1 and *2/*2, *1/*1 and *4/*4, *1/*2 and *1/*1, *1/*2 and*1/*3 and *1/*2 and *1/*4 respectively for *CYP2C9* and *VKORC1* genes were not found.

4. Discussion

Warfarin and acenocoumarol are highly effective for the prevention and treatment of various thromboembolic disorders (*1*). Although warfarin is most used coumarin, acenocoumarol is also commonly used in many countries (*15*). It is well known that an individual's response to oral anticoagulants depends on several factors. These include genetic factors, non-genetic factors, ethnic factors and yet unknown factors (*1,2*).

The known genetic factors contributing to the variability in acenocoumarol dosing requirements include mainly *CYP2C9* and *VKORC1* gene polymorphisms with minor contributions from APOE and *CYP4F2* genes (*15*). In the current study two genetic polymorphisms of *CYP2C9* gene *i.e. CYP2C9*2* (C430T) and *CYP2C9*3* (A1075C) and three genetic polymorphisms of *VKORC1* gene *i.e. VKORC1*2* (G1639A), *VKORC1*3* (G9041A) and *VKORC1*4* (C6009T) were analysed. These polymorphisms are known to have a significant effect on the acenocoumarol dose requirements. To the best of our knowledge, this is the first study that has evaluated 5 single nucleotide polymorphisms in two genes, simultaneously evaluating their allele and genotypic frequencies and the role of two of these gene polymorphisms in influencing acenocoumarol dose requirements in north Indian patients.

The non-genetic factors contributing to the variability in acenocoumarol dosing requirements include age, gender, BMI, vitamin K intake, concurrent medications and patient compliance (*15*). Age has a varied impact on the dosage of acenocoumarol. Some studies have shown that the activity of the cytochrome (P450) enzyme system decreases with age and that dose requirements fell with advancing age, decreasing by 0.5 to 0.7 mg per decade between the ages of 20 to 90 years irrespective of genotype and patient's height (*16*) while, others have demonstrated the opposite trend (*17*). In the current study though the dosage decreased with age, the difference was not statistically significant (p = 0.49). It was also observed that gender did not associate significantly with acenocoumarol dose requirements in (p = 0.97). Though, some studies have shown that the daily maintenance dose of acenocoumarol for females was significantly higher than the males (*16*), others did not find any differences (*18*). Drug-drug interactions have also been associated with variations in the acenocoumarol dose requirements to obtain stable anticoagulation (*15*). The concurrent medications which were essential as a part of the treatment required by the patients were not excluded and their effect on acenocoumarol dosage was studied. In our study the bivariate analysis model did not show any significant differences in acenocoumarol dose requirement in patients receiving concomitant amiodarone (p = 0.12) or atorvastatin (p = 0.5), however considerable caution is to be taken in the interpretation of this observation since the numbers of patients on these drugs were very small. Some reports suggest that the use of statins together with acenocoumarol has led to a slight decrease in the average daily dose of the latter (*16*), whereas others did not show any significant differences between patients receiving concomitant medications and those without the medication (*19*). Excessive consumption of vitamin K-rich diets (*e.g.* green vegetables) reduces the anticoagulation effect of coumarin derivatives (*20*) and in contrast, the administration of certain antibiotics that interfere with the production of vitamin K by gut flora have been suggested to exaggerate the anticoagulation response to coumarins (*21*). In the current analyses, the dietary consumption of vitamin K was not considered, it was assumed that all patients had relatively stable vitamin K consumption, given that they had a stable INR. The BMI has been included as a parameter in various algorithms that predict acenocoumarol dose requirements (*15,19*). In the current analysis BMI was correlated with the acenocoumarol dose requirements by categorizing the patients into high and low dose groups. No significant correlation was found between BMI of the patients and acenocoumarol dose requirements (p = 0.6). Compliance is yet another factor that effects acenocoumarol dose requirement. Drug dosage required to achieve an anticoagulation response may vary in a non-compliant patient and hence, the time required to achieve stable INR is more (*9*). All patients enrolled in our study had an apparently good compliance as they had a stable INR. This may be the result of good patient counseling well before starting anticoagulation therapy by the clinicians. The effect of non-genetic factors including age, gender, BMI and concurrent medications did not significantly relate to the drug dosage in this study group.

The genetic factors such as *CYP2C9* and *VKORC1* gene polymorphisms account for 5% and 20% variability respectively in the dosing of acenocoumarol (*3,6*). The prevalence of these polymorphisms varies across different ethnic groups. In the current study, the allele frequencies of *CYP2C9*1,*2,*3* were 0.773, 0.056, 0.169, respectively. The allelic frequencies were in Hardy-Weinberg equilibrium. The allele frequency of *CYP2C9*2* gene polymorphism in north Indian patients was higher than in other Asian countries (0.029), African-Americans (0.028) and south Indians (0.025) but was lower than the Caucasians (0.151) (*22,23*). The allele frequency of *CYP2C9*3* (A1075C) gene polymorphism in north Indian patients was higher than the other Asian countries (0.039), African-Americans (0.020), south Indians (0.083) and Caucasian population (0.057) (*22,23*). The allele frequency was comparable to that of Romanians (0.155) and also Indians residing in Singapore (0.18) (*14,24*). Further, in north Indian patients the allele frequency of *CYP2C9*3* gene polymorphism (0.169) was more than the allele frequency of *CYP2C9*2* gene polymorphism (0.056). A study from India showed similar findings, while, other two from the same region showed the reverse trend (*7,8,10*).

It was found that the carriers of *CYP2C9*3* alleles had the lowest dose requirement followed by carriers of *CYP2C9*2* alleles. This observation is in concordance with other similar studies on the effects of *CYP2C9*2* and *CYP2C9*3* polymorphisms on acenocoumarol (*8,17*). Of the total 53 subjects enrolled in our study, 62% (33/53) constituted the low dose group while 38% (*20*) patients required a higher dose of acenocoumarol

to maintain a stable INR. The patients with *CYP2C9*2* or *CYP2C9*3* alleles required a lower dose of acenocoumarol than patients without this variant. A similar trend was observed on 113 Spanish patients on acenocoumarol (*25*). Thus, it appears that the presence of the variant allele *CYP2C9*2* or *CYP2C9*3* in our patients necessitates a lower dose of acenocoumarol. An anticipated side effect of fixed dosage administration protocols is an increased risk of bleeding. None were documented in the course of this study.

Three different polymorphisms of *VKORC1* gene were studied, of which two, *VKORC1*3* (G9041A) and *VKORC1*4* (C6009T) have not previously been reported from north India. The allele frequency of *VKORC1*2* gene polymorphism was 0.15, similar to one reported in Malaysian Indians (0.14) (*26*). This was higher than the allele frequencies obtained in African-Americans (0.108) but lower than the Chinese (0.95), Caucasians (0.40) and the Israelis (0.41) (*14,23,27*). Among other studies from northern India the frequency of this polymorphism varied from 0.13-0.17 which was comparable to our study (8,10,11). The frequency of this polymorphism was lower at 0.079 in a study from South India while another study involving South Indian patients had results similar to this study at 0.14 (*10,22*). The presence of this gene polymorphism is associated with a lower dose of anticoagulants (*28*). *VKORC1*3* gene polymorphism was the most frequent SNP prevalent in our study with an allele frequency of 0.72. This polymorphism is also prevalent in the Tamil population (0.83) of southern India and is the most frequent one in the African population (0.43) and is also common among Caucasians (0.38) and Israelis (0.37) however, it is less prevalent in Chinese population (0.04) (*9,25,27,29*). Patients with this polymorphism are fast acetylators hence, would require a higher dose (*9*). The allele frequency of *VKORC1*4* gene polymorphism was 0.11. This SNP is less common in Chinese (0.01), 0.20 in Caucasians and 0.18 in Israelis (*14,27,28*). The patients with this polymorphism are also fast acetylators hence, would require a higher dose. Currently there is paucity of data available on the latter two and it would be of interest to determine similarities or differences in the diverse Indian population. We were unable to demonstrate significant differences studied, though it is known that the categorization into a low-dose and a high-dose haplotype group is clinically helpful to prevent the risk of under or over anticoagulation (*6,28*).

The effect of the *VKORC1* gene polymorphisms on drug dosage was studied. The presence of *VKORC1*2* gene polymorphism, was associated with lower doses of acenocoumarol whilst patients with *VKORC1*3* or *VKORC1*4* alleles required a higher dose of acenocoumarol than patients without this variant. In this study stepwise regression analysis model showed that *VKORC1*2* contributed to 7.6% to the variation of acenocoumarol dosage (r^2 = 0.076, p = 0.025). A study involving Caucasians has shown that *VKORC1*2* explained 17.6% of the dose variations of acenocoumarol (*16*). The first acenocoumarol dosing algorithm involved *VKORC1* and *CYP2C9* gene variants and clinical factors such as age, BMI and interacting drugs and also included *CYP4F2* and *APOE* gene variants and explained 60.6% of the total variability in the acenocoumarol dose needed to obtain a stable INR (*15*).

The limitations of this study were small sample size, exclusion of real life variables like pregnancy, smoking, alcohol intake, the inclusion of a restricted INR range and absence of data pertaining to other genetic polymorphisms that affect the metabolism of acenocoumarol and therefore, its dosage. Nevertheless, this preliminary study adds to the prevalence data of the *VKORC1* gene polymorphisms G9041A and C6009T that have hitherto not been reported from north India.

In the current study involving *CYP2C9* and *VKORC1* gene polymorphisms in north Indians on acenocoumarol with mechanical prosthetic valves, the *2 (C430T) polymorphism of *CYP2C9* and *3 (G9041) polymorphism of *VKORC1* was the most common. The high prevalence of *VKORC1* 9041A gene polymorphism in this sample population is a novel finding. As has been previously described, low dosage were associated with wild types of *VKORC1*2* and mutant types of *CYP2C9*2* and *CYP2C9*3*. Significant dose differences were not seen among the haplotypes and with respect to non-genetic factors. Further studies on larger populations are required to confirm the findings obtained in this North Indian cohort.

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