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VKORC1 and *CYP2C9*3* Polymorphisms and Their Impacts to Acenocoumarol Dosage in Vietnamese Heart Valve Replacement Patients[†]

Thuy Thi PHAM[1], Pham Thi Hong NHUNG[2], Van Thanh TA[3], Long Doan DINH[2], Kien Trung PHAM[2] and Thom Thi VU[2,*]

[1]*Thai Nguyen University of Medicine and Pharmacy, Ha Noi, Vietnam*
[2]*School of Medicine and Pharmacy, Vietnam National University Hanoi, Ha Noi, Vietnam*
[3]*Ha Noi Medical University, Ha Noi, Vietnam*

([*]Corresponding author's email: thomtbk5@gmail.com)

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Abstract

Acenocoumarol therapy has been widely used for heart valve replacement (HVR) patients in Vietnam to improve dose management of this drug. The variety of responses among patients to this drug are driven by genetic background. Hence, aim of the study is to explore the relation between acenocoumarol dosages and genetic polymorphisms of CYP2C9*3, VKORC1-1173 C>T and VKORC1-1639 G>A genes. One hundred fifty HVR patients was enrolled in this study. Blood samples were collected and analyzed using PCR and Sanger's sequencing. The result showed that there was no variant homozygous genotype (CC) of CYP2C9*3 observed, whereas wild-type (AA) and heterozygous (AC) were most abundant with 95.3, and 4.7 %, respectively. In contrast, variant homozygous genotypes of VKORC1-1173 C>T and -1639 G>A accounted for 70.7 and 87.3 % of Vietnamese HVR patient population while wildtype homozygous was not seen. Interestingly, there was significant difference in acenocoumarol doses between 2 genotypes of VKORC1-1173 C>T, but this result was not observed for CYP2C9*3. Patients with the variant homozygous genotype of VKORC1-1173 C>T have the lower dose of acenocoumarol in comparison with heterozygous genotype ($p = 0.001$). In conclusion, polymorphism of VKORC1-1173 C>T not CYP2C9*3 contributeseemed to relate to acenocoumarol dose responses of Vietnamese HVR patients.

Keywords: Acenocoumarol, CYP2C9, Heart valve replacement patients, VKORC1

Introduction

Oral acenocoumaric anticoagulant drugs such as warfarin, phenprocoumon and acenocoumarol are used to manage the diseases related to blood coagulation in heart valve replacement, deep vein thrombosis, atrial fibrillation patients [1-3]. In Vietnam, acenocoumarol was the most frequent anticoagulant drug for the treatment of the heart valve replacement patients in hospitals. Acenocoumarol has two isoforms called R (+) and S (-), in which R (+) form has higher anticoagulant activity than S (-) form that acts via vitamin K dependent manner [4]. Acenocoumarol has a short half-life so the maximum drug concentration in patient plasma to reach optimal prothrombin time is from 24 to 30 h. Acenocoumarol is used for long-term treatment, however it is difficult to manage the therapy. The efficacy of the treatment is dose-dependent and various from patients to patients. Moreover, this

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anticoagulant shows many side effects on the patients, for example the over-dose therapy could cause hemorrhagic whereas the under-dose therapy could lead to thrombosis and heart valve jam [5-8]. A reliable indicator for clinicians to evaluate the effectiveness of anticoagulants in the patients on mechanical valve replacement is the International Normalized Ratio (INR). This test assesses the extent of blood clot formation with the use of acenocoumarol, according to international recommendations. The target INR in patients with a mechanical heart valve is from 2.0 to 3.5 [6]. However, to maintain the INR goal is a challenge due to the influence of many factors including foods, drugs, accompanying diseases, and genetics.

Recently, many studies reported the effect of genetic background on the drug responses. For acenocoumarol, CYP2C9 and VKORC1 genes showed an important role as drug metabolite enzyme and drug targeted enzyme [9,10]. CYP2C9 belongs to p450 gene family located in 10q24 chromosome with more than 50 single nucleotide polymorphisms (SNPs) encoded for enzyme of the cytochrome P450 system. VKORC1, located in 16p11.2 chromosome, encodes for vitamin K epoxide reductase whichis the target enzyme for acenocoumarol [11]. Among various SNPs, CYP2C9*2, *3 and VKORC1-1173 and -1639 are well known for high prevalence and association with anticoagulant drug dose. The studies in Hungarian, Romanian, Lebanese, South Indian people demonstrated that patients with variant allele of CYP2C9*2, *3 and VKORC1*2 required low dose of acenocoumarol and had high risk of blood bleeding than people who carried wildtype allele [12-15]. Other studies also reported the impact of CYP2C9, VKORC1 on warfarin dosage response in Han-Chinese population [16,17]. Indeed, Gaikwad *et al*. reviewed the distribution of VKORC1 and CYP2C9 genotype for people in Asian countries. In which, the variant CYP2C9*2 allele was rare in East and Southeast Asia population such as China [16,17], Indonesia, Japan, Korea, Malaysia, Taiwan, and Thailand [16-18] whereas the wildtype allele of CYP2C9 and variant CYP2C9*3 allele were widespread and variant alleles of VKORC1 (-1639 or -1173) showed remarkable high allele frequencies from 0.74 to 0.92. Interestingly, these observations on VKORC1 and CYP2C9 genotype and allele frequencies were totally opposite to these in West and Central Asia population as well as European population [18], indicating the high level of divergence of these genes. Hence, a question arisen is whether Vietnamese population has difference in distribution of those polymorphisms on VKORC1 and CYP2C9 or not? Our study here for the first time demonstrated the allele and genotype frequencies of VKORC1 and CYP2C9 and their roles for acenocoumarol responses in Vietnamese patients with heart valve replacement.

Materials and methods

Study population and design

This was a cross-sectional study which selected 150 patients from Hanoi Heart Hospital, Vietnam. The inclusion criteria were the mechanical heart valves replaced patients using acenocoumarol with Internal Normal Ratio (INR) from 2 to 3.5 and the difference of acenocoumarol doses used for patients in 3 months should be less than 20 percent. The exclusion criteria were patients having renal or liver dysfunction, diabetes mellitus, Human Immunodeficiency Virus (HIV), tuberculosis (TB), gastritis, hepatitis B or C viruses (HBV or HCV). The study was approved by Ethic Committee of Vietnam National University Hanoi (IRB-VN01016) prior to this study.

Genotype analysis

Blood samples were drawn into 2 ml Ethylenediaminetetraacetic acid (EDTA) containing tubes and stored at −20 °C. DNA was extracted using the DNA Mini Kit (Omega Bio-Tek, GA, USA). CYP2C9*3 and VKORC1-1173 was amplified by using primers (IDT, USA) with sequences for CYP2C9*3: (F): GCATCTGTAACCATCCTCTC; (R): GTGTCAAGATTCAGTTCTTTCC); VKORC1-1173: (F): GGTGCCTTAATCCCAGCTACTC; (R): AAAGACTCCTGTTAGTTACCTCCC, and VKORC1-1639: (F): TACACTCCCATCATGCCTG; (R): GACCATCGTCAATCTCTACC. PCR was performed using following conditions:initial denaturation of 98 °C for 3 min; 35 cycles with 95 °C for 10 s, annealing at 63 °C for 30 s for both CYP2C9*3; VKORC1-1639 and -1173; the extending period at 72 °C for 5 min was used for all 2 SNPs. Sequencing results were obtained by using 3500 Automatic DNA Segmentation

Analyzer with BigDye Kit Terminator v3.1 cycle sequencing (Applied Biosystems, California, USA), analyzed with BioEdit version 7.1.9 software.

Statistical analysis

The data was edited and entered using SPSS 16.0 software (IBM, NewYork, USA). Analysis of variances test (ANOVA) was used to compare continuous variables while Chi-square analysis was applied for comparison of categorical variables among groups. Categorical variables were performed as n (%) and continuous variables as mean ±SD. The analysis was of statistical significance with a p-value < 0.05.

Results

General characteristics of the study population presented in Table 1. Patients had nearly equal gender ratio with 40.7 % male and 59.3 % female; the average age of 50.81±8.54 years old. Patients were normal in BMI, INR, hematology and biochemistry parameters. Rheumatic fever was the main causes of valve replacement with 70 % and mitral valve replacement was 68.7 %. Thirty-six percent (36 %) of patients had bleeding in the skin and only 14 % of patients had hypertension. The patients used acenocoumarol for more than 2 years and there was the concomitant medication with aspirin (4.7 %), digoxin (6 %) observed.

Table 1 Characteristics of the study population.

Medical history of patients		**Mean ± SD or N (%)**	**Paraclinical Parameters**	**Mean ± SD**
Age (years)		50.81 ± 8.54	INR1	2.70±0.42
BMI		21.45 ± 2.71	INR2	2.69±0.45
Male/Female (n, %)		61 (40.7) / 89 (59.3)	INR3	2.69±0.44
Hypertension (n, %)		21 (14.0)	RBC (n=114, ^12/L)	4.66±0.49
Causes of valve replacement (n, %)	Infective endocarditis	8 (5.3)	HGB (n=114, g/dL)	13.232±1.498
	Rheumatic fever	105 (70.0)	HCT (n=114, %)	41.00±3.80
	Valve deformation	21 (14.0)	PLT (n=114, ^9/L)	222.14±77
	Other causes	16 (10.7)	MPV (n=114, fL)	9.66±1.28
Valve replacement position (n, %)	Mitral valve	103 (68.7)	PDW (n=114, %)	11.52±9.31
	Aortic valve	26 (17.3)	PCT (n=114, %)	0.28±0.72
	Dual valves	21 (14.0)	WBC (n=114, ^9/L)	7.87±6.55
History of bleeding (n, %)	No bleeding	66 (44)	Ure (n=120, mmol/L)	5.25±1.68
	Skin	54 (36)	Glucose (n=120, mmol/L)	5.8±4.3
	Urology	1 (0.7)	Creatinin (n=120, mmol/L)	70.1±17.34
	Tooth	4 (2.7)	AST (n=120, UI/L)	28±8.9
	Skin and tooth	19 (12.7)	ALT (n=120, UI/L)	23±14.9
	Urology and digestion	3 (2)	Cholesterol (n=108, mmol/L)	4.68±1.15
	Epistaxis	1 (0.7)	Triglycerid (n=108, mmol/L)	1.18±1.97

Medical history of patients		**Mean ± SD or N (%)**	**Paraclinical Parameters**	**Mean ± SD**
History of thrombosis (n, %)	No thrombosis	145 (96.7)	HDL-C (n=108, mmol/L)	1.24±0.38
	Thrombosis of limb	1 (0.7)	LDL-C (n=108, mmol/L)	2.81±2.56
	Cerebral infarction	2 (1.3)	Na^+ (n=121, mmol/L)	139.88±2.88
	Prosthetic valve thrombosis	2 (1.3)	K^+ (n=121, mmol/L)	4.06±0.34
Other drug (n, %)	Aspirin	7 (4.7)	Cl^- (n=121, mmol/L)	99.54±9.28
	Digoxin	9 (6)		
Duration of drug use (years)		2.11±1.3		

BMI: body mass index; INR 1, 2, 3: International Normal Ratio 1st month, 2nd month, 3rd month; RBC: Red blood cell; HGB: Hemoglobin; HCT: Hematocrit; PLT: Platelet; MPV: Mean platelet volume; PDW: Platelet distribution width; PCT: Plateletcrit; WBC: White blood cell; ALT: Alanine transaminase; AST: Aspartate transaminase; HDL-C: High density lipoprotein- cholesterol; LDL-C: Low density lipoprotein- cholesterol.

To investigate the correlation of acenocoumarol doses and the genetic background of patients, *CYP2C9*3* and *VKORC1-1173* were genotyped and the results of allele and genotype frequencies of these SNPs were shown in **Table 2**. For *CYP2C9**3 allele, wild-type genotype (AA) accounted for 95.3% of patient population, heterozygous genotype (AC) was 4.7 %, and without variant homozygous genotype (CC) was observed in this study. For *VKORC1-1173 C>T* and *-1639 G>A,* the proportion of patients with wild-type (CC), heterozygous (CT), variant homozygous (TT) genotypes were 0; 29.3; 70.7 and 2.0; 10.7; 87.3, respectively. In contrast to *CYP2C9*3*, there was neither wild-type genotype (CC) of *VKORC1-1173 C>T* nor wild-type genotype (GG) of *VKORC1-1639 G>A* observed whereas variant homozygous of *VKORC1-1173 C>T* waswidespread. Frequency of mutant alleles of *CYP2C9**3, *VKORC1-1173C >T* and *VKORC1-1639 G>A* was 0.023, 0.853, and 0.927 respectively.

Table 2 The result of genotypes and frequency of alleles.

Gene SNP	**Genotypes (N, %)**			**Frequency of alleles**	
	Wildtype Homozygous	**Heterozygous**	**Variant Homozygous**	**Wildtype allele**	**Variant allele**
*CYP2C9*3*	AA	AC	CC	A	C
	143 (95.3 %)	7 (4.7 %)	0 (0 %)	0.977	0.023
VKORC1-1173C>T	CC	CT	TT	C	T
	0 (0 %)	44 (29.3 %)	106 (70.7 %)	0.147	0.853
VKORC1-1639G>A	GG	GA	AA	G	A
	3(2,0)	16(10,7)	131(87,3)	0,073	0,927

SNP: Single nucleotide polymorphism; N: Number of patients; %: percentage

In clinical practice, it is difficult for the clinician to address optimal dose of acenocoumarol despite the fact that there is a common international guideline for therapy. Due to high variant in the prevalence of *CYP2C9*3* and *VKORC1-1173C>T* gene polymorphisms between ethnic groups all over the world, the correlation between gene polymorphism and drug treatment dose should be addressed individually for each group. **Table 3** was mean of dosage in mg per week cross different genotype groups of each gene. *CYP2C9*3* genotype showed no effect on the dosage of acenocoumarol, while *VKORC1* presented the clearly significant difference between genotypes. Patients with heterozygous genotype (CT) of *VKORC1-1173* had to use the higher amount of acenocoumarol (15.57±4.79 mg/week) than patients with variant homozygous (10.68±3.52 mg/week) to archive the target INR value. Interestingly, with respect to *VKORC1-1639,* the acenocoumarol doses exhibited the genotype-dependent manner. Wild-type genotype (GG) required the highest dose of 18.50±2.50 mg/week, then heterozygous genotype (GA) was in the middle which was equal to the heterozygous genotype of *VKORC1-1173* with 15.97±5.97 mg/week, the variant homozygous genotype (AA) got the lowest dose of 11.49±3.99 mg/week.

Table 3 Genotypes and mean dosages of acenocoumarol in the patients.

Gene	Genotype	Mean Dosage X±SD (mg/week)	p-value
*CYP2C9*3*	*1*1 (n=143)	12.08±4.39	P = 0.790
	*1*3 (n=7)	12.81±6.94	
VKORC1-1173C>T	CT (n=44)	15.57±4.79	P = 0.001
	TT (n=106)	10.68±3.52	
VKORC1-1639G>A	GG (n=3)	18.50±2.50	P < 0.001
	GA (n=16)	15.97±5.97	
	AA (n=131)	11.49±3.99	

To have an overview of each patient genotype and to examine the potential relationship or additive effects between 2 genes, we combined the genotype of both *CYP2C9*3* and *VKORC1* and recorded the acenocoumarol dose using for treatment. Most patients (68 %) had wild-type *CYP2C9*3* and variant homozygous *VKORC1* combined genotype. It is observed that acenocoumarol doses between patients with *CYP2C9*3* wild-type and *VKORC1* wild-type or heterozygous (15.74±4.81 mg/week) and patients with *CYP2C9*3* wildtype and *VKORC1* variant homozygous (10.53±3.28 mg/week) were significant different (p = 0.001) (**Figure 1)**. It indicated that only *VKORC1* genotypes, both *-1173 C>T* and *-1639 G>A* singularly, played a role in acenocoumarol response, while *CYP2C9*3* had no function on that drug response. In addition, there was no additive correlation between these 2 genes on acenocoumarol dosages. For this reason, the interactive effect of 2 SNPs of *VKORC1-1173* and *-1639* on acenocoumarol doses was explored and presented in **Figure 1** below.

Among 150 patients, there was no genotype combination of the variant homozygous genotype of *VKORC1-1173 C>T* (TT) and wild-type genotype of *VKORC1-1639 G>A* (GG). The major combination was TT and AA, 2 variant homozygous genotypes with frequency of 69.4 %. In **Figure 1**, the highest acenocoumarol dose was observed in group with the combination of *VKORC1-1173* heterozygous (CT) and *VKORC1-1639* wildtype (GG) as 18.50±2.50 mg/week. Other combinations (CT+GA) and (CT+AA) of these 2 variants *VKORC1-1173* and *-1639* required acenocoumarol doses of 17.25±5.20 and 14.37±4.47 (mg/week), respectively. However, there was no significant difference between these 3

groups (p > 0.05). In addition, these 3 groups had significantly higher doses than the 2 other groups containing variant homozygous of *VKORC1-1173 C>T* (TT) as (GA+TT) and (GG+TT) with less than 10.75±3.52 mg/week drug dose (p < 0.05). It indicated that *VKORC1-1173 C>T* might have stronger modulating effect on acenocoumarol dose than *VKORC1-1639 G>A* in the combination genotypes.

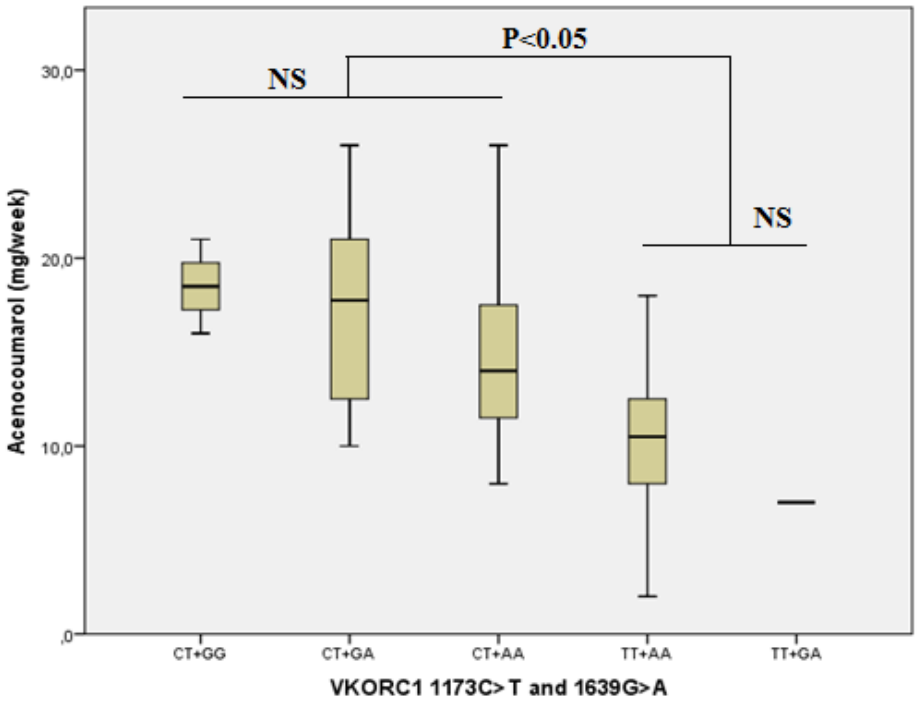

Figure 1 Mean dosages of patients with combined genotypes of *VKORC1-1173 C>T* and *-1639 G>A*. NS: Not significant; P < 0.05: significant difference

Discussion

This study shared the first data of genotypes and alleles frequency of *CYP2C9* and *VKORC1* genes and their roles in acenocoumarol responses in polulation of 150 heart valve replacement patients in Vietnam. The results showed that wild-type allele of *CYP2C9*3* (97.7 %) and variant alleles of *VKORC1-1173* (85.3 %) and *VKORC1-1639* (92.7 %) were widespread whereas the variant allele of *CYP2C9*3* and wildtype alleles of *VKORC1-1173* (14.7 %) and *-1639* (7.3 %) were rare. These distributions *of CYP2C9*3*, *VKORC1-1173* and *VKORC1-1639* allele in Vietnamese population in this study were similar to these of East Asia people, for example Han-Chinese (95; 81.2 and 90 %), Japanese (95.8; 81.9 and 90.3 %), Korea (90.8; 85.5 and 92 %) and Taiwan (95.4; 80.5 and 89.0 %), respectively [18]. However, this prevalence was significantly different with other populations, including South, West and Central Asian, Caucasians, South American where the wild-type allele of *CYP2C9*3* (60 - 85 %) and variant alleles of *VKORC1-1173* and *VKORC1-1639* (14 - 56 %) were rather lower [14]. Among Southeast Asian countries, Vietnamese had close *CYP2C9*3* wild-type allele distribution to Thais, Malaysian, Indonesian. However, variant alleles of *VKORC1-1173* and *-1639* in these countries were in range of 64 to 70 %, lower than that of Vietnamese (85.3 and 92.7 %, respectively) [14,18].

During the last 5 years, Hanoi Heart Institute has carried on the long-term treatment mainly with acenocoumarol for more than 1422 patients annually undergoing heart valve replacement. Metabolism and pharmacokinetics of acenocoumarol were variable from patient to patient. For instance, low

concentration of the drug could cause the intra vessel clotting or heart jam but hemorrhage or thromboembolism were observed in the high dose treatments complications such as [19]. Therefore, it is very important but challenge for the clinician to manage the drug doses for each patient to optimize the efficacy of treatment.

Polymorphisms of *CYP2C9* and *VKORC1* genes were well-known for manipulating acenocoumarol dose. *CYP2C9* enzyme is responsible for both R (+) and S (-) acenocoumarol form metabolism. As the result, variant genotypes of *CYP2C9* led to the reduction of *CYP2C9* enzyme activity that caused the accumulation of acenocoumarol in patient plasma [4]. *VKORC1* enzyme activated proteins to form a blood clot. By this function, variant genotypes of *VKORC1* such as *-1173 C>T* and *-1639 G>A* could induce the inhibition of blood clotting, as the consequence they needed lower dose of the anticoagulation drug [10]. In 2013, Buzoianu et al reported that both *CYP2C9*3* and *VKORC1-1639 G>A* variant alleles played an important role on acenocoumarol dose response of Romanian patients [15]. Other studies on the North and the South Indian patients with mechanical heart valve showed that the patients carried the combination of variant alleles of *CYP2C9*2, *3* and *VKORC1-1639* and *-1173* received lower dose of acenocoumarol treatment to reach INR from 2.5 to 3.0 [11,14]. Similar conclusions were proposed by Hungarian [12], Lebanese [13], Romanian [15], Pakistani [20]. A difference in our study to previous studies was that *CYP2C9* polymorphism was not related to acenocoumarol dose in Vietnamese patients. However, in line with most published studies, *VKORC1-1173* and *-1639* polymorphisms significantly contributed to lower the dose of this drug. The combination of these 2 *CYP2C9* and *VKORC1* genes did not give the additive effect on anticoagulant drug dose. However, when combination of 2 SNP genotypes of *VKORC1,* the SNP *VKORC1-1173* seemed to have more power to lower the dose of acenocoumarol in comparison to SNP *VKORC1-1639*. From genetic background point of view, *CYP2C9*3, VKORC1-1639* and *-1173* allele and genotype distribution in Vietnamese patient population were close to prevalence of North East Asian such as Chinese, Korean, Japanese. Zhang [16] and Jia [17], found that in Chinese population, patients carried the combination of *CYP2C9*3* variant allele and *VKORC1-1173* and *-1639* variant allele required significantly lower dose of warfarin compared to wild-type carrier. In line with this study, Korean people with variant alleles of *CYP2C9*3* and *VKORC1-1173* were recommended a lower initial and maintenance dose of warfarin [21]. The most similarity to our findings was the study performed by Miyagata in 2011 on Japanese population where no variant allele of *CYP2C9*2* and **3* appeared and *VKORC1-1173* variant allele associated with a significantly lower mean warfarin maintenance dose [23]. Currently, in Hanoi Heart Institute, there was only acenocoumarol therapy and absence of warfarin therapy. However, based on our present data on relation of *VKORC1* polymorphism and acenocoumarol dose, we could predict the response of patients for other anticoagulant drugs that share similar pharmacokinetics and pharmacogenetics to acenocoumarol.

Conclusions

In Vietnamese heart valve replacement patients, variant allele of *CYP2C9*3* expressed with low frequency while variant alleles of *VKORC1-1173 C>T* and *-1639 G>A* was very abundant. The *CYP2C9*3* genotype showed no effect on acenocoumarol dose, on the other hand, patients with the variant homozygous genotype of *VKORC1-1173 C>T* and *-1639 G>A* singularly had the significant lower dose in comparison with heterozygous genotype of this. Combined genotypes of *VKORC1-1173 C>T* and *-1639 G>A* modulated the acenocoumarol dose response of patients and *VKORC1-1173 C>T* seemed to have more contribution on that effect than *-1639 G>A*.

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