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Effects of *VKORC1* Genetic Polymorphisms on Warfarin Maintenance Dose Requirement in a Chinese Han Population

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Background: *VKORC1* is reported to be capable of treating several diseases with thrombotic risk, such as cardiac valve replacement. Some single-nucleotide polymorphisms (SNPs) in *VKORC1* are documented to be associated with clinical differences in warfarin maintenance dose. This study explored the correlations of *VKORC1*–1639 G/A, 1173 C/T and 497 T/G genetic polymorphisms with warfarin maintenance dose requirement in patients undergoing cardiac valve replacement.

Material/Methods: A total of 298 patients undergoing cardiac valve replacement were recruited. During follow-up, clinical data were recorded. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was applied to detect *VKORC1*–1639 G/A, 1173 C/T and 497 T/G polymorphisms, and genotypes were analyzed.

Results: Correlations between warfarin maintenance dose and baseline characteristics revealed statistical significances of age, gender and operation methods with warfarin maintenance dose (all $P<0.05$). Warfarin maintenance dose in *VKORC1*–1639 G/A AG + GG carriers was obviously higher than in AA carriers ($P<0.001$). As compared with patients with TT genotype in *VKORC1* 1173 C/T, warfarin maintenance dose was apparently higher in patients with CT genotype ($P<0.001$). Linear regression analysis revealed that gender, operation method, method for heart valve replacement, as well as *VKORC1*–1639 G/A and 1173 C/T gene polymorphisms were significantly related to warfarin maintenance dose (all $P<0.05$).

Conclusions: *VKORC1* gene polymorphisms are key genetic factors to affect individual differences in warfarin maintenance dose in patients undergoing cardiac valve replacement; meanwhile, gender, operation method and method for heart valve replacement might also be correlate with warfarin maintenance dose.

MeSH Keywords: Genotype • Polymorphism, Genetic • Warfarin

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Background

Warfarin, as an anticoagulant, is frequently applied to prevent thromboembolism in cases of deep vein thrombosis, atrial fibrillation (AF) and cardiac valve replacement [1]. Warfarin is a racemic mixture of R-warfarin and S-warfarin, the pharmacologically active enantiomer of which are metabolized by the CYP3A4 and CYP1A2 systems and the *CYP2C9* enzyme, respectively, to their inactive forms [2]. Therefore, when one enzyme system is inhibited or induced by an interacting drug, a clinically significant interaction may or may not occur, which is associated with warfarin exhibiting stereo-selective and regio-selective metabolism [2]. In elderly patients, on the other hand, warfarin may lead to comorbidities such as kidney disease, diabetes and risk of bleeding [3]. Warfarin maintenance dose is difficult to control with inadequate anticoagulation causing embolism while overdosed warfarin resulting in bleeding, which is mainly attributed to narrow anticoagulant therapeutic index and extremely individual differences in clinical efficacy and adverse reactions [4]. Currently, the effectiveness and safety in warfarin management is associated with prothrombin time and the improvement in thromboplastin reagents, which is expressed as the international normalized ratio (INR) [5]. Warfarin maintenance dose is protean resulted from multiple clinical and environmental factors, including age, gender, body weight, dietary intake, comorbidities, co-medications and variations in pharmacokinetics and pharmacodynamics [6]. Additionally, the focus of researches has altered from environmental factors to the genetic determinants of warfarin maintenance dose requirements [7–9]. Some studies have revealed the modulation of warfarin by polymorphisms in the *vitamin K epoxide reductase complex 1* (*VKORC1*) gene [10–12].

VKORC1 functions to recycle vitamin K 2, 3-epoxide, activating vitamin K hydroquinone, which plays a vital role in the activation of vitamin K-dependent clotting factors [13]. In addition, VKORC1 is reported to be the target of vitamin K anticoagulants, and is capable of treating several diseases with thrombotic risk, such as AF, myocardial infarction, cardiac valve replacement, stroke and venous thrombosis [14]. Some single nucleotide polymorphisms (SNPs) in *VKORC1* have been documented to may cause amino acid changes of VKORC1 protein, which is associated with clinically significant differences in warfarin maintenance dose requirement [15]. Five common polymorphisms, 3730 G>A in the 3'-untranslated region, 1173 C>T in intron 1, –1639 G>A in the *VKORC1* gene promoter, 1542 G>C, and 2255 T>C have been reported to have a role in the inter-individual differences of warfarin maintenance dose requirement [10,16–19].

While rare, clinically significant prolonged prothrombin time and potentially life threatening bleeding can occur when amoxicillin/clavulanate is concomitantly administered with warfarin; therefore, prompt recognition and intervention is necessary to avoid life threatening complications from warfarin-amoxicillin/clavulanate interaction [20]. In our present study, we explored the correlations of *VKORC1*–1639 G/A, 1173 C/T and 497 T/G genetic polymorphisms, which are common in Chinese Han population, with warfarin maintenance dose requirement in Chinese Han population, providing a helpful clinical indicator for the safe dosage of warfarin.

Material and Methods

Subjects

A total of 298 patients undergoing cardiac valve replacement were recruited at the Department of Cardiothoracic Surgery of Xiangyang Central Hospital, Affiliated Hospital of Hubei College of Arts and Science between June 2010 and June 2014. All patients were required to receive warfarin anticoagulation therapy. Inclusion criteria for the patients were as follows: (1) Chinese Han patients treated with cardiac valve replacement and long-term warfarin anticoagulation therapy; (2) patients with age ≥18 years; (3) patients administrated with stable dose of warfarin for ≥3 months; (4) patients with normal liver and kidney function; (5) patients with an INR of 2.0–3.0; (6) patients without complications such as hemorrhage and embolism; (7) patients without abnormal prosthetic valves after the surgery by cardiac ultrasonography; (8) no sibship or history of intermarriage among all patients. Exclusion criteria included: (1) patients with abnormal liver, kidney and thyroid function; (2) patients with an allergy to warfarin; (3) patients administrated with amiodarone, rifampin, or barbitone which may influence the pharmacokinetic effect of warfarin; (4) patients administrated with aspirin, clopidogrel, heparin, or vitamin K which may influence INR value; (5) patients with miocardial infarction, infective endocarditis, or active peptic ulcer within 1 month after the surgery; (6) patients with malignant tumors or hematological diseases, or during gestation period. This study was approved by the Xiangyang Central Hospital, Affiliated Hospital of Hubei College of Arts and Science. All eligible participants provided written informed consent for individual drug testing of warfarin.

Data collection

During the first follow-up, clinical data including gender, age, body mass index (BMI, classified into low BMI group, normal BMI group, and high BMI group according to the guidelines for prevention and control of overweight and obesity in Chinese adults) [21], body surface area (BSA), operation method, method for heart valve replacement (biological valve or mechanical valve), prescribed dosage of warfarin and combined medication were recorded. Subsequently, follow-up was conducted

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Table 1. PCR primers for *VKORC1*–1639 G/A, 1173 C/T and 497 T/G.

Site	Sequence
VKORC1 –1639 A/G	Upstream primer: 5'-GCCAGCAGGAGAGGGAAATA-3' Downstream primer: 5'-AGTTTGGACTACAGGTGCCT-3'
VKORC1 1173 C/ T	Upstream primer: 5'-TAGGACTGT-CAACCCAGT-3' Downstream primer: 5'-AGTGACATG-GAATCCTGA-3'
VKORC1 497 T/G	Upstream primer: 5'-ATGGCAAGGCTGGTATAACG-3' Downstream primer: 5'-AGGATGGCGGTAGAGATTGA-3'

PCR – polymerase chain reaction.

once a month. Average daily warfarin dose and average INR value were recorded. Warfarin maintenance dose refers to the mean dose of warfarin with INR value ranging from 2.0 to 3.0 2–3 times in a row, which was recorded every ≥7 days.

Sample collection

Fasting venous blood (5 mL) was obtained from each patient 15 h after medicine taking, and drawn into ethylene diamine tetraacetic acid (EDTA) anticoagulant. Two mL of the venous blood was utilized to measure INR values; and the left 3 mL was centrifuged at 4000 r/min for 15 min, with upper plasma collected and preserved at –80°C. Genomic DNA samples for genotyping were fast extracted by using a DNA extraction kit (Beijing SBS Genetech Co., Ltd., China), and preserved at –20°C.

Genotyping

The genotyping was performed by using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, and the primers for *VKORC1* were provided by Invitrogen Trading Co. Ltd., Shanghai, China (Table 1). The reaction system (25 μl) included 2.0 μl template DNA, 0.5 μl 100 μmol/L upstream and downstream primers for –1639 G/A (1μl 100 μmol/L upstream and downstream for 1173 C/T and 497 T/G), 2.5 μl 10×PCR buffer (containing Mg^{2+}), 2 μl 2.5 mmol/L dNTP, 0.25 μl TaqDNA polymerase and double distilled water with a pH value of 8.2. PCR started with initial denaturation at 95°C for 5 min and 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s (annealing at 55°C for 30 s for 1173 C/T) and extension at 72°C for 45 s, followed by a final extension at 72°C for 7 min. The PCR products of –1639 G/A, 1173 C/T and 497 T/G were digested by Msp I, Hinf I and Hph I restriction enzyme, respectively. PCR product (8 μl), 2 μl buffer, 8.5 μl dH2O and 1.5 μl 10 U/μl restriction enzymes were incubated at 37 °C overnight. The digested products (9 μl) were added in the sample wells of the negative-pole end of the 2.5% w/v agarose gel electrophoresis and electrophoresed at 120V for 30 min (Bromophenol blue as an indicator and ethidium bromide as a staining agent), and observed under an ultraviolet light.

Genotype analysis

The length of PCR amplification products of *VKORC1*–1639 A/G was 290 bps. The wild type homozygote GG was digested by Msp I, producing two fragments of 168 bps and 122 bps. The mutant heterozygote AG yielded three fragments of 290 bps, 168 bps and 122 bps after digestion, while the mutant homozygote AA produced only one fragment of 290 bps due to a lack of Msp I restriction site. The length of PCR amplification products of *VKORC1* 1173 C/T was 150 bps. The wide homozygote (CC type) cannot be digested by Hinf I and had only one fragment (150 bps), the mutant heterozygote (TC type) was partly cut and had three fragments (150, 100 and 50 bps), and the mutant homozygote (TT type) was cut into two fragments (100 and 50 bps). The length of PCR amplification products of *VKORC1* 497 T/G was 259 bps. The wild type homozygote TT was digested by Hph I, producing two fragments of 212 bps and 47 bps, the mutant heterozygote TG yielded three fragments (259, 212 and 47 bps) after digestion, while the mutant homozygote GG was not detected.

Statistical analysis

Measurement data were presented as mean ± standard deviation (SD), and enumeration data were presented as frequency. The χ^2 test was used to detect if the gene distribution accorded with the Hardy-Weinberg equilibrium, and to compare genotype and allele frequency between groups. The comparisons of clinical characteristics and warfarin maintenance dose between different genotypes were conducted by using the *t* test or variance analysis. Linear regression method was used to analyze the relevances between warfarin maintenance dose and variables. A two-tailed significance level was used in all tests and a *P* value less than 0.5 was taken as statistically significant. All the analyses were performed using software SPSS17.0 (SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics

A total of 278 patients who underwent cardiac valve replacement were enrolled to this study, including 117 males and 161 females, with age ranging from 18 to 75 years (mean age, 42.50±13.89 years). Among them, 100 patients (35.97%) aged

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Table 2. Distribution of genotype and allele frequencies for *VKORC1*–1639 G/A, 1173 C/T and 497 T/G.

Gene	Genotype	Frequency (%)
VKORC1–1639 G/A	AA	237 (85.25)
	AG	38 (13.67)
	GG	3 (1.08)
	A	254 (91.37)
	G	24 (8.63)
VKORC1 1173 C/ T	TT	231 (83.09)
	CT	47 (16.91)
	CC	0 (0.00)
	C	22 (7.91)
	T	256 (92.09)
VKORC1 497 T/G	TT	275 (98.92)
	TG	3 (1.08)
	GG	0 (0.00)
	T	272 (97.84)
	G	6 (2.16)

≥50 years and 178 patients (64.03%) aged <50 years. Average BMI was 18.37±3.59 kg/m^2 for all the enrolled patients, with 217 patients (78.06%) in the low BMI group, 41 patients (14.75%) in the normal BMI group, and 20 patients (7.19%) in the high BMI group. Average BSA was 1.54±0.15 m^2, with 171 patients (61.51%) with BSA ≥1.5 m^2, and 107 patients (38.49%) <1.5 m^2. Seventeen patients (6.12%) underwent Bentall surgery, 60 patients (21.58%) underwent aortic valve replacement (AVR), 86 patients (30.94%) underwent mitral valve replacement (MVR), and 115 patients (41.37%) underwent double valve replacement (DVR). On the other hand, 21 patients (7.55%) received biological valve replacement, and 257 patients (92.45%) revived mechanical valve replacement. Average warfarin maintenance dose was 3.20±1.08 mg.

Genotype and allele frequencies

The genotype and allele frequencies of *VKORC1*–1639 G/A, 1173 C/T, and 497 T/G showed no deviation from Hardy-Weinberg equilibrium (all $P>0.05$), suggesting the representativeness of all samples. The distribution frequencies of genotype AA, AG and GG in *VKORC1*–1639 G/A were 85.25% (237/278), 13.67% (38/278) and 1.08% (3/278), respectively, and the frequencies of allele A and G in *VKORC1*–1639 G/A were 91.37% and 8.63%. The distribution frequencies of genotype TT, CT and CC in *VKORC1* 1173 C/T were 83.09% (231/278), 16.91% (47/278) and 0.00% (0/278), respectively and the frequencies of allele C and T in *VKORC1* 1173 C/T were 7.91% and 92.09%. The distribution frequencies of genotype TT, TG and GG in *VKORC1* 497 T/G were 98.92% (275/278), 1.08% (3/278) and 0.00% (0/278), respectively and the frequencies of allele T and G in *VKORC1* 497 T/G were 97.84% and 2.16% (Table 2).

Genotype and allele frequencies and ethnicity

Results of genotyping demonstrated that genotype AA (TT) in *VKORC1*–1639 G/A displayed the highest frequency, which was in line with the results of the distribution of genotype and allele frequencies in *VKORC1*–1639 G/A by Miao et al. in the Chinese population [22]; while different from the results in the foreign populations, such as European populations [23,24] and Caucasian populations [25]. In addition, compared with the distribution frequencies of genotype AA, AG and GG in *VKORC1*–1639 G/A in the Chinese Uygur population, which showed a higher frequency of genotype AG [26], our results displayed an obvious ethnic difference (Table 3). As for the distribution frequencies of genotype in *VKORC1* 1173 C/T, the genotyping results in our study revealed the highest frequency of genotype TT, which was consistent to the results of Yang et al. who also evaluated the distribution frequencies of genotype in *VKORC1* 1173 C/T in the Chinese population [27,28]; while

Table 3. Distribution of genotype and allele frequencies for *VKORC1*–1639 G/A in different races.

Race	*VKORC1*–1639 A/G Genotype frequency			*VKORC1*–1639 A/G Allele frequency	
	AA	AG	GG	A	G
Taiwan Han population	0. 82	0. 18	0. 00	0. 91	0.09
Chinese Han population [22]	0.83	0.15	0.02	0.93	0.07
Caucasian population [25]	0.14	0.47	0.39	0.38	0.72
Chinese Uygur population [26]	0.33	0.58	0.09	0.62	0.38
English population [23]	0.25	0.56	0.19	0.47	0.53
French population [24]	0.43	0.35	0.22	0.42	0.58
Present study	0.85	0.14	0.01	0.91	0.09

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Table 4. Distribution of genotype and allele frequencies for *VKORC1* 1173C/T in different races.

Race	*VKORC1* 1173 C/T Genotype frequency			*VKORC1* 1173 C/T Allele frequency	
	AA	AG	GG	A	G
Chinese Han population [27,28]	0.85	0.14	0.01	0.08	0.92
Caucasian population [27,28]	0.17	0.49	0.34	0.58	0.42
African Americans [29]	0. 01	0.19	0.80	–	–
European Americans [29]	0.12	0.50	0.38	–	–
Present study	0.83	0.17	0.00	7.91	92.09

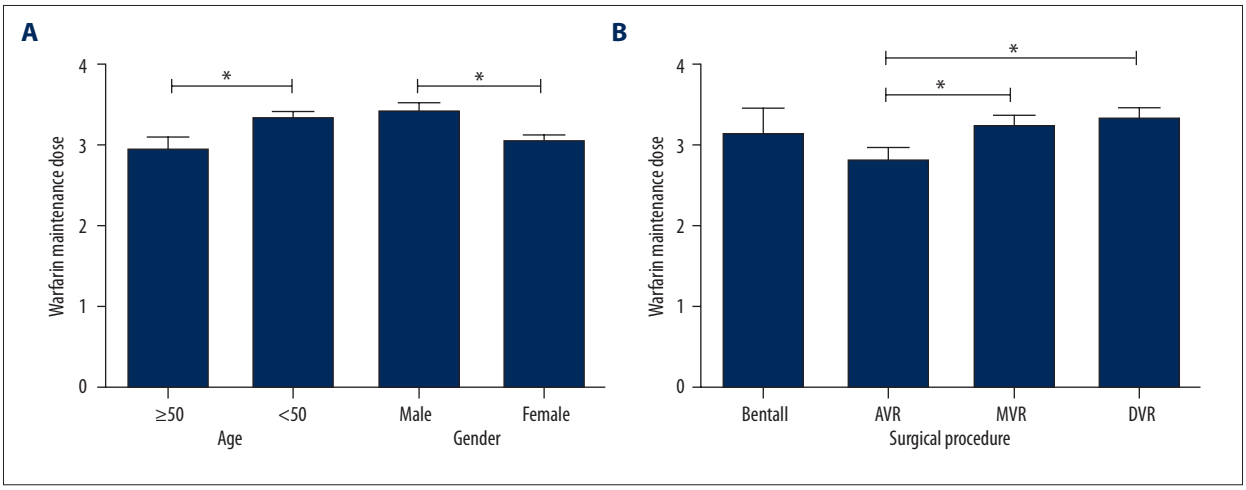

Figure 1. (**A**) Comparisons of warfarin maintenance dose between patients with age ≥50 years and patients with age <50 years, and between male patients and female patients; * $P<0.05$. (**B**) Comparisons of warfarin maintenance dose among patients with different operation methods; * compared with AVR, $P<0.05$; AVR, aortic valve replacement; MVR, mitral valve replacement; DVR, double valve replacement.

obviously different from the results in the foreign populations, such as Caucasian populations [28], African Americans, and European Americans [29], which showed a higher frequency of genotype CT and CC, suggesting an apparent ethnic difference, and a low warfarin maintenance dose requirement in Chinese Han population (Table 4). The *VKORC1* 497 T/G is non-polymorphic (497TT) in all the populations except for the Indian population, in which 20% of the population has 497 T/G genotype. The 497G allele is very rare in the East-Asian populations (frequency <1%) [30]. The transitions at 497 T/G in intron 1 appeared to be present in Caucasians at a low frequency, and significant differences in allelic frequencies of 497 T/G were found between the Caucasian and Japanese groups, as well as between the Caucasian and African-American groups [31]. However, we failed to obtain the original data about the genotype and allele frequencies; therefore, the distribution frequencies of genotype and allele in *VKORC1* 497 T/G still required supports from studies with large sample size.

Warfarin maintenance dose and baseline characteristics

Results showed statistical difference in warfarin maintenance dose between patients with age ≥50 years and patients with age <50 years (2.98±1.09 *vs.* 3.32±1.05, $P=0.010$). Compared with female patents, warfarin maintenance dose in male patients was evidently higher (3.42±1.02 *vs.* 3.04±1.09, $P=0.003$) (Figure 1A). Warfarin maintenance dose requirement in groups with different operation methods also showed statistical differences (MVR *vs.* AVR *vs.* DVR: 3.26±1.07 *vs.* 2.82±1.12 *vs.* 3.36±0.01, all $P<0.05$) (Figure 1B). No statistical significance existed in warfarin maintenance dose among patients with different BMI, BSA, as well as methods for heart valve replacement (all $P>0.05$).

Warfarin maintenance dose and genotypes

In patients with *VKORC1*–1639 G>A polymorphism, warfarin maintenance dose in AG + GG carriers was obviously higher

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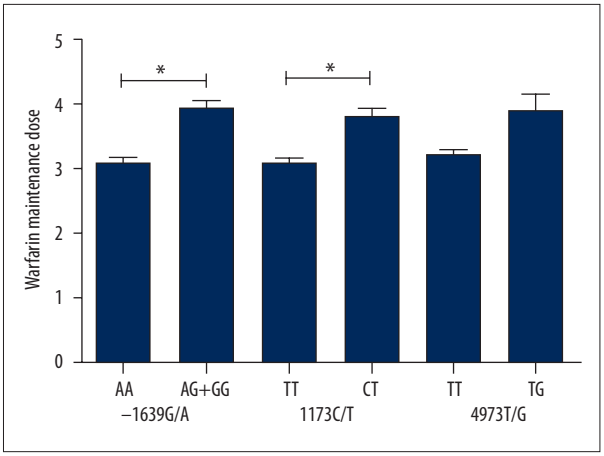

Figure 2. Comparisons of warfarin maintenance dose among patients with different genotypes in *VKORC1*–1639 G/A, 1173 C/T and 497 T/G; * *P*<0.05.

than that in AA carriers (3.92±0.83 *vs.* 3.07±1.06, *P*<0.001). As compared with patients with TT genotype in *VKORC1* 1173 C/T, warfarin maintenance dose was apparently higher in patients with CT genotype in *VKORC1* 1173 C/T (3.81±0.92 *vs.* 3.07±1.07, *P*<0.001). There was no statistical significance in warfarin maintenance dose between patients with TT genotype in *VKORC1* 497 T/G and patients with TG genotype in *VKORC1* 497 T/G (3.19±1.08 *vs.* 3.87±0.44, *P*>0.05) (Figure 2).

Linear regression analysis for warfarin maintenance dose

Linear regression method was used to analyze the correlations of gender, age, BMI, BSA, operation method, method for heart valve replacement, genotypes in *VKORC1* with mean warfarin maintenance dose requirement. Gender, operation method, method for heart valve replacement, as well as *VKORC1*–1639 G/A and 1173 C/T gene polymorphisms were significantly related to the warfarin maintenance dose requirement (all *P*<0.05). However, no correlations of age, BMI, BSA, or *VKORC1* 497 T/G gene polymorphism with warfarin maintenance dose requirement were detected (all *P*>0.05) (Table 5).

Discussion

The most important results in our present study showed significant correlations of *VKORC1*–1639 G/A and 1173 C/T genetic polymorphisms with warfarin maintenance dose requirement, implying that –1639 G/A and 1173 C/T polymorphisms in *VKORC1* gene may be predictors of safe warfarin maintenance dose. The average maintenance dose of warfarin in the *VKORC1*–1639 G/A AG + GG genotype carriers was significantly higher than that in the AA genotype carriers; the warfarin maintenance dose in *VKORC1* 1173 C/T CT genotype carriers was significantly higher than that in the TT carriers. The potential mechanisms by which *VKORC1*–1639 G/A and 1173 C/T genetic polymorphisms play a role in warfarin maintenance dose requirement may be explained by that, warfarin exerts its inhibiting effect on vitamin K to form coagulation factors II, VII, IX and X in the liver, and thereby warfarin dose requirement may be related to the genes encoding the related proteins in the vitamin K cycle pathway [32,33]. VKORC1, as a key vitamin K metabolism enzyme, is able to prevent the production of reduced vitamin K, reducing the formation of vitamin K dependent coagulation factors to achieve an anticoagulant effect [34,35]. *VKORC1*–1639 G>A, located in the promoter, is

Table 5. Linear regression analysis for warfarin maintenance dose.

Model	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. error	Beta		
Gender	0.431	0.177	0.198	2.436	0.015
Age	–0.029	0.147	–0.013	–0.196	0.845
BMI	–0.048	0.149	–0.026	–0.320	0.749
BSA	0.029	0.122	0.013	0.234	0.816
Operation method	0.209	0.088	0.182	2.383	0.018
Method for heart valve replacement	–0.609	0.253	–0.150	–2.407	0.017
–1639 G/A	0.357	0.134	0.227	2.655	0.008
1173 C/ T	0.659	0.270	0.230	2.442	0.015
497 T/G	0.269	0.601	0.026	0.448	0.654

BMI – body mass index; BSA – body surface area; Std – stabdard; Sig – significance, *P* value.

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capable of regulating the expression of VKORC1, thus may affect the warfarin maintenance dose requirement. In this study, higher warfarin maintenance dose in the AG + GG genotype carriers than the AA genotype carriers showed that the mutation of the promoter in *VKORC1* were related with low warfarin dose and high warfarin sensitivity. In addition, the correlation of *VKORC1* 1173 C/T intronic polymorphism with warfarin maintenance dose requirement revealed that 1173 TT genotype had the lower average daily dose of warfarin than the CT genotype [25,29]. In line with our studies, Liang et al. also found that AG genotype in the *VKORC1*–1639 G>A SNP was associated with a higher maintenance dose than those with the AA genotype [7]. D'Andrea et al. also demonstrated the mean required warfarin dose was higher among patients with the *VKORC1* 1173 CC genotype than those of patients carrying the CT or the TT genotype [36].

The results of our linear regression analysis further confirmed the importance of *VKORC1*–1639 G/A and *VKORC1* 1173 C/T in individual differences of warfarin maintenance dose. As for other environment factors for the maintenance dose of warfarin, gender, operation method, and method for heart valve replacement might also be independent factors for the warfarin dose differences. Our study revealed a higher warfarin maintenance dose in male patients than female patients, which is line with a previous study of Kabagambe et al. demonstrating that inclusion of vitamin K intake in the model slightly improved the amount of variance explained by gender [37]. Daniel et al. have predicted that the operation method would provide an INR value within the therapeutic range 65–80% of the time once three or more INR observations are available, making this a useful tool for clinicians and warfarin clinics [38].

In addition, our study further extends the current observation that ethnicity affects warfarin maintenance dose requirements independent of previously identified variables. The causes of the observed difference in warfarin maintenance dose requirements among the ethnic groups have several possible explanations. Differential protein binding has been proposed to contribute to the variability in drug response [7,8,39]. Moreover, genetic differences in drug-metabolizing capacity across ethnic groups may account for the variable response observed with warfarin [6,40]. Dang et al. also documented that warfarin dose maintenance requirements vary across ethnic groups, even when adjusted for confounding factors, suggesting that genetic variation contributes to inter-patient variability [41].

Our study also has several limitations. First, the genotype distribution in *VKORC1* gene may be influenced by ethnicity. In our study, only Chinese subjects were included due to geographical restrictions and we detected the genotypes and alleles of *VKORC1* polymorphisms among Chinese populations, which may have resulted in subject selection bias. Thus, it is essential to perform related studies to confirm the associations of *VKORC1* polymorphisms with warfarin dose maintenance in patients undergoing cardiac valve replacement in other ethnicities. Second, the genetic studies might be affected by random variation due small sample size, therefore, further studies should be conducted to evaluate *VKORC1* polymorphisms with warfarin dose maintenance in patients undergoing cardiac valve replacement with a larger sample size to achieve a more accurate and systematic outcome. Third, the *CYP2C9* status was not taken into account which may also have effects on warfarin dose maintenance requirement. In this study, only the association between *VKORC1* gene polymorphisms and warfarin dose maintenance requirement was examined. Thus, further studies are required to confirm our results. Finally, the effect of *VKORC1* polymorphisms on conferring a predictive role in warfarin dose maintenance may be significantly influenced by polymorphisms in other related genes, and this may have influenced the results in our study.

Conclusions

VKORC1–1639 G/A and *VKORC1* 1173 C/T gene polymorphisms were significantly associated with the maintenance dose of warfarin, and the *VKORC1*–1639 G/A AG + GG genotype carriers are linked with higher warfarin maintenance dose than the AA genotype. In addition, CT patients in *VKORC1* 1173 C/T has significantly higher warfarin maintenance dose than that in the TT patients. *VKORC1* gene mutation could be the most important factor in the differences of warfarin doses, as well as gender, operation method, and method for heart valve replacement were also independent factors for warfarin maintenance dose.

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Competing interests

All authors in our study have no conflict of interest.

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