Impact of VKORC1, CYP2C9, CYP1A2, UGT1A1, and GGCX polymorphisms on warfarin maintenance dose
Exploring a new algorithm in South Chinese patients accept mechanical heart valve replacement

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Abstract

Background: Warfarin is the most recommended oral anticoagulant after artificial mechanical valve replacement therapy. However, the narrow therapeutic window and varying safety and efficacy in individuals make dose determination difficult. It may cause adverse events such as hemorrhage or thromboembolism. Therefore, advanced algorithms are urgently required for the use of warfarin.

Objective: To establish a warfarin dose model for patients after prosthetic mechanical valve replacement in southern China in combination with clinical and genetic variables, and to improve the accuracy and ideal prediction percentage of the model.

Methods: Clinical data of 476 patients were tracked and recorded in detail. The gene polymorphisms of VKORC1 (rs9923231, rs9934438, rs7196161, and rs7294), CYP2C9 (rs1057910), CYP1A2 (rs2069514), GGCX (rs699664), and UGT1A1 (rs887829) were determined using Sanger sequencing. Multiple linear regressions were used to analyze the gene polymorphisms and the contribution of clinical data variables; the variables that caused multicollinearity were screened stepwise and excluded to establish an algorithm model for predicting the daily maintenance dose of warfarin. The ideal predicted percentage was used to test clinical effectiveness.

Results: A total of 395 patients were included. Univariate linear regression analysis suggested that CYP1A2 (rs2069514) and UGT1A1 (rs887829) were not associated with the daily maintenance dose of warfarin. The new algorithm model established based on multiple linear regression was as follows: $Y = 1.081 - 0.011$ (age) + 1.532 (body surface area)-0.807 (rs9923231 AA) + 1.788 (rs9923231 GG) + 0.530 (rs1057910 AA)-1.061 (rs1057910 AG)-0.321 (rs699664 AA). The model accounted for 61.7% of individualized medication differences, with an ideal prediction percentage of 69%.

Conclusion: GGCX (rs699664) may be a potential predictor of warfarin dose, and our newly established model is expected to guide the individualized use of warfarin in clinical practice in southern China.

Abbreviations: ACEI = angiotensin converting enzyme inhibitors, BSA = body surface area, DNA = deoxyribonucleic acid, INR = international standard ratio, IWPC = International Warfarin Pharmacogenetics Consortium, PCR = polymerase chain reaction, PE = pulmonary embolism, SNP = single nucleotide polymorphisms.

Keywords: Chinese, maintenance dose, pharmacogenetic algorithm, polymorphisms, warfarin

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1. Introduction

Warfarin is an anticoagulant that is prescribed for life to patients after artificial valve replacement. However, it interferes with the metabolism of the vitamin K.[1] Warfarin has a narrow therapeutic window and large differences in terms of efficacy in different individuals; its safety has also been a concern. Overdosing or under-dosing warfarin has been reported to cause serious complications such as bleeding or thromboembolism, respectively.[2–4] Thus, warfarin dose must be personalized.[5] In 2009, the International Warfarin Pharmacogenetics Federation established the most famous warfarin drug delivery prediction model based on clinical and pharmacogenetic variables,[6] providing ideas for accurate and rapid prediction methods.

Warfarin exerts its anticoagulant effect by inhibiting vitamin K epoxide reductase complex subunit 1 (VKORC1). Single nucleotide polymorphisms (SNPs) in VKORC1 can affect its transcription, thereby disturbing warfarin pharmacodynamics.[7,8] VKORC1-1639G > A (rs9923231) in the promoter and 1173C > T(rs9934438) in its introns are the most common variables in the existing regression models, both of which are associated with a reduced warfarin dose,[9,10] whereas rs7196161 and rs7294 located in the untranslated region are associated with increased warfarin dose.[11] CYP2C9 catalyzes the conversion of S-warfarin to its active metabolite. Common SNPs in CYP2C9 include * 2 (rs1799853) and * 3 (rs1057910), which have a significant effect on the dose requirement of warfarin,[12–15] and are responsible for approximately 9.6% to 20.6% of the differences in individualized medications.[11]

CYP1A2 is a member of the cytochrome P450 superfamily; its SNP rs2069514 accounts for approximately 3.7% of the differences in individualized warfarin doses.[16] γ-Glutamyl carboxylase encoded by GGCX is a key enzyme in vitamin K metabolism; it carboxylates the inactive vitamin K-dependent prothrombin II, VII, IX, and X precursors into active clotting factors.[11,17,18] The SNP rs699664 affects the dose of warfarin.[19] Uridine diphosphate glucuronyl transferase, encoded by UGT1A1, catalyzes the second-phase biotransformation of warfarin,[20,21] and its SNP rs 887829 accounts for approximately 5.5% of individual differences.[22] Studies on the predictive modeling of warfarin administration involving these 3 genes are still lacking.

Relevant clinical variables can also have an important impact on the demand for warfarin, and non-genetic factors can explain approximately 9.4% to 22% of individualized differences.[21,23] Factors that reduce the demand for warfarin include old age,[24,25] lack of exercise,[26] diets low in vitamin K,[27,28] and concomitant use of drugs such as amiodarone,[29] simvastatin,[30] betaloc,[10] and angiotensin converting enzyme inhibitors (ACEI).[31] Body surface area (BSA), history of thrombotic diseases, and smoking history are also factors that increase the dose of warfarin.

Considering the above-mentioned genetic and clinical variables, scholars have established a dose algorithm model based on multiple linear regression analysis to predict the daily maintenance dose of warfarin. Unfortunately, however, most of these model studies lack a verification group or only a few studies have set a verification group.

Therefore, in the present study, we aimed to establish a warfarin drug delivery model, with improved accuracy and prediction percentage, for patients undergoing artificial valve replacement in Southern China by considering multiple genes and loci and combining clinical variables.

2. Materials and Methods

2.1. Patient selection

The Ethics Committee of the Second Affiliated Hospital of Nanchang University approved this study (number 2016 [027]). Between August 2013 and January 2017, patients who underwent artificial mechanical valve replacement and received warfarin treatment after surgery were recruited from the Department of Cardiovascular and Vascular Surgery of the Second Affiliated Hospital of Nanchang University. A total of 476 patients volunteered to participate. The inclusion criteria were as follows: aged between 18 and 70 years; preoperative coagulation function and platelet count within the normal range; patients willing to sign informed consent; patients who can be followed-up regularly for at least 3 months during warfarin treatment; patients in whom a stable dose was achieved (international standard ratio [INR] was maintained between 1.8 and 2.5 after 6 consecutive monitoring[32–34]). The exclusion criteria were as follows: patients with liver or kidney dysfunction or chronic liver and kidney diseases associated with chronic renal insufficiency, chronic hepatitis, or cirrhosis; patients with hematological diseases; patients who cannot take warfarin as instructed or cannot be followed-up; patients with missing clinical data or failed tissue specimen deoxyribonucleic acid (DNA) sequencing; occurrence of bleeding or death; atrial or valvular thrombosis detected by echocardiography during follow-up; use of a combination of antiplatelet or non-steroidal anti-inflammatory drugs; and vegans.

2.2. Group division

Eligible subjects were randomly divided into 2 groups. The data from cohort 1 were used to establish the algorithm model, whereas cohort 2 was used to verify the new model and calculate the ideal prediction percentage (defined as the predicted dose within ±20% of the actual dose[33]). More than 100 patients should be enrolled in cohort 2.

2.3. Clinical data collection

The collected clinical data included patient sex, age, height, weight, BSA, warfarin daily maintenance dose, smoking history, presence of concomitant diseases (such as hypertension, diabetes, and history of thrombosis), and drug combination use (such as amiodarone, ACEI, and Betaloc). BSA (m²) = 0.0061 × height (cm) + 0.0128 × weight (kg) – 0.1529. The daily maintenance dose of warfarin was the individualized oral warfarin dose of the patient, with the INR being maintained between 1.8 and 2.5 for more than 6 consecutive monitoring sessions, during which the warfarin dose did not change. Deep vein thrombosis, cerebral infarction, transient ischemic attack, and pulmonary embolism (PE) were predictors of a history of thrombotic diseases.[10] The ACEIs used in this study mainly included enalapril and valsartan.

2.4. Sampling

To avoid the pain of venous blood collection, 2 tubes of 10 mL arterial blood were extracted from the extracorporeal circulation machine for each patient after anticoagulation before transfusion treatment on the day of surgery, numbered, and then stored in a deep cryogenic refrigerator.

2.5. DNA extraction and gene sequencing

DNA was extracted from the blood samples using a DNA extraction kit, and forward and reverse primers were designed for SNP determination and then amplified by polymerase chain reaction (PCR) (9700 PCR Amplifier, ABI Corporation, CA, USA). The PCR products were verified by 1% agarose electrophoresis (150 V, 100 mA for 20 minutes).

Gene sequencing was performed using Sanger sequencing. After the PCR products were purified, the samples were run on a 3730XL sequencer (ABI), and Run 3730 data collection V3.0 software was run at the same time. The final sequencing

Table 1

Summary of clinical characteristics of study population.

Clinical variables	Cohort 1 (n = 295)	Cohort 2 (n = 100)	P value
Daily stable dose (mg)	2.608 ± 0.630	2.609 ± 0.873	.218
Age (y)	51.690 ± 10.454	51.100 ± 13.080	.870
Weight (kg)	54.270 ± 9.251	54.300 ± 9.762	.829
Height (cm)	158.386 ± 7.969	158.320 ± 8.030	.824
Body surface area (m²)*	1.510 ± 0.150	1.508 ± 0.143	.934
Sex (n [%])			
Male	111 (37.62)	34 (34)	.017
Female	184 (62.38)	66 (66)	
History (n [%])			
Previous thromboembolism†	40 (13.6)	14 (14)	.086
Smoke	27 (9.2)	9 (9)	.145
Hypertension	54 (18.3)	18 (18)	.094
Diabetes mellitus	6 (2.0)	2 (2)	.029
Concomitant medications (n [%])			
ACEI‡	11 (3.73)	4 (4)	.029
β-blocker (metoprolol)	70 (23.72)	25 (25)	.014
Amiodarone	17 (5.76)	4 (4)	.091

ACEI = angiotensin converting enzyme inhibitors.

*Body surface area = 0.0061 × height(cm) + 0.0128 × weight (kg)-0.1529.

†The previous thromboembolism were found in our patient population included deep vein thrombosis, cerebral infarction, transient ischemic attack, and pulmonary embolism.

‡Angiotensin converting enzyme inhibitors. In our patient population, this drug class included enalapril and valsartan.

results were assessed using Chromas or SeqMan software, and sequence alignment was completed using SeqMan software.

2.6. Collection of genetic variables

The genetic variables of the samples were determined. Specific SNPs included VKORC1 (rs9923231, rs9934438, rs7196161, and rs7294), CYP2C9 (rs1057910), CYP1A2 (rs2069514), GGCX (rs699664), and UGT1A1 (rs887829).

2.7. Statistical analysis

We conducted a descriptive statistical analysis of the clinical variables of the tested population. Numerical variables such as age and height are presented as means ± standard deviation and non-numeric variables were expressed as percentages. For the 2 cohort clinical variables, a *t* test or non-parametric test was used to compare the differences in baseline characteristics of the data. For the gene polymorphisms, we calculated the genotype frequency and allele frequency and performed Hardy-Weinberg equilibrium measurements. The method was used to calculate the actual frequency of each genotype, and the *P* value was calculated using the chi-square test or Fisher exact test (meaningful α = 0.05). Differences were considered statistically significant at $P > .05$, and the Hardy–Weinberg genetic equilibrium was met. In addition, the *D′* and values were calculated using the corresponding formula to evaluate whether there is a linkage disequilibrium among the SNPs.

Univariate linear regression was used to analyze the relationship between clinical and genetic variables, and the daily maintenance dose of warfarin and the independent sample *t*-test or *F* test (analysis of variance) was used to verify the effect of each genotype of the same SNP on the dose of warfarin. Multiple linear regression was used to analyze the gene polymorphisms and the contribution of clinical data variables. The variables that caused multicollinearity were screened stepwise and excluded to establish a regression model to calculate the daily maintenance dose of warfarin. Finally, the final multiple linear regression model was tested using Pearson correlation analysis. All

3. Results

3.1. Participant characteristics

In total, 476 patients were recruited for this study. After rigorous screening, 81 subjects were excluded, of which 49 patients had severe DNA degradation; 18 patients failed to follow-up regularly, and the data on maintenance dose of warfarin and INR were missing; 7 patients had incomplete clinical data; 5 patients died of heart failure or severe pneumonia during hospitalization; and 2 patients were diagnosed with left atrial thrombosis during follow-up. Finally, the clinical data and characteristics of the 395 patients were complete and met the standards for the model derivation and verification process. Among them, 295 were randomly selected as the modeling group (cohort 1) and the remaining 100 subjects were used as the model validation group (cohort 2). There were no statistical differences in baseline characteristics between the 2 groups. Clinical information of both the cohorts is summarized in Table 1, and the genetic variables are listed in Table 2.

3.2. Genotyping

The genotyping results confirmed that the allele frequency distributions of all SNPs were consistent with the Hardy–Weinberg equilibrium. While verifying linkage disequilibrium, we found that VKORC1 rs9923231 (-1639G > A) showed strong linkage disequilibrium with the other 3 SNPs of VKORC1: rs9934438 ($D″ > 0.8$, $r^2 > 0.9$), rs7196161 ($D″ > 0.8$, $r^2 > 0.9$), and rs7294 ($D′ > 0.8$, $r^2 > 0.9$). Therefore, only rs9923231 was used as a genetic factor to represent VKORC1 in the model derivation program.

3.3. Warfarin dose correlation analysis

Univariate regression analysis revealed statistically significant effects of VKORC1 (rs9923231, rs9934438, rs7196161, and rs7294), CYP2C9 (rs1057910), GGCX (rs699664), age, height, weight, BSA, and amiodarone on the daily stable dose of warfarin. Height ($R = 0.244$), weight ($R = 0.317$), and BSA ($R = 0.336$) were positively relevant factors (increasing the demand for warfarin), whereas age ($r = –0.181$) and amiodarone use ($r = –0.115$) were negatively correlated factors (reducing the demand for warfarin). We also found that the effects of CYP1A2 (rs2069514), UGT1A1 (rs887829), sex, history of thrombosis, hypertension, diabetes, smoking history, ACEI, and β-acceptor blockers on the daily stable warfarin dose were not statistically significant, as shown in Table 3.

The daily stable dose of warfarin in the VKORC1 rs9923231 (-1639G > A) AA group was 2.434 ± 0.501 mg, which was significantly lower than that in the AG group (3.347 ± 0.838 mg; $P < .001$) and GG group (5.025 ± 0.413 mg, $P = .003$). The daily stable dose of warfarin in the VKORC1 rs9934438 (1173C > T) TT group was 2.475 ± 0.557 mg, which was significantly lower than that in the CT (3.109 ± 0.844 mg; $P < .001$) and CC (4.015 ± 1.581 mg; $P = .021$) groups. The warfarin requirement of VKORC1 rs7196161 and rs7294 carriers was 85.98% and 87.26% higher than those in the AA group, and the dose requirement for carriers with each A allele increased by 25.23% and 26.57%, respectively. The daily stable dose of warfarin in the CYP2C9 rs1057910 GG group was 0.875 ± 0.177 mg, which was significantly lower than that in the GA (1.838 ± 0.595 mg; $P = .044$) and AA (2.682 ± 0.656 mg; $P < .001$) groups. In addition, the daily stable dose of warfarin in the GGCX rs699664 GG group was

Table 2

Distribution of alleles and genotypes in the study population.

Genetic variables	Cohort 1 (n = 295)				Cohort 2 (n = 100)			
	Genotype (%)		Allele (%)		Genotype (%)		Allele (%)	
VKORC1	AA	243 (82.4%)	A	(91%)	AA	82 (82%)	A	(90.5%)
rs9923231	A/G	50 (16.9%)			A/G	17 (17%)		
	GG	2 (0.7%)	G	(9.2%)	GG	1 (1%)	G	(9.5%)
VKORC1	TT	238 (80.7%)	T	(89.8%)	TT	81 (81%)	T	(90%)
rs9934438	T/C	54 (18.3%)			T/C	18 (18%)		
	CC	3 (1%)	C	(10.2%)	CC	1 (1%)	C	(10%)
VKORC1	AA	3 (1%)	A	(8.1%)	AA	1 (1%)	A	(8%)
rs7196161	A/G	42 (14.2%)			A/G	14 (14%)		
	GG	250 (84.7%)	G	(91.9%)	GG	85 (85%)	G	(92%)
VKORC1	AA	3 (1%)	A	(9.2%)	AA	1 (1%)	A	(9%)
rs7294	A/G	48 (16.3%)			A/G	16 (16%)		
	GG	244 (82.7%)	G	(90.8%)	GG	83 (83%)	G	(91%)
CYP2C9	AA	271 (91.9%)	A	(95.8%)	AA	92 (92%)	A	(95.5%)
rs1057910	A/G	23 (7.8%)			A/G	7 (7%)		
	GG	1 (0.3%)	G	(4.2%)	GG	1 (1%)	G	(4.5%)
CYP1A2	AA	17 (5.8%)	A	(28.1%)	AA	6 (6%)	A	(28.5%)
rs2069514	A/G	132 (44.7%)			A/G	45 (45%)		
	GG	146 (49.5%)	G	(71.9%)	GG	49 (49%)	G	(71.5%)
UGT1A1	AA	7 (2.4%)	A	(11.0%)	AA	2 (2%)	A	(10.5%)
rs887829	A/G	51 (17.3%)			A/G	17 (17%)		
	GG	237 (80.3%)	G	(89.0%)	GG	81 (81%)	G	(89.5%)
GGCX	AA	25 (8.5%)	A	(30.2%)	AA	9 (9%)	A	(30.5%)
rs699664	A/G	128 (43.4%)			A/G	43 (43%)		
	GG	142 (48.1%)	G	(69.8%)	GG	48 (48%)	G	(69.5%)

Table 3

Relationship between variables and stable dose of warfarin under univariate regression analysis.

Variable	P value	R^2	Adjusted R^2	r
Sex	.230	0.004	0.000	-
Age	<.001	0.033	0.030	−0.181
Height	<.001	0.059	0.057	0.244
Weight	<.001	0.100	0.098	0.317
Body surface area	<.001	0.113	0.111	0.336
Amiodarone	.005	0.020	0.017	−0.115
Metoprolol	.327	0.002	<0.001	-
ACEI	.451	0.001	−0.001	-
Previous thromboembolism	.466	0.001	−0.001	-
Smoke	.726	<0.001	−0.002	-
Hypertension	.204	0.004	0.002	-
Diabetes mellitus	.404	0.002	−0.001	-
rs9923231	<.001	0.325	0.324	-
rs9934438	<.001	0.172	0.170	-
rs7196161	<.001	0.174	0.172	-
rs7294	<.001	0.196	0.194	-
rs1057910	<.001	0.134	0.132	-
rs2069514	.213	0.004	0.001	-
rs887829	.528	0.001	−0.002	-
rs699664	.004	0.021	0.019	-

ACEI = angiotensin converting enzyme inhibitors.

2.691 ± 0.737 mg, which was higher than that in the AA group (2.313 ± 0.566 mg; P = .004). The differences in warfarin dose among the rs9923231, rs1057910, and rs699664 carriers are shown in Figure 1A–C, respectively.

3.4. Dosing algorithm model establishment

We included all the parameters into the regression model for calculation, and the final regression model obtained through stepwise regression screening and by excluding the variables causing multicollinearity are summarized in Table 4 and the deleted variables are summarized in Table 5. The predictive factors included in the final model accounted for individualized dose differences (R^2 = 61.7 %). Figure 1B shows the warfarin regression normalized residual histogram and normal P–P plot, indicating that the new regression model meets the normality assumption.

While using our model to determine warfarin dosage for a patient, clinicians must complete the following equations based on patient-specific clinical characteristics and genetic variability:

Y = 1.081 − 0.011 (age) + 1.532 (BSA) − 0.807 (rs9923231 AA) + 1.788 (rs9923231 GG) + 0.530 (rs1057910 AA) − 1.061 (rs1057910 AG) − 0.321 (rs699664 AA).

Cases in parentheses are recorded as 1, otherwise recorded as 0. Age is in years, body weight in kg, and BSA in m^2.

3.5. Validation of the dosing model

Figure 3 shows the results of comparison between the predicted dose for cohort 2 and the actual observed dose, and Pearson's correlation analysis revealed a strong correlation between them (Pearson R = 0.884; P < .001). Figure 4 shows a scatter plot of the predicted and actual dose residuals of the model. When the predicted dose is within ±20% of the actual dose, it is considered an ideal prediction (i.e., the model validation is effective). The ideal prediction for cohort 2 was 69%, for the low estimated group (the predicted dose was 20% less than the actual dose), it was 13%, and for the overestimated group, it was 18% (the predicted dose was 20% higher than the actual dose). The model tended to overestimate the dose in the low-dose group and underestimated the dose in the high-dose group.

4. Discussion

Warfarin is a coumarin anticoagulant and it has been discovered for more than 60 years. In the past 50 years, coumarin drugs were the only oral anticoagulants available to clinicians,[35] it was widely used in the treatment of non-valvular atrial fibrillation, mechanical valve replacement, deep vein thrombosis, PE, etc. However, warfarin has several disadvantages, such
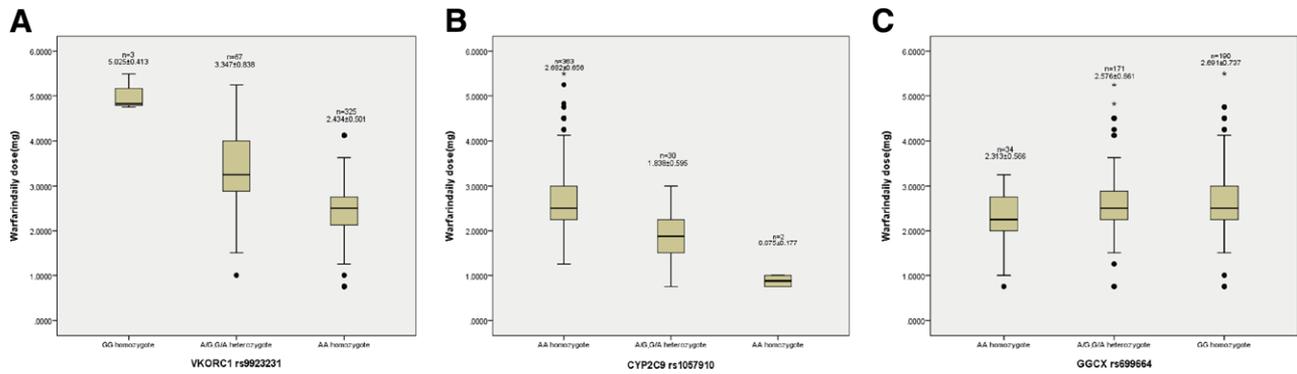
Figure 1. (A) (VKORC1 rs9923231(-1639G > A)polymorphisms), (B) (CYP2C9 rs1057910 polymorphisms), and (C) (GGCX rs699664 polymorphisms): boxplots describing the relationship between genetic polymorphisms and mean daily maintenance dose of warfarin (mg/d) in the study population n = 395. Median maintenance dose for each polymorphisms are shown as is the interquartile range.

as narrow therapeutic windows, a high incidence of bleeding or thrombotic events, and repeated venous blood collection increases patient suffering. With the advent of new oral anticoagulants, warfarin has been gradually replaced by dabigatran or rivaroxaban in the treatment of atrial fibrillation, deep venous thrombosis, or PE.[36–38] For anticoagulation after mechanical valve replacement, warfarin is still the only anticoagulant with the most reliable effect.[39] Therefore, considering the differences and risks of individualized warfarin medications, gene-based algorithm model is considered to be a faster, safer, and more economical method of systemic administration than fixed model.[31,40] And it is still of great significance to establish a drug delivery prediction model in the population after artificial mechanical valve replacement.

In this study, we designed and evaluated a regression algorithm model to predict the stable therapeutic dose of warfarin in a Southern Chinese population who underwent artificial valve replacement. Our model considers genetic and clinical variables, which can account for approximately 61.7% of individualized medication differences, and it is similar to the 57.8%[31] and 60.2%[11] reported in previous studies, but higher than the results of a large retrospective study (49.4%) published by the International Warfarin Commission on Pharmacogenetics (IWPC),[31] which may be attributed to the fact that the IWPC's research involved multiple races, had fewer gene types, and ignored the drug combination and underlying diseases.

When analyzing genetic factors, we found that genetic variables contributed much more to the model than clinical variables, owing to their large parameter values and large R^2 values. In our study, VKORC1 rs9923231 and CYP2C9 rs1057910 explained 43.4% of the individual differences, which is similar to the results reported by Wattanachai et al,[21] Liang et al,[41] and Johnson.[42] It is worth noting that the 4 SNPs of VKORC1,

rs9923231, rs9934438, rs7196161, and rs7294 were shown to have strong linkage disequilibrium, and the variations in rs9923231 (promoter region) and rs9934438 (intron region) could cause reduction of warfarin demand, whereas the variations in rs7196161 and rs7294 in the untranslated region could increase the demand for warfarin, which was consistent with the studies by Indian scholars[11] and Arab scholars.[43] Considering the strong linkage disequilibrium of the multiple SNP loci of VKORC1, we only selected rs9923231 as a representative for model derivation. Therefore, we strongly suggest that it is inappropriate to sequence VKORC1 for multiple loci to avoid unnecessary resource wastage. Furthermore, our study found that the effect of GGCX rs699664 on the daily stable dose of warfarin was statistically significant, which is consistent with the findings of Jiang et al.[19] We first retained this SNP in the final model after stepwise screening, which accounted for 2% of the individual differences. GGCX rs699664 may be an important predictor in the warfarin dose algorithm, but further research is needed in the future.

Our study confirmed that, under univariate linear regression analysis, the effects of CYP1A2 rs2069514 and UGT1A1 rs887829 on the daily stable dose of warfarin were not statistically significant. Meanwhile, when they were added to the multiple linear regression as variables, they were ultimately removed from the model due to insufficient significance or multicollinearity. For

Table 4

Final regression model obtained by screening and excluding the variables that cause multicollinearity through stepwise regression.

Variable	Partial R^2	P value	Parameter estimate
Intercept	-	-	1.081
Age	0.033	<.001	−0.011
Body surface area	0.130	<.001	1.532
rs9923231 AA	0.313	<.001	−0.807
rs9923231 GG	0.053	<.001	1.788
rs1057910 AG	0.010	.009	−1.061
rs1057910 AA	0.058	<.001	0.530
rs699664 AA	0.020	<.001	−0.321
The best regression model	0.617	<.01	-

Table 5

Variables that were removed from the procedure for the derivation of the final regression model.

Variable	Partial R^2	P value	Collinearity statistics tolerance
Sex	0.095	.107	0.815
Weight	0.108	.326	0.083
Height	−0.044	.326	0.505
Previous thromboembolism	0.003	.918	0.984
Amiodarone	0.002	.961	0.938
β-blocker (metoprolol)	0.044	.167	0.979
ACEI	−0.057	.075	0.961
Smoke	−0.045	.162	0.964
Hypertension	−0.040	.226	0.930
Diabetes mellitus	−0.018	.578	0.949
rs9923231 AG	−0.817	.065	0.052
rs1057910 GG	0.117	.342	0.066
rs2069514 AA	−0.047	.141	0.977
rs2069514 AG	−0.010	.764	0.991
rs2069514 GG	0.031	.325	0.985
rs887829 AA	−0.044	.163	0.998
rs887829 AG	−0.048	.135	0.992
rs699664 GG	0.062	.051	0.994
rs699664 AG	−0.014	.668	0.912

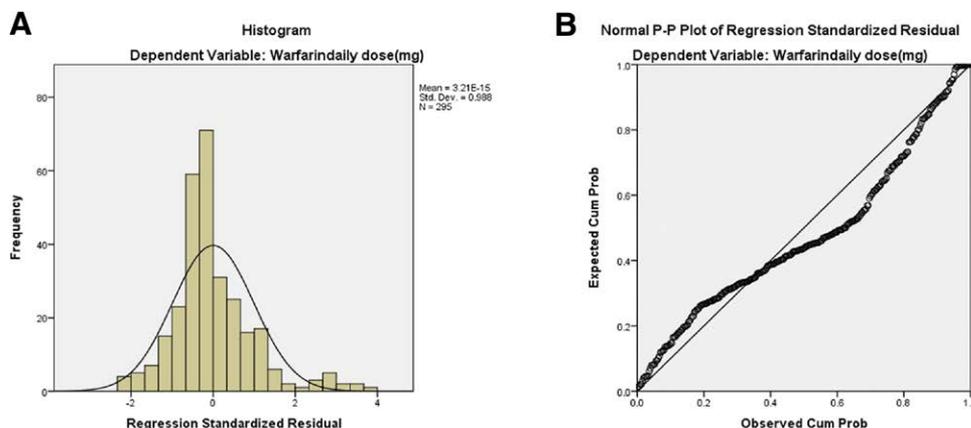ACEI = angiotensin converting enzyme inhibitors.

Figure 2. (A) The histogram of standardized residuals of warfarin regression, showing that the residuals follow a normal distribution of equal variances. (B) The normal P-P plot of the residuals, revealing the distribution of the data along the diagonal and diagonal directions, indicating that the newly established regression model in this study meets the normality assumption.

CYP1A2 rs2069514, our results were inconsistent with those of Liu et al,[16] which may be due to the fact that their study subjects were from the Northern Chinese population. Regarding UGT1A1 rs887829, Thai scholars reported results that were consistent with ours and also confirmed its lack of contribution to the model,[19] which was contrary to the findings of Korean scholars whose research also focused on Asians[20] and the specific reasons remain to be discussed.

In the analysis of clinical variables, we found that under the univariate linear regression, the effect of amiodarone on the daily stable dose of warfarin was statistically significant, and was negatively correlated with the dose of warfarin, which was consistent with previous studies.[21,23,44] However, amiodarone was excluded from the model after multiple linear regressions, possibly because of the small sample size (only 17 cases). Furthermore, we also found that smoking may not be associated with the dose of warfarin, which is contrary to the results of numerous studies that have confirmed that smoking is an important predictor of warfarin dose[45]; however, a few studies have finally applied this variable to the model. Similarly, Betaloc, ACEI, and history of thrombosis were included in the model because of their effects on the daily stable dose of warfarin, as confirmed in previous studies.[46] However, in our study, neither

unilinear regression nor multiple linear regression could make these variables important predictors, and larger-sample studies are needed for further verification.

In the verification study, the comparison between the predicted dose and the actual observed dose (Fig. 1C) revealed discrepancies but had a strong correlation ($R = 0.884$), which tended to overestimate the dose in the low-dose group (<2.0 mg/d) and underestimate the dose in the high-dose group (>4.0 mg/d); these phenomena were also observed in other studies from IWPC.[47] The model had the best prediction for the middle-dose group (2.0–4.0 mg/d) with 13% in the underestimated group and 18% in the overestimated group. Although the ideal percentage predicted by our model is as high as 69%, it is still within the range reported in previous studies (62.5%,[41] 71.6%[34]).

A limitation of our study is that we were unable to evaluate some clinical factors of patients, such as physical activity and diet structure, which may affect the daily stable dose of warfarin.[27,48] In addition, CYP4F2 rs2108622 was considered to have important predictive value in numerous studies.[10] However, we could not perform subsequent experiments due to its small individual variability in early sequencing. Finally, our algorithm model is only suitable for predicting the daily stable dose of 0.75 to 5.25 mg/d; thus, predictions beyond this range may be unreliable.
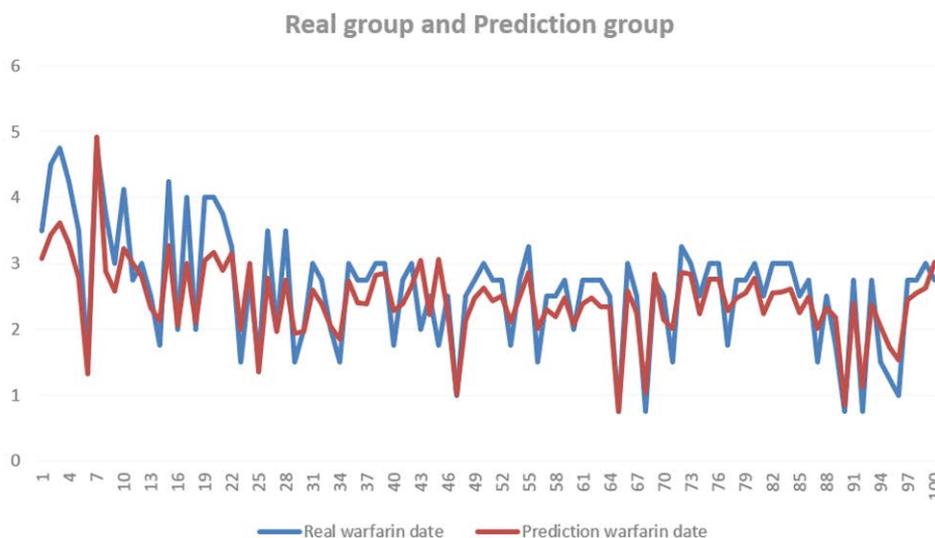

Figure 3. A comparison diagram of the relationship between the model's predicted dose (red curve) and the actual observed dose (blue curve) of cohort 2. The ordinate is the warfarin dose, and the horizontal is the serial number of the cohort 2 subjects. The 2 curves are in good agreement.
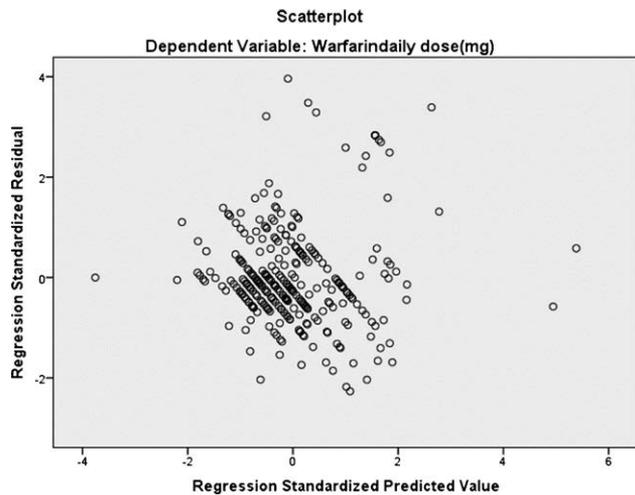
Figure 4. The residual scatter plot. Most of the scatter points are in the interval (−2, 2), indicating that the data has normality and homogeneity of variance. The regression equation can explain most of the predicted values, and the regression model is valid.

In summary, we developed a predictive model to determine appropriate doses of warfarin after artificial mechanical valve replacement in the Southern Chinese population. Preliminary studies showed that the model is useful for predicting maintenance doses with a high percentage of ideal predictions. In addition, we found that GGCX rs699664 might be an important predictor of warfarin dose calculation, and further studies are required to validate these results.

Author contributions

Jin Li: conceptualization, methodology, software, writing-original draft preparation; Tao Chen: validation, formal analysis, resources, data curation, investigation; Fangfang Jie: supervision, formal analysis, validation, software; Lidong Wu: conceptualization, writing - reviewing, supervision; Yanhua Tang: methodology, writing-reviewing, supervision, funding acquisition; Haiyan Xiang: investigation, methodology; Li Huang: investigation, resources; Hongfa Jiang: investigation, data curation, resources; Fei Lu: investigation, data curation, resources; Shuqiang Zhu: investigation, data curation.

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