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Development and comparison of a new personalized warfarin stable dose prediction algorithm in Chinese patients undergoing heart valve replacement

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Background: Pharmacogenetics-based algorithms would be especially desirable for patients undergoing heart valve replacement (HVR), who are particularly sensitive to warfarin during the initial treatment phase following surgery. We aimed to derive a warfarin dosing algorithm from data of Chinese patients undergoing HVR, and to compare it with previously published dosing algorithms as applied to our HVR patients.
Methods: 641 Chinese HVR patients on stable maintenance dose of warfarin were enrolled from a single clinic site. Data of 321 patients were used to derive a warfarin dosing algorithm using stepwise multiple linear regression analysis. Previously published algorithms were selected from Pubmed database for comparison. The performance of all the algorithms was characterized according to mean absolute error (MAE) and percentage of predicted doses falling within ± 20% of clinically observed doses (percentage of ideal prediction) in the other 320 patients.
Results: The newly developed algorithm included eight factors: VKORC1–1639G > A, CYP2C9*3, BSA, age, number of increasing INR drugs, smoking habit, preoperative stroke history and hypertension. Our algorithm accounted for 56.4% of variations in the inter-patient warfarin stable doses. All the algorithms showed better performance in a medium-dose (1.88–4.38 mg/day) and high-dose (≥ 4.38 mg/day) groupings than in a low-dose (≤ 1.88 mg/day) grouping. Compared with the 14 previously published algorithms, our algorithm had the lowest MAE (–0.07 mg/day) and the highest percentage of ideal prediction (62.8%) in the total validation cohort.
Conclusions: Our warfarin dosing algorithm is potentially useful for patients whose population profiles are similar to those of our patients.

1. Introduction

Warfarin is the most widely used oral anticoagulant worldwide for patients following heart valve replacement (HVR) to prevent thrombosis. Patients who undergo mechanical HVR (MHVR) need lifelong anticoagulation and those with bioprosthetic valves (BHVR) require a minimum of 3 months' anticoagulant treatment (Keeling et al. 2011). Warfarin has a narrow therapeutic index and exhibits extensive drug and food interactions (Well et al. 1994). Patients' responses to warfarin vary widely, both interindividually and interethnically. The response is particularly unpredictable in the initiation phase of warfarin therapy. Close monitoring of prothrombin time standardized by the international normalized ratio (INR) is routinely carried out in clinical practice. Nevertheless, warfarin was reported to be one of ten drugs with the largest number of serious adverse event reports submitted during the 1990 and 2000 decades by the US Food and Drug Administration's Adverse Event Reporting System (Wysowski et al. 2007).

Warfarin is a mixture of R and S enantiomers. S-Warfarin is about 3 to 5 times more potent than the R isomer for anticoagulation effect. CYP2C9 is the major enzyme metabolyzing S-warfarin. The various polymorphisms in CYP2C9 may alter the pharmacokinetics of warfarin to different degrees. *CYP2C9*2* and **3* are the most prevalent alleles. The enzymatic activity of CYP2C9*2/*2 and *3/*3 are 20% and 80%, respectively, lower than that of the common wild type (*1/*1) (Lindh et al. 2009). Thus patients carrying either *CYP2C9*2* or **3* alleles generally reach a desired INR range and consequently warfarin stable dosage (WSD) at a lower level of warfarin dosage (Takahashi et al. 2006).

Warfarin exerts its anticoagulant effects by inhibiting vitamin K epoxide reductase complex subunit 1 (VKORC1), which recycles vitamin K 2,3-epoxide to vitamin K hydroquinone (Wallin and Martin 1985). Vitamin K hydroquinone is a crucial cofactor in posttranslational activation of clotting factors II, VII, IX, and X by gamma-glutamyl carboxylase (GGCX). Polymorphisms in *VKORC1* may change the pharmacodynamics of warfarin. It's

reported that *VKORC1-1639G > A* (or *3673G > A,* rs9923231*)* and *1173C > T* (or *6484C > T*, rs9934438) individually account for the greatest variations in warfarin dose requirement and these two loci show almost complete linkage disequilibrium (LD) in all races (Limdi et al. 2010). Patients carrying the −1639 G allele and the 1173 C allele require much higher WSD (Yuan et al. 2005).

Numerous genotype-guided algorithms have been derived to predict WSD, most of which are based on multiple linear regression analysis by using patients' *VKORC1* and *CYP2C9* genotypes and various non-genetic data. These algorithms generally explain only about 50% of the variations in clinically observed WSD (Sconce et al. 2005; Wen et al. 2008; Gage et al. 2008; Huang et al. 2009; You et al. 2011).

It is known that HVR patients are particularly sensitive to warfarin during the initial treatment phase following surgery (Rahman et al. 2006). Therefore, effective genotype-guided algorithms would be especially desirable for HVR patients. It has been reported that a genotype-guided algorithm significantly shortened the time to reach WSD ($P = 0.001$) and increased the percentage of patients who attained WSD within 50 days ($P = 0.013$) as compared to an initial standard dose procedure for Chinese MHVR patients (Huang et al. 2009).

Chinese patients are known to have 40% to 50% lower dose requirement than Caucasians (Wen et al. 2008; Huang et al. 2009; You et al. 2011). Rheumatic heart disease affects at least 2 million adults in China, many of whom require HVR surgery as their disease progresses (Zhimin et al. 2006). Though numerous algorithms have been published, few of them were specifically derived from data of HVR patients (Kim et al. 2009; Cen et al. 2010). As of now, no study has compared the performance of previously published algorithms using data of Chinese HVR patients.

In this study, we aimed to develop an algorithm specifically from data of Chinese HVR patients, and to compare the performance of our algorithm with previously published algorithms using our HVR patients' data.

Only two SNPs were considered in our study: *CYP2C9*3* and *VKORC1-1639G > A*. Because currently only *CYP2C9*2, *3,* and *VKORC1-1639G > A*, or its equivalent, are universally recognized as biomarkers for warfarin dose response (Shin and Cao 2011). However, since *CYP2C9*2* is seldom present in the Chinese population, we did not include it in creating our algorithm. We decided that to include additional genetic factors would require multiple genotype analyses that might inhibit the adoption of our algorithm in clinical practice.

2. Investigations and results

2.1. Patient enrollment

From May 2011 to February 2012, 641 Chinese patients undergoing HVR on stable maintenance dose of warfarin were enrolled from the Department of Cardio-thoracic Surgery of the Second Xiangya Hospital. All the eligible patients had been on warfarin therapy for at least 6 weeks. WSD was defined as the same daily dose over 2 consecutive INR measurements performed at an interval of more than 7 days and the INR values were within the target ranges (Huang et al. 2009). The target INR ranges were 1.7–2.2 for Aortic Valve Replacement (AVR), 2.0–2.5 for Mitral Valve Replacement (MVR) and 2.5–3.0 for Tricuspid Valve Replacement (TVR), respectively. If a patient had undergone a double or triple valve replacement surgery, the target INR range was defined as the highest one (e.g. target INR range for patients undergoing AVR + MVR was defined as 2.0–2.5). In addition, the target INR range for BHVR patients was 0.2–0.3 lower than that for MHVR patients. The study was approved by the Ethics Committee of the Institute of Clinical Pharmacology at Central South University (CTXY-110005) and was registered at the Chinese Clinical Trial Register (ChiCTR-ONC-11001532). Written informed consent was obtained from each subject.

All the eligible patients were at least 18 years old with satisfactory hepatic and renal functions as well as platelet counts. Patients were excluded from the study if they had cancer, pregnancy, history of severe bleeding, heart failure, cardiomyopathy or concomitant administration of herbal medications (ginseng, danshen, etc).

The initial standard warfarin dosing was 2.5 mg/day on the day following surgery. Subsequent dose adjustment was done according to results from INR tests. All patients took warfarin manufactured by the same pharmaceutical company (2.5 mg per pill, Shanghai Xinyi Pharmaceutical Co., Shanghai, China).

2.2. Demographic and clinical data collection

Data were collected from patients' medical records and direct questioning. If a patient took INR interacting drugs for more than 7 days during the stable maintenance period, details of drug uses were recorded. The interacting drugs were defined based on previously published literature (Holbrook et al. 2005; Klein et al. 2009; Zhong et al. 2011). As only a few of our patients were taking interacting drugs, we divided the drugs into two categories for further analysis: INR increasing drugs (including amiodarone, statins, thyroxine) and INR decreasing drugs (including carbamazepine and phenytoin). Detailed information is listed in Table 1.

2.3. DNA extracting and genotyping

Peripheral venous blood (2 ml) was collected from each subject. Genomic DNA was extracted. Genotype analyses of *VKORC1-1639G > A* and *CYP2C9*3* were done by a standard pyrosequencing method.

2.4. Derivation of the XY warfarin dosing algorithm

The function RAND in microsoft Excel was used to randomize the dataset. 321 patients were randomly designated as the "derivation cohort" to derive a WSD prediction model to be identified as XY algorithm (XY, abbreviation for Xiangya Hosptital). The other 320 patients were designated as the "validation cohort" to test the performance of the XY and the previously published algorithms in our HVR patients.

In the derivation cohort, a univariate regression analysis was carried out to evaluate the association of each variable with WSD (demographic, clinical and genetic factors). Variables with P values < 0.10 were entered the stepwise multiple linear regression analysis and only those with P values < 0.05 were remained in the final algorithm.

2.5. Selection of previously published algorithms

The previously published algorithms were selected based on the following criteria: 1) Precise equations to predict WSD. 2) Non-genetic variables routinely collected in clinical practice. 3) Only two SNPs consisting of *VKORC1-1639G>A* (or *VKORC1 1173C > T*) and *CYP2C9*3* (and/or **2, *11, *13, *14*). 4) Published in English.

Searches were performed from Pubmed database by various combinations of MeSH terms: warfarin, algorithm, polymorphism, VKORC1 and CYP2C9. The searches were limited to

Table 1: Baseline of patient demographic, clinical and genetic characteristics

Variable	Total (n = 641)	Derivation Cohort (n = 321)	Validation Cohort (n = 320)	*P*-value
Male	258(40.2%)	132(41.1%)	126(39.4%)	0.652
Age (years)	46.6 ± 10.9	46.1 ± 11.0	47.1 ± 10.9	0.248
Height (cm)	160.60 ± 8.08	160.62 ± 8.04	160.58 ± 8.13	0.956
Weight (kg)	56.38 ± 9.31	55.77 ± 9.35	56.99 ± 9.23	0.096
BSA (m^2)	1.55 ± 0.15	1.54 ± 0.15	1.56 ± 0.15	0.208
Smoking	67(10.5%)	33(10.3%)	34(10.6%)	0.887
Drinking	33(5.1)	17(5.3%)	16(5.0%)	0.865
Warfarin Stable Dose (mg/day)	3.17 ± 1.03	3.16 ± 1.07	3.18 ± 1.00	0.541
Han-Chinese	619(96.6%)	308(96.0%)	311(97.2%)	0.390
HVR type				
BHVR	135(21.1%)	67(20.9%)	68(21.3%)	0.907
MHVR	506(78.9%)	254(79.1%)	252(78.8%)	
Target INR Range				0.548
1.7–2.2	110(17.2%)	58(18.1%)	52(16.3)	
2.0–2.5	494(77.1%)	242(75.4%)	252(78.8%)	
2.5–3.0	37(5.8%)	21(6.5%)	16(5.0%)	
HVR + Thrombectomy	55(8.6%)	17(5.3%)	29(9.1%)	0.065
Preoperative Stroke	34(5.3%)	11(5.4%)	17(5.3%)	0.243
Hypertension	49(7.6%)	27(8.4%)	22(6.9%)	0.464
Coronary Heart Disease	8(1.2%)	5(1.6%)	3(0.9%)	0.479
Diabetes	16(2.5%)	7(2.2%)	9(2.8%)	0.608
Atrial Fibrillation	207(32.3%)	104(32.4%)	103(32.2%)	0.954
Number of Increasing INR drugs	21(3.3%)	11(3.4%)	10(3.1%)	0.830
Number of Decreasing INR drugs	2(0.3%)	1(0.3%)	1(0.3%)	0.998
VKORC1-1639G>A				0.724
GG	6(0.9%)	4(1.2%)	2(0.6%)	
GA	122(19.0%)	62(19.3%)	60(18.8%)	
AA	513(80.8%)	255(79.4%)	258(80.6%)	
CYP2C9				0.720
*1/*1	582(90.8%)	289(90.0%)	293(91.6%)	
*1/*3	56(8.7%)	30(9.3%)	26(8.1%)	
*3/*3	3(0.5%)	2(0.6%)	1(0.3%)	

PubMed publications dating 1 January, 2004 to 29 February, 2012. 14 algorithms were selected, of which 4 were derived from ethnically mixed populations: Gage's (Gage et al. 2008), IWPC's (Klein et al. 2009), Takahashi's (Takahashi et al. 2006) and Anderson's (Anderson et al. 2007); 7 were from single East-Asians: Wen's (Wen et al. 2008), Huang's (Huang et al. 2009), You's (You et al. 2011), Miao's (Miao et al. 2007), Ohno's (Ohno et al. 2009), Kim's (Kim et al. 2009) and Cho's (Cho et al. 2011); 3 were from single Caucasians: Zhu's (Zhu et al. 2007), Sconce's (Sconce et al. 2005) and Wadelius' (Wadelius et al. 2009).

2.6. Validation of all the algorithms

Data from our validation cohort (n = 320) were entered into the XY and 14 previously published algorithms to generate predicted WSD. As the SNPs in VKORC1 rs9923231 and rs9934438 are almost in complete LD, the rs9923231 genotype was used for VKORC1 in all the algorithms. Since *CYP2C9*2, *11, *13, *14* are rare in the Chinese population, we substituted the wild type *CYP2C9*1/*1*for these rare alleles in the various equations. Gage's algorithm is the only one which includes the target INR, thus we defined the target INR for our patients as follows: 1) BHVR patients: AVR = 1.9, MVR = 2.1, and TVR = 2.6; 2) MHVR patients: AVR = 2.1, MVR = 2.3, and TVR = 2.8.

Performance of all the algorithms was determined using 2 metrics. 1) Mean absolute error (MAE), defined as the mean of the predicted dose minus the observed dose (Shin and Cao 2011). 2) Percentages of under, ideal and over predictions. Ideal was defined as a predicted dose falling within ± 20% of the observed dose. Under was defined as a predicted dose being more than 20% lower than the observed dose. Over was defined as a predicted dose being more than 20% higher than the observed dose (Klein et al. 2009; Shin and Cao 2011).

Algorithms showing both less than ± 1 mg/day of MAE and more than 40% of ideal prediction were further assessed in the sensitivity analysis. We elected to divide our patient WSD data into 3 dose groups: low-dose group (≤ 1.88 mg/day, n = 26), medium-dose group (1.88–4.38 mg/day, n = 250) and high-dose group (≥ 4.38 mg/day, n = 44). Because MAE and percentage of ideal prediction were inversely correlated, we determined the performance of these algorithms in the sensitivity analysis according to percentages of under, ideal and over predictions as previously defined.

As the dose adjustment was commonly made in quarter pill steps at this hospital, we set 1.88 mg (0.75 pill) and 4.38 mg (1.75 pills) as the threshold of low- and high-dose, respectively.

2.7. Statistical analyses

Genotype distribution of *VKORC1-1639 G > A* and *CYP2C9*3* polymorphisms were assessed for deviations from the Hardy–Weinberg disequilibrium using Chi square test. The normal distribution of continuous variables was checked using Shapiro–Wilk test. Square root transformation of daily WSD was performed to meet better normality and fulfill the requirement for linear regression analysis. Data regarding demographic, clinical and genetic characteristics of the patients were presented as mean ± standard deviation or counts (%), as appropriate. The baseline data in the two cohorts were compared by Student's t-test, Mann-Whitney U test, Pearson Chi

Table 2: Effects of *VKORC1-1639G > A* and *CYP2C9*3* genotypes on warfarin stable dosage (n = 641)

Genotype	n	Warfarin dose (mg/day)	*P*-value
VKORC1-1639			< 0.001
GG	6	5.57 ± 0.94	0.009[a]
GA	122	4.25 ± 1.14	< 0.001[b]
AA	513	2.88 ± 0.77	< 0.001[c]
*CYP2C9*3*			< 0.001
**1/*1*	582	3.27 ± 1.00	< 0.001[d]
**1/*3*	56	2.20 ± 0.74	0.023[e]
**3/*3*	3	1.04 ± 0.65	0.010[f]

Pair-comparisons are: a. GG vs GA; b. GA vs AA; c. AA vs GG; d. *1/*1 vs *1/*3; e. *1/*3 vs *3/*3; f. *1/*1 vs *3/*3

square test, Fisher's exact test or Monte Carlo Chi square test, as appropriate. *Krus*kal Wallis one-way AN*OVA* (*k s*amples) analysis was used to compare the difference in WSD among three genotypes of *VKORC1-1639G > A* and *CYP2C9*3*. Mann-Whitney U test was used to compare the difference in WSD between each of the two *VKORC1-1639G > A* genotypes carriers and *CYP2C9*3* genotypes carriers. Pearson's correlation analysis was used to assess the correlation between the predicted doses by XY algorithm and the observed doses of warfarin in the validation cohort. Correlation of MAE and percentage of ideal prediction was also assessed by Pearson's correlation analysis. The percentages of ideal prediction between the XY algorithm and the published algorithms were pair-compared using McNemar's chi square test. All statistical tests were two-sided with a *P* value < 0.05 significance. All analyses were performed using the Statistical Package for Social Science (SPSS 16.0, SPSS Science, Chicago, IL).

2.8. Results

2.8.1. Patients' characteristics

The characteristics of all 641 patients are listed in Table 1. The mean age was 46.6 ± 10.9 years (18 to 76 years). The mean WSD was 3.17 ± 1.03 mg/day (0.31 to 7.50 mg/day). The allele frequencies of *VKORC1-1639 G > A* and *CYP2C9*3* were in accordance with Hardy–Weinberg equilibrium. The frequencies of these two SNPs were similar to previous studies in Chinese patients. There were no significant differences in demographic, clinical or genetic characteristics between the derivation cohort (n = 321) and the validation cohort (n = 320) (all *P* > 0.05).

*2.8.2. Effects of VKORC1-1639G > A and CYP2C9*3 genotypes on WSD*

As shown in Table 2, for *VKORC1-1639G > A,* the mean WSD (mg/day) was 5.57 ± 0.94 for GG, 4.25 ± 1.14 for GA and 2.88 ± 0.77 for AA carriers, respectively. There were significant differences in WSD among the three genotypes of *VKORC1-1639G > A*. Significant difference in WSD between each of the two *VKORC1-1639G > A* genotype carriers was observed (all *P* < 0.01). For *CYP2C9*3,* the mean WSD (mg/day) was 3.27 ± 1.00 for **1/*1*, 2.20 ± 0.74 for **1/*3* and 1.04 ± 0.65 for **3/*3* carriers, respectively. There were significant differences in WSD among the three genotypes of *CYP2C9*3*. Significant differences in WSD between each of the two *CYP2C9*3* genotype carriers was also observed (all *P* < 0.05). The effects of the 7 different combinations of *VKORC1-1639G > A* and *CYP2C9*3* genotypes on WSD are illustrated in Fig. 1.

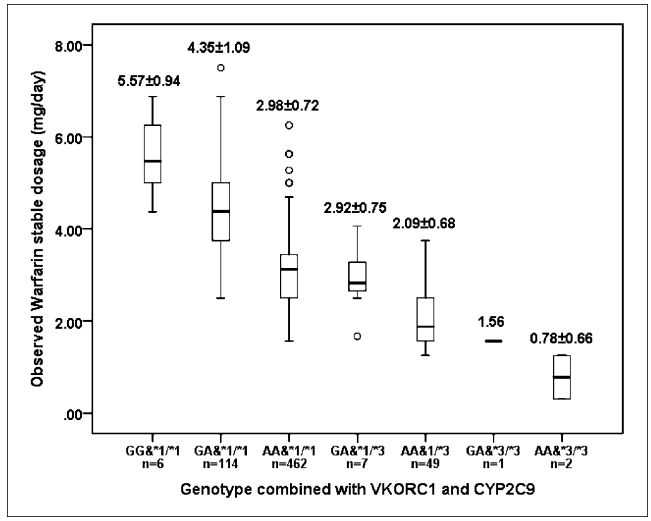

Fig. 1: The boxplot shows the distribution of clinically observed warfarin stable doses in patients with different combined genotype of VKORC1-1639G>A and CYP2C9*3. Boxes indicate the median and interquartile ranges. Vertical lines above and below boxes indicate the minimum and maximum values, respectivley. The numbers above the whiskers show mean ± SD values. Outlier is denoted with a circle

2.8.3. Regression analyses and the XY algorithm derivation

After the stepwise multiple linear regression analysis, 8 factors were selected for the final equation: *VKORC1-1639G > A*, *CYP2C9*3*, body surface area (BSA), age, number of increasing INR drugs, smoking habit, preoperative stroke history and hypertension, as shown in Table 3. The regression equation accounted for 56.4% (R^2) of the inter-patient variations in WSD. Because of failing to reach statistical significance, the following factors were not included in the derivation of our regression analysis: gender, Han-Chinese, drinking habit, target INR range, heart valve type, combination thrombectomy surgery, diabetes, coronary heart disease, atrial fibrillation and number of decreasing INR drugs.

Our final algorithm, identified as XY algorithm, for predicting WSD was:

Warfarin stable dose (mg/day) = [2.140 − 0.370 × (*VKORC1-1639G > A*) − 0.332 × (*CYP2C9*3*) + 0.324 × (BSA) − 0.004 × (age) − 0.231 × (number of increasing INR drugs) + 0.105 × (smoking habit) − 0.135 × (preoperative stroke history) − 0.108 × (hypertension)]^2

The coding was as follows: *VKORC1-1639G > A* genotype: input 0 for *GG*, 1 for *GA*, and 2 for *AA*; *CYP2C9*3* genotype: input 0, 1, or 2 for the number of **3* alleles; BSA(m^2) = 0.0061 × height(in cm) + 0.0128 × weight(in kg) − 0.1529; age in years; increasing INR drugs were defined according to previously published literature (Holbrook et al. 2005; Klein et al. 2009; Zhong et al. 2011); smoking habit, preoperative stroke history and hypertension were coded as 1 if present and 0 if absent.

2.8.4. Correlation assessment of the XY algorithm

Data from our validation cohort (n = 320) were entered into the XY algorithm to generate predicted WSD. The mean predicted WSD was 3.11 ± 0.75 mg/day and the mean clinically observed WSD was 3.18 ± 1.00 mg/day. As shown in Fig. 2, the predicted stable doses correlated positively with the clinically observed stable doses (Pearson's correlation analysis, r = 0.638, *P* < 0.001).

Table 3: Stepwise multiple linear regression analysis with the daily warfarin stable dosage as dependent variable

Independent variables	Coefficient	SE	R^2	Adjusted R^2	*P* value
Intercept	2.140	0.137	–	–	< 0.001
VKORC1-1639G > A	−0.370	0.025	0.341	0.339	< 0.001
*CYP2C9*3*	−0.322	0.034	0.446	0.443	< 0.001
BSA	0.324	0.076	0.485	0.480	< 0.001
Age	−0.004	0.001	0.514	0.508	< 0.001
Number of increasing INR drugs	−0.231	0.061	0.533	0.526	< 0.001
Smoking habit	0.105	0.038	0.546	0.537	0.007
Preoperative stroke history	−0.135	0.050	0.555	0.545	0.008
Hypertension	−0.108	0.042	0.564	0.553	0.010

Table 4: Performance of all the algorithms in the validation cohort (n = 320)

Model	Under (%)	Ideal (%)	Over (%)	*P* value	MAE (95% CI)
XY	18.1	62.8	19.1	–	−0.07 (−0.16–0.01)
Gage	13.8	56.9	29.4	0.020	0.07 (−0.02–0.15)
IWPC	8.8	54.4	36.9	0.006	0.28 (0.20–0.37)
Anderson	3.1	40.6	56.3	< 0.001	0.69 (0.60–0.78)
Takahashi	2.8	35.9	61.3	< 0.001	0.83 (0.74–0.92)
Wen	19.4	62.5	18.1	1.000	−0.15 (−0.24–0.06)
Huang	26.3	60.0	13.8	0.289	−0.30 (−0.38–0.22)
You	9.7	54.1	36.3	0.005	0.26 (0.17–0.35)
Miao	79.7	19.1	1.3	< 0.001	−1.09 (−1.17–1.00)
Ohno	11.6	55.0	33.4	0.007	0.13 (0.05–0.22)
Kim	1.3	25.6	73.1	< 0.001	1.09 (1.00–1.18)
Cho	88.8	9.4	1.9	<0.001	−1.35 (−1.45–1.25)
Zhu	11.3	54.1	34.7	0.002	0.20 (0.11–0.29)
Sconce	8.4	47.5	44.1	< 0.001	0.44 (0.33–0.55)
Wadelius	0.6	15.3	84.1	< 0.001	1.49(1.38–1.60)

2.8.5. Performance of all the algorithms

MAE and percentages of under, ideal and over predictions for each algorithm are listed in Table 4. The MAE for the XY algorithm was −0.07 mg/day. 11 algorithms had a MAE less than ± 1 mg/day (−0.07−0.83 mg/day). Our XY algorithm showed 62.8% of ideal prediction, which was the highest of all the algorithms. 10 algorithms showed the percentage of ideal prediction higher than 40% (40.6%–62.8%). 12 of the 14 published algorithms showed significantly lower percentage of ideal prediction than that of the XY algorithm (all $P < 0.05$). The percentage of over prediction was higher than the percentage of under prediction for most algorithms. MAE was inversely correlated with percentage of ideal prediction (r = −0.977), which was consistent with the previous study (Shin and Cao 2011).

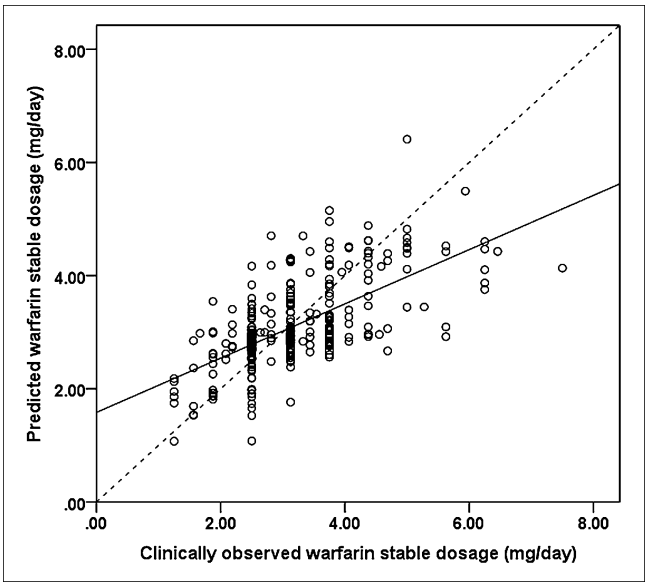

Fig. 2: The boxplot shows the distribuScatterplot of predicted versus clinically observed warfarin stable doses (n=320). The solid line is the linear regression, r=0.638, P < 0.001

2.8.6. Sensitivity analysis

More than ± 1 mg/day of MAE or less than 40% of ideal prediction disqualified 5 algorithms from the sensitivity analysis. As shown in Table 5, all the 10 algorithms which were further assessed in the sensitivity analysis showed better performance in the medium-dose (1.88–4.38 mg/day) and high-dose (≥ 4.38 mg/day) ranges than in the low-dose (≤ 1.88 mg/day) range. Generally, algorithms derived from East-Asian and racially mixed populations tended to perform better than algorithms derived from Caucasians in the medium-dose range. Algorithms derived from Caucasians and racially mixed populations tended to perform better than algorithms derived from East-Asians in the high-dose range. For the low-dose range, all the algorithms showed less than 40% of ideal prediction, and all of them tended to over predict the stable doses. The best performer in this range was our XY algorithm, which showed 38.5% of ideal prediction. For the high-dose range, the XY algorithm showed 50% of ideal prediction, which was not significantly different from all the other algorithms with one exception. For the medium-dose range, the XY algorithm showed 67.6% of ideal prediction, which was the second highest of all the algorithms. Overall, the XY algorithm performed well across all three dose ranges.

Table 5: Sensitivity analysis based on three dose ranges

Model	Dose ≤ 1.88 mg/day (n = 26)				Dose 1.88 to 4.38 mg/day (n = 250)				Dose ≥ 4.38 mg/day (n = 44)			
	Under	Ideal	Over	*P* value	Under	Ideal	Over	*P* value	Under	Ideal	Over	*P* value
XY	0	38.5	61.5	–	14.8	67.6	17.6	–	47.7	50.0	2.3	–
Gage	0	23.1	76.9	0.125	9.2	61.6	29.2	0.049	47.7	50.0	2.3	1.000
IWPC	0	19.2	80.8	0.063	4.8	57.2	38.0	0.004	36.4	59.1	4.5	0.289
Anderson	3.8	0	96.2	NA	0	39.2	60.8	< 0.001	20.5	72.7	6.8	0.013
Wen	0	26.9	73.1	0.453	14.4	70.0	15.6	0.345	59.1	40.9	0	0.289
Huang	3.8	30.8	65.4	0.625	22.4	66.8	10.8	0.883	61.4	38.6	0	0.125
You	0	19.2	80.8	0.063	6.0	56.8	37.2	0.004	36.4	59.1	4.5	0.289
Ohno	0	7.7	92.3	0.008	5.6	61.6	32.8	0.091	52.3	45.5	2.3	0.625
Zhu	0	11.5	88.5	0.016	7.2	58.8	34.0	0.009	40.9	52.3	6.8	1.000
Sconce	0	19.2	80.8	0.063	4.8	48.8	46.4	< 0.001	34.1	56.8	9.1	0.581

3. Discussion

Estimating an appropriate warfarin dosing is challenging due to warfarin's narrow therapeutic index and large inter-individual variability in drug responses. It has been suggested that genotype-guided warfarin dosing algorithms might be faster and safer than current use of standard initial dosing in the establishment of WSD (Anderson et al. 2007; Wen et al. 2008; Huang et al. 2009). Using data of 321 Chinese patients undergoing HVR, we have developed a new warfarin stable dosage prediction algorithm, identified as the XY algorithm. Our XY algorithm accounted for 56.4% of the variations in the observed WSD. In addition, we compared the performance of the XY algorithm with that of 14 previously published genotype-guided algorithms in 320 Chinese HVR patients recruited in our study. To our knowledge, this is the first attempted external validation of these published algorithms using data of Chinese HVR patients.

In our study, we confirmed that the *VKORC1-1639G > A* and *CYP2C9*3* genotypes are the major determinants affecting WSD. In our study we found that the mean WSD for *VKORC1-1639 GG* and *GA* carriers were significantly different ($P = 0.009$). The mean WSD for *CYP2C9*1/*3* and **3/*3* carriers were also significantly different ($P = 0.023$). Should these results be confirmed in further studies, it would be inappropriate to combine the data of *GG* and *GA* carriers or **1/*3* and **3/*3* carriers, in creating a warfarin dosing model, as has been done in some instances.

Age and indicators of body size (e.g. height, weight or BSA) appeared in all the selected algorithms except for the Wadelius' algorithm. Our XY algorithm included not only age and BSA, but also several other non-genetic factors: number of increasing INR drugs, smoking habit, preoperative stroke history and hypertension, which were consistent with previously published algorithms (Gage et al. 2008; Wen et al. 2008; Wadelius et al. 2009; Lenzini et al. 2010).

Compared to the 14 previously published algorithms, our XY algorithm had the lowest MAE (–0.07 mg/day) and highest count of predicted doses falling within ± 20% of clinically observed (62.8%). In the sensitivity analysis, our XY algorithm performed well across all three dose ranges. Our study implies that our algorithm is potentially useful for patients whose population profiles are similar to those of our patients.

The sensitivity analysis showed that all the algorithms had poor prediction performance using our low-dose group patients' data. This phenomenon might be explained as follows: 1) The scatter plot figures of these algorithms presented in the originally published papers showed that the linear ranges extended approximately from 2.0 to 6.0 mg/day. Our clinically observed WSD values in the low-dose range were outside of these linear ranges. 2) Other genetic factors, including *CYP2C9 C-65* (rs9332127) mutation (Chern et al. 2006; Wang et al. 2008), a newly identified *CYP2C9* haplotype (Lee et al. 2010), and SNPs (*−173C > A, −208C > T* and rs2868177) in *POR* (Cytochrome P450 oxidoreductase, the obligate electron donor to all microsomal cytochrome P450 enzymes) (Zhang et al. 2011), have been reported to result in lower dose requirement of warfarin.

There are several limitations in our study. 1) We used only two SNPs in our study. Other genetic factors may be responsible for warfarin dose variability: polymorphisms in *CYP4F2*, *GGCX, APOE, PROC, CALU, ORM1, Factors II* and *VII*. 2) Our non-genetic data was more limited than some of the previously published algorithms. Some previously published algorithms included INR values, S-warfarin in the blood or dietary Vitamin K information.

Improvement of warfarin dosing management could be important in clinical practice. Clinical management should emphasize that physicians closely follow guidelines regarding oral anticoagulation with warfarin. Patients should be made aware of the important effects of the non-genetic factors on warfarin response. It's especially important to improve patients' compliance and their appearance for the INR test appointment. Unlike in the West where warfarin is supplied in several sizes, in China warfarin is dispensed in only two sizes (2.5 mg and 3.0 mg). Chinese patients have to use a knife to create different doses of warfarin to satisfy the prescription. This probably results in inaccurate warfarin dosing.

In conclusion, we have developed and validated a new warfarin stable dosage prediction algorithm based on data of Chinese patients undergoing HVR. Our algorithm is potentially useful in clinical practice. As suggested by previous studies (Roper et al. 2010; Cho et al. 2011), it may be important to consider the characteristics of each dosing algorithm and the nature of a population in choosing the most appropriate dosing algorithms.

4. Experimental

4.1. DNA extracting

Genomic DNA was extracted using a DNA Purification Kit (Promega, CA, USA) according to the manufacture's protocol.

1) Whole blood (300 µl) was transferred to a 1.5 ml tube. 2) 900 µl Cell Lysis Solution was added to the blood, mixed by inversion, and incubated for 10 minutes at room temperature. 3) Centrifuged at 13,000× g for 20 s, discarded supernatant and vortexed pellets. 4) 300 µl Nuclei Lysis Solution was added, and then mixed by inversion. 5) 100 µl Protein Precipitation Solution was added, and then votexed for 20 s. 6) Centrifuged at 13,000 g for 3 min, and transferred the supernatant to a new tube containing 300 µl isopropanol. 7) Centrifuged at 13,000 g for 1 min, discarded supernatant, and added 70% ethanol. 8) Centrifuged at 13,000 g for 1 min, aspirated the ethanol and air-dried the pellet. 9) 100 µl DNA Rehydration Solution was added to rehydrate the DNA, and incubated for 1 h at 65 °C.

4.2. Genotyping

4.2.1. PCR amplification

PCR was amplified in a 50 μl reaction mixture containing 5 μl of 10 × PCR buffer, 2.5 mM dNTP mixture, 200 ng DNA, 0.5 unit Taq polymerase (TaKaRa, Japan) and 5 pM of each oligonucleotide primer. The cycling profile consisted of 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 57 °C (for *VKORC1*) or 50 °C (for *CYP2C9*) for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min.

Primers for PCR amplification were as follows: *VKORC1-F, 5′*- TGTTGGCCAGGCTTGTCTTAAACT-3′, *VKORC1-R, 5′*-Biotin-CCAGAAGGGTAGGTGCAACAGTA-3′, *CYP2C9-F, 5′*-Biotin-ATGCAAGACAGGAGCCACATG-3′, *CYP2C9-R, 5′*-GGGACTTCGAAAACATGGAGTTG-3′.

4.2.2. Pyrosequencing analysis

1) Biotinylated PCR products (40 μl) were immobilized on streptavidin-coated Sepharose beads in binding buffer for 10 minutes at room temperature. 2) Beads with bound DNA were separated by the Vacuum Prep Tool (Biotage AB, Uppsala, Sweden) and were treated sequentially with 70% ethanol for 5 s, denatureation buffer for 5 s and washing buffer for 10 s, and were released to a PSQ 96 plate. 3) Beads were resuspended in 40 μl annealing buffer containing 3 μl sequencing primers (10 pM), heated to 80 °C for 2 min and cooled to room temperature. 4) The sequence was analyzed using a PyroMark Q96 ID System (Qiagen, CA, US) with a SNP reagent kit. Primers for pyrosequencing are: *VKORC1-F,* 5'-GCGTGAGCCACCGCA-3', *CYP2C9-R,* 5'-TGGGGAGAAGGTCAA-3'. 5% samples were randomly selected and subjected to direct DNA sequencing by an independent commercial company and the genotyping results were 100% consistent with the pyrosequencing results.

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