

Development and Comparison of a Warfarin-Dosing Algorithm in Chinese Han Patients with Atrial Fibrillation

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Abstract

Objective: The objective of this study was to develop a new warfarin dosing algorithm based on polymorphisms in CYP2C9 and VKORC1 gene with other demographic variables from a retrospective study for accurate prediction of maintenance dosage in Han subjects with AF in China.

Methods: A total of 131 Chinese AF patients with steady warfarin daily dosage (INR reach target range) were recruited from Beijing Hospital during January to November 2011. Blood samples were taken to detect genotype distribution and allelic frequencies of target SNPs: rs1057910 and rs9934438 by direct gene sequencing technique. Demographics variables were recorded during regular visit. All variables for multivariate regression analysis were those significant predictors derived from the univariate analysis, thereafter a stepwise multiple regression analysis was performed to deduce a new dosing algorithm.

Results: Four significant predictors (CYP2C9 A1075C, VKORC1 C1173T, age and BSA) derived from univariate analysis were carried to step-wise multiple regression analysis and a new algorithm was deduced ($R^2=0.436$). Their weights for predicting of warfarin dosage were 20.8%, 11.5%, 4.5% and 6.8%, respectively.

Conclusion: For Chinese Han patients with AF who accepted oral anticoagulation drug therapy, age, BAS, CYP2C9 A1075C and VKORC1 C1173T are the predictors which highly correlated to variation of warfarin dosage. In dosing prediction system, the weight of pharmacogenetic factors is more robust than that of clinical variables. The interpretation of our algorithm could account for nearly half of heterogeneity of individualized warfarin dosage.

Keywords: Warfarin pharmacogenomics; CYP2C9; VKORC1; Atrial fibrillation

Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia and one of the most potent independent risk factors for ischemic stroke. It is associated with remarkably increased cardiovascular morbidity, mortality and disability, oral anticoagulation therapy with warfarin, a vitamin K antagonist, is the first choice for stroke prevention in patients with AF [1,2]. Owing to a narrow therapeutic index, a highly individual variation in dose needed, a long time periodic International Normalized Ratio (INR) monitoring needed and a high prevalence of bleeding complications from inappropriate dosage chose, the prescription rate of warfarin in AF patients is extremely low in China, despite its convinced efficacy. Personalized warfarin therapy became one of the most important challenges in China for prognosis improvement in AF population. A great advantages in pharmacogenomics in recent 2 decades indicated that polymorphisms in CYP2C9 and VKORC1 acted as dominant role for high-degree variability in warfarin dose from one to another [3,4]. A simple, economical and fairly accurate warfarin dosing algorithm is still expected in clinical practice of real world.

The objective of this study was to identify the correlation between polymorphisms in CYP2C9 and VKORC1 gene and individualized steady dosage of warfarin in Han subjects with AF in China, to develop a new warfarin dosing algorithm based on pharmacogenetic and demographic variables from a retrospective study for accurate prediction of maintenance dosage, comparing with some existed algorithms.

Materials and Methods

Subjects and patient selection

The protocol of our study was approved by the Ethics Committee

of Beijing Hospital, Ministry of Health. The study conformed to the principles outlined in the declaration of Helsinki. From January 2011 to December 2011, all patients with AF who accepted warfarin (3 mg a pill, Orion Corporation, Finland) therapy for CHADS2 score ≥ 1 followed up in outpatient department of Beijing Hospital were screened for their eligibilities. Only 131 Chinese Han AF patients who were 18 years old or above, received warfarin therapy targeting INR 2.0~3.0, on a stable maintenance dose of warfarin and provided written informed consent before their participations were included. Stable warfarin maintenance dose was achieved when the patient's INR monitoring were within therapeutic range (with a variation $\leq 30\%$) by a stable mean daily dose over two consecutive clinic visits separated by at least 1 week.

Exclusion criteria included:

1. Allergic to warfarin
2. Patients with hemorrhagic disorders or abnormal coagulation function
3. Patients with severe liver or kidney dysfunction.

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Data related to age, gender, height, weight, body surface area (BSA), current smoking and drinking status, type of AF, CHADS2 score, concurrent drugs and serum creatinine value were recorded during regular visit.

Genotyping

Approximately, 5 ml of peripheral blood of each patient was obtained during his first routine follow-up of INR monitoring. DNA was extracted from white blood cells using a salting-out method, and the final concentration of DNA used for PCR amplification was within 25~50 ng/µl.

The CYP2C9*3 (rs1057910) and VKORC1 C1173T (rs9934438) specific primers of PCR to amplify the extracted DNA were listed in Table 1.

The genotyping process was performed in a total volume of 25 µl containing 25 ng of genomic DNA, 1.25 U LA Taq (Takara Bio, Shiga, Japan), 1 × GC Buffer I, 2.5 mM MgCl₂, 0.4 mM dNTP and 0.2 mM of each primer. The cycling stages were 95°C for 1 min, followed by 30–35 cycles at 95°C for 30 s, 50–60°C for 30 s, 72°C for 0.5–4 min and a final extension at 72°C for 5 min. After being verified by agarose gel electrophoresis, successfully amplified PCR products were digested with 4.8 U of shrimp alkaline phosphatase (Promega, Madison, WI, USA) and 1.5 U of exonuclease I (New England Biolabs, Beverly, MA, USA) at 37°C for 30 min, followed by heat inactivation at 85°C for 15 min. The products were sequenced using the ABI Prism BigDye Terminator Cyler Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on an Applied Biosystems 3730xl DNA Analyzer or using the CEQ DTCS Quick Start Kit (Beckman Coulter, Brea, CA, USA) on the CEQ 8000 Genetic Analysis System.

Statistical analysis

All data were presented as mean ± SD deviation or frequency (%). Stable warfarin dose were logarithmic transformed to obtain a normal distribution, allowing parametric tests to be performed. Association between stable warfarin dose and demographic factors or genotypes was tested by Spearman-rank correlation, and all variables for multivariate regression analysis were those significant predictors derived from the univariate analysis, thereafter a step-wised multiple regression analysis was performed and a new dosing algorithm were deduced. Statistical analyses were done using SPSS 16.0 (SPSS Inc., Chicago, IL). A p-value<0.05 was considered statistically significant.

Results

Patient characteristics

The patients' demographics and clinical characteristics are shown in Table 2. We found 95 patients (72.5%) were male and the mean age was 61.27 ± 10.75 years. The most common type of AF for warfarin therapy was paroxysmal AF (46.6%), 79 patients (60.3%) with a CHADS2 score of 1point, 31 patients (23.7%) were current smokers, and 20.6% patients accepted a combined therapy of amiodarone. There are 20 (15.3%) patients with comorbidity of coronary artery disease (CAD) accepted antiplatelet therapy at the same time, just 5 of them accepted dual antiplatelet regimen for stent implantation due to acute coronary syndrome. The rate of statin medication is higher to 31%. The median dosage of warfarin therapy was 3.25 ± 0.96 mg/d. Figure 1 shows the frequency distribution of stable warfarin dose of all 131 patients, it ranged from 0.75 mg~9 mg daily.

Genotyping results

Genotype frequencies for CYP2C9*3 (rs1057910) and VKORC1 C1173T (rs9934438) for the study population are shown in Table 2.

	Primers	Region	Fragment size
CYP2C9*3 (rs1057910)	F(5'-3') : GGGTGGAAACCAGGTTAGGAC R(5'-3') : GGGTCCAGGGCAAAGATAAT	Exon 7	383bp
VKORC1 C1173T (rs9934438)	F(5'-3') : TCCAGGAAGAGATTGAACGTG R(5'-3') : GGAGAACACACACTGCCAGA	Intron 1	661bp

F: Forward primer; R: Reversed primer.

Table 1: PCR primers.

Variables	[n(%) or X ± SD]
age (year)	61.27 ± 10.75
Male [n(%)]	95 (72.5)
weight (Kg)	73.72 ± 12.48
height (cm)	169.56 ± 7.78
BSA (m ²)	1.83 ± 0.19
Current smoker [n(%)]	31 (23.7)
Drinker [n(%)]	32 (24.4)
Paroxysmal AF[n(%)]	61 (46.6)
Persistent AF[n(%)]	44 (33.6)
Chronic AF[n(%)]	26 (19.8)
CHADS ₂ Score	1.87 ± 0.84
≥ 2[n(%)]	52 (39.7)
<2[n(%)]	79 (60.3)
serum creatinine (µmol/L)	92.33 ± 32.08
Stable warfarin maintenance dose (mg/d)	3.25 ± 0.96
Concurrent antiplatelet agent [n(%)]	
aspirin only	11 (8.4)
clopidogrel only	4 (3.0)
dual antiplatelet therapy	5 (3.8)
Concurrent amiodarone [n(%)]	27 (20.6)
Concurrent statins [n(%)]	31 (23.7)
CYP2C9	
*1*1	122 (93.1)
*1*3	9 (6.9)
TT	113(86.3)
VKORC1	
CT	16(12.2)
CC	2(1.5)

*According to Stevenson's equation, BSA(m²)=0.0061×height (cm)+0.0128×weight (Kg)-0.1529

Table 2: Demographic, or clinical factors, genotype of CYP2C9 and VKORC1.

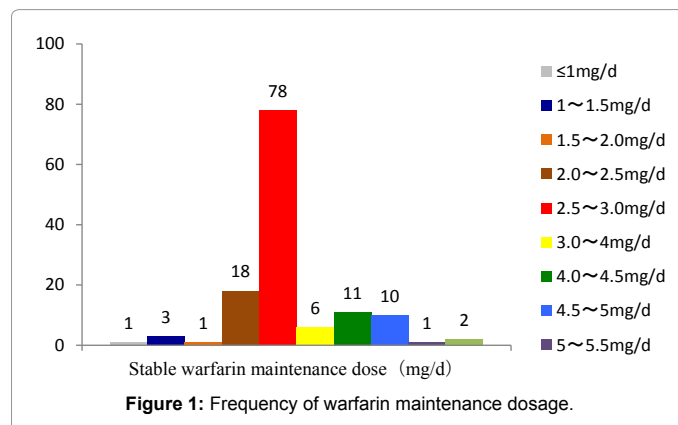


Figure 1: Frequency of warfarin maintenance dosage.

Two alleles of CYP2C9 were detected: CYP2C9*1 and CYP2C9*3 (allelic frequencies: 96.6% vs. 3.4%). And two genotypes of CYP2C9 were identified: CYP2C9*1*1 and CYP2C9*1*3 (genotype distribution : 93.1% vs. 6.9%). Two alleles of VKORC1 were found: VKORC1-T and VKORC1-C (allelic frequencies : 93.1% vs. 6.9%). And three genotypes of VKORC1 were detected: VKORC1-TT, VKORC1-CT and VKORC1-CC (genotype distribution: 86.3% vs. 12.2% vs. 1.5%). All genotypes were in Hardy-Weinberg equilibrium.

Association of demographics and genetic variables with stable warfarin dose

Univariate analysis was conducted to evaluate the association of all the clinical, demographic and genetic variables. It indicated that the logarithmic transform of stable maintenance dose was highly significantly correlated with BSA ($r=0.216$, $p=0.013$, Pearson correlation coefficient), height ($r=0.223$, $p=0.010$), and weight ($r=0.191$, $p=0.029$), and age ($r=0.15$, $p=0.05$). The predictive value of BSA, height, weight and age for warfarin dosage prediction were 5.3%, 7.9%, 3.6%, and 2.0%, respectively. The current smokers required a slightly lower warfarin dose than those who were not (3.16 ± 0.79 mg/d vs. 3.54 ± 1.32 mg/d, but this difference was not statistically significant ($p=0.054$).

Stable maintenance dose of warfarin in patients with CYP2C9*3 allele is significantly lower than those homozygous CYP2C9*1 carriers (2.14 ± 0.79 mg vs. 3.33 ± 0.92 mg, $P<0.001$). And stable dosage in patients carried VKORC1-TT genotype is significantly lower than those with VKORC1-CT or VKORC1-CC (3.10 ± 0.78 mg vs. 4.09 ± 1.35 mg, $P<0.001$) (Table 3). The weight of CYP2C9 A1075C and VKORC1 C1173T for warfarin dosage prediction was 10.1% and 15.2%, respectively (Table 4).

Four single predictors (age, BAS, CYP2C9 A1075C and VKORC1 C1173T) were carried to step wise multiple regression analysis and a new algorithm was deduced:

$Y = \exp(0.958 + 0.0026 \times \text{Age} + 0.26 \times \text{BSA} - 0.456 \times \text{CYP2C9}^*1^*3 + 0.214 \times \text{VKORC1-CT} + 0.442 \times \text{VKORC1-CC})$. The final model exhibited an R2 value of 0.436 ($P<0.001$) (Table 5).

Discussion

The risk of ischemic stroke in patients with non-valvular AF is 5-fold higher than that in general population, oral anticoagulation drug therapy is the key point for stroke prevention [1,5]. The narrow therapeutic index of warfarin and high individual variation in dose needed is a challenge in clinical care. The CHADS2 risk scores in all patients involved in our study are ≥ 1 (42%, ≥ 2), and nearly 10% of them are older than 80, therefore the indication of warfarin treatment is indubitable. The median dose of warfarin we chose to maintain INR within 2.0~3.0 was 3.15 ± 0.96 mg/d, it was significantly lower than average dosage in Caucasian, and 80% patients in study cohort accepted warfarin less than 3 mg daily [6].

Genotype		n(%)	stable warfarin maintenance dose (mg/d)	F	p
CYP2C9	*1*1	122 (93.1)	3.33 ± 0.92	14.49	<0.001
	*1*3	9 (6.9)	2.14 ± 0.79		
VKORC1	TT	113 (86.3)	3.10 ± 0.78	23.08	<0.001*
	CT	16 (12.2)	4.09 ± 1.35		
	CC	2 (1.5)	4.87 ± 1.59		

*Comparison between VKORC1 1173 CC/TC and CC by analysis of variance

Table 3: VKORC1 and CYP2C9 genotypes and stable warfarin maintenance dose (n = 131) 1.

CYP2C9	VKORC1	n(%)	stable warfarin maintenance dose (mg/d)
*1*1	TT	100 (76.9)	3.03 ± 0.54
	TC	19 (14.6)	3.79 ± 1.47
	CC	1 (0.8)	3
*1*3	TT	9 (6.9)	2.74 ± 0.75
	TC	1 (0.8)	2

Table 4: VKORC1 and CYP2C9 genotypes and stable warfarin maintenance dose (n = 131) 2.

Independent variables	Regression model	R ²	p
CYP2C9	$Y = \exp(1.688 - 0.495 \times \text{CYP2C9}^*1^*3)$	0.208	<0.001
CYP2C9 and VKORC1	$Y = \exp(1.594 - 0.458 \times \text{CYP2C9}^*1^*3 + 0.23 \times \text{VKORC1-CT} + 0.421 \times \text{VKORC1-CC})$	0.323	<0.001
CYP2C9, VKORC1 and BSA (m ²)	$Y = \exp(1.162 + 0.228 \times \text{BSA} - 0.441 \times \text{CYP2C9}^*1^*3 + 0.218 \times \text{VKORC1-CT} + 0.43 \times \text{VKORC1-CC})$	0.391	<0.001
CYP2C9, VKORC1, BSA (m ²) and Age (year)	$Y = \exp(0.958 + 0.0026 \times \text{Age} + 0.26 \times \text{BSA} - 0.456 \times \text{CYP2C9}^*1^*3 + 0.214 \times \text{VKORC1-CT} + 0.442 \times \text{VKORC1-CC})$	0.436	<0.001

Table 5: Stepwise regression modeling stable warfarin maintenance dose.

Based on the study data, we developed an algorithm of stable warfarin maintenance dose prediction, but the time to reach steady-state concentrations of a drug depends on its half-life. The average half-life of warfarin varies between 36~42 h. This means that it takes us at least one week to reach a pharmacokinetic steady state when starting on a maintenance dose we calculated. This strategy is safe with respect to over-anticoagulation, but some patients who should have taken higher doses than average dosage we predicted will be under-anticoagulated for a large part of time during the induction phase.

This study is a first cross-sectional investigation of the impact of demographic, genetic, and clinical factors on stable warfarin dose requirement in patients with AF in China reported up to date. The influence of some demographic variables on variability of stable warfarin dose, the predictive value of BSA, height, weight and age for warfarin dosage prediction were just 5.3%, 7.9%, 3.6%, and 2.0%, respectively. This is consistent with data previously reported. We found a 0.5 mg/d reduction of stable warfarin dose for every 10 years aged. Moreover, we observed that current smokers tended to require a lower daily warfarin dose than non-smokers, which may be due to the influence of polycyclic aromatic compounds contained in cigarette on hepatic drug-metabolizing enzymes and blood coagulation system [7].

In the process of variable selection, the concurrently used drug, such as amiodarone or statins, were not included in this algorithm according to the $p \leq 0.05$ significance rule. The effect of both variables could actually be the same to BSA, height, weight or age, but the precision of the estimate could be different due to proportion of taking these two drugs was fairly low.

Compared with demographic, or clinical factors, genotype mutation of CYP2C9 and VKORC1 acted as more important predictors for warfarin dose forecasting (predictive value in algorithm: 32.3% vs. 11.3%, $p<0.01$). Recently, meta-analyses identified CYP2C9 and VKORC1 polymorphism accounts for approximately 12% (4%~20%) and 27% (15%~45%) interindividual variability in stable warfarin dose requirement [8,9]. In our study, the influence of CYP2C9 on maintenance warfarin dose requirement was higher in our AF cohort (in which it accounted for 20.8% of variability), meanwhile the predictive weight of VKORC1 (11.5%) in our algorithm is a little bit

Investigators	Ethnicity	Indication	Sample (n)	R ²
Our study	Han Chinese	AF	131	43.6 (20.8/11.5)
Zhang W, et al. [15]	Han Chinese	DVT/PE	297	37.4 (20.3/3.4)
Wen MS, et al. [16]	Han Chinese	AF, DVT, stroke and cardiac valve replacement	108	48.2 (4.5/28.8)
You JHS, et al. [17]	Han Chinese in Hongkong	AF, DVT, and cardiac valve replacement	100	68 (5/47)
Cho HJ, et al. [18]	Korean	AF	130	62 (3/18)
Kim SH, et al. [19]	Korean	cardiac valve replacement	239	56 (/22.6/19.8)
Yoshizawa M, et al. [20]	Japanese	AF, DVT/PE, cardiac valve replacement, and artificial vessel	259	33.2
Klein TE, et al. [21]	Caucasians, Chinese and African Americans	AF, DVT, PE, cardiac valve replacement, stroke, and cardiomyopathy/LV dilation	3,234	47 (13.3/7.2)
Gage BF, et al. [22]	Caucasians and African Americans	AF and DVT prevention	1,015	53.1 (25/11)
Wadelius M, et al. [23]	Caucasians	AF, DVT, PE, cardiac valve replacement, stroke /TIA, and cardiomyopathy/heart failure	1,496	58.7
Zhu Y, et al. [24]	Caucasians	AF	65	61 (22/27)
Palacio L, et al. [25]	Hispanics	AF, DVT, and cardiac valve replacement	191	38.2 (11.3/7.1)

AF=Atrial Fibrillation, DVT=Deep Venous Thrombosis, PE=Pulmonary Embolism, TIA=Transient Ischemia Attack, LV=Left Ventricle

Table 6: Comparing the coefficient of different algorithms which contained CYP2C9 and VKORC1 genotype data.

lower, lower than those reported in Japanese or Korean population, but still significantly higher than the value from African Americans [10-12].

The results indicated that a considerable proportion (43.6%) of the interindividual variability in stable warfarin dose requirement in patients with AF could be attributed to variances of CYP2C9, VKORC1, age, and BSA (BSA was transformed by height and weight with Stevenson's). To accommodate the influence of all these variables, different pharmacogenomic algorithms were developed for optimizing warfarin dose. The reported pharmacogenomics-guided dosing algorithms explained warfarin dose variation over a wide range (from about 33% to over 68%) (Table 6), and the coefficient of determination in our new algorithm is similar to most of existed both in China and abroad. The remaining currently unexplained of the interindividual variability in warfarin dose requirement may relate to differences of dietary vitamin K intake, variations of VII factor, factor, prothrombin, APOE and polymorphism of CYP4F2 [13,14].

Our study has certain limitations. First of all, there were only 131 patients' INR level reached target therapeutic range among 300 patients recruited at the beginning of our study, the sample size was relatively small. Secondly, we didn't test the equation for its accuracy to predict warfarin maintenance dose in another unrelated patient population.

Although our study showed that prediction of pharmacogenomics-guided warfarin dosing algorithm was highly accurate, large scale prospectively controlled trails should be undergone to evaluate the clinical benefit of pharmacogenetic dosing models.

Conflict of Interest

None declared.

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