BJCP British Journal of Clinical Pharmacology

DOI:10.1111/j.1365-2125.2010.03698.x

CYP4F2 rs2108622: a minor significant genetic factor of warfarin dose in Han Chinese patients with mechanical heart valve replacement

Han-Jing Cen,[1,3]* Wu-Tao Zeng,[2]* Xiu-Yu Leng,[2] Min Huang,[1] Xiao Chen,[2] Jia-Li Li,[1] Zhi-Ying Huang,[1] Hui-Chang Bi,[1] Xue-Ding Wang,[1] Yan-Ling He,[3] Fan He,[3] Rui-Na Zhou,[1] Qi-Shan Zheng[1] & Li-Zi Zhao[1]

[1]School of Pharmaceutical Sciences, Sun Yat-sen University, [2]The First Affiliated Hospital of Sun Yat-sen University, [3]Guangzhou Women and Children's Medical Center, Guangzhou, China

Correspondence
Dr Li-Zi Zhao, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, China.
Tel.: +86 133 2281 3456
Fax: +86 20 3994 3000
E-mail: zhaolizi818@yahoo.com.cn
and
Professor Min Huang, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, China.
Tel.: +86 136 0978 4669
Fax: +86 20 3994 3000
E-mail: huangmin@mail.sysu.edu.cn

*These authors contributed equally to this work. Both authors are first author.

Keywords
CYP2C9, *CYP4F2*, Chinese, mechanical heart valve replacement, *VKORC1*, warfarin

Received
6 August 2009
Accepted
22 April 2010

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Genetic polymorphisms of *VKORC1* and *CYP2C9* are known to influence warfarin dosage.
- Recent studies among Caucasians showed that polymorphisms of *CYP4F2* also play a role in warfarin pharmacogenetics.
- The contribution of *CYP4F2* variants to the variability inwarfarin dose requirement in Chinese subjects remains to be investigated.

WHAT THIS STUDY ADDS

- This research was to study the effect of *CYP4F2* variants on warfarin requirements in the Han Chinese population.
- This study developed a multiple regression model including *CYP2C9*, *VKORC1* 3673G>A, *CYP4F2* genotypes and age, weight, combination use of amiodarone which could explain 56.1% of the individual variability in warfarin dose *CYP4F2* could explain 4% of the variance in warfarin dose.
- We found that one novel genotypic polymorphism 5417G>T for Asp36Tyr, which was identified as an important marker of warfarin resistance, was absent in the Han Chinese population in our study.

AIMS

The objective of this study was to assess the effect of the *CYP4F2* on the daily stable warfarin dose requirement in Han Chinese patients with mechanical heart valve replacement (MHVR).

METHODS

From March 2007 to November 2008, 222 Han Chinese MHVR patients were recruited in our study. *VKORC1* 3673G>A, 5417G>T, *CYP2C9* *3 and *CYP4F2* rs2108622 were genotyped by using the polymerase chain reaction restriction fragment length polymorphism method (PCR-RFLP). Polymorphisms of *VKORC1* 9041G>A were detected by direct sequencing. Multiple linear regression analysis was used to investigate the contribution of *CYP4F2*.

RESULTS

The *CYP4F2* rs2108622 CT/TT group took a significantly higher stable warfarin dose (3.2 mg day^{-1}) than the CC group (2.9 mg day^{-1}, 95% CI 0.2, 1.0, $P = 0.033$). The multiple linear regression model included *VKORC1* 3673G>A, *CYP2C9*, *CYP4F2* genotypes and clinical characteristics. The model could explain 56.1% of the variance in stable warfarin dose in Han Chinese patients with MHVR. *CYP4F2* contributed about 4% to the variance in the warfarin dose. There was no variation in the SNPs of *VKORC1* 5417G>T.

CONCLUSION

CYP4F2 is a minor significant factor of individual variability in the stable warfarin dose in Han Chinese patients with MHVR. The effect of *CYP2C9* and *VKORC1* genotypes on variability in the stable warfarin dose had also been confirmed.

Introduction

Warfarin, a coumarin derivative, exerts its anticoagulant effect by inhibiting vitamin K epoxide reductase (VKORC1), hence preventing vitamin K 2,3- epoxide reducing to vitamin K hydroquinone which is essential for the activation of vitamin K-dependent clotting factors [FII (or (pro-)thrombin), FVII, FIX, and FX]. It is the most commonly used oral anticoagulant for patients with mechanical heart valve replacement (MHVR) to prevent thromboembolism. However, due to the narrow therapeutic index and the wide inter-individual variation, it usually takes a long time to reach the target international normalized ratio (INR). To minimize the adverse events and to shorten the time to reach the target INR, algorithms including patients' clinical characteristics and genetic polymorphism information have been explored.

It has been reported that genetic variants in the gene encoding *VKORC1* result in altered sensitivity to warfarin [1]. A promoter polymorphism, 3673 G>A was detected in the upstream region of *VKORC1* and *VKORC1* 3673 A has been found to be associated with warfarin-'sensitive' individuals who required lower warfarin doses [1]. Recently, a novel genotypic polymorphism 5417G>T for Asp36Tyr has been identified as an important marker of warfarin resistance [2]. This polymorphism has been detected in Ashkenazi and Sephardi Jewish populations (allele frequency 4.2% and 0.6%, respectively) [3] and also Ethiopian populations (allele frequency 15%) [4], but has not yet been identified in other populations.

Warfarin is mainly metabolized by CYP2C9. The common variants of *CYP2C9* (*CYP2C9**2 and *CYP2C9**3) are known to decrease warfarin maintenance dose requirement in patients undergoing anticoagulation therapy, and detecting the polymorphism of *CYP2C9* has been proved to be helpful in optimizing the administration of warfarin in several studies [5, 6].

In the past few years, research on the pharmacogenetics of warfarin has mostly focused on *VKORC1* and *CYP2C9*. Together with weight, age and other clinical factors, about half of the interindividual variability of warfain response can be explained. However, there is still a large part of warfarin response variability that cannot be explained.

CYP4F2 is a member of cytochrome P450. It is involved in the ω-hydroxylation of arachidonic acid and vitamin E [7, 8]. Recently, McDonald *et al* [9] reported that CYP4F2 is involved in VK1 metabolism, and the polymorphism of *CYP4F2* rs2108622 could affect VK1 oxidase activity. In a comparison of VK1 oxidase activity in genotyped human liver microsomes (HLM), the HLM-CC pool (*CYP4F2* CC) exhibited the highest VK1 oxidase activity, while the HLM-TT pool (*CYP4F2* TT) exhibited a 75% reduction and the HLM-CT pool displayed an intermediate activity [9]. Therefore, the *CYP4F2* rs2108622 T carriers are likely to have higher hepatic levels of VK1, and thus require a higher warfarin dose [9]. The effect of *CYP4F2* in clinic has been studied in several studies. It was reported that a polymorphism of *CYP4F2* rs2108622 could affect warfarin dose requirement and explain approximately 2%~7% of the variability [10, 11]. A genome-wide association study (GWAS) with 1 053 Swedish subjects also considered *CYP4F2* as a minor predictor of warfarin dose [12]. These studies proved that *CYP4F2* rs2108622 T carriers had a higher warfarin dose. *CYP4F2* may be the third genetic predictor for warfarin dose.

The consideration of pharmacogenetics may help optimize the warfarin dose and minimize the risk of adverse reactions. However, because of the different genetic backgrounds and environment, the same genetic factor may exert different effects in different populations. The Chinese population is known to be warfarin sensitive, and thus needs lower warfarin doses than other populations. It is important to know the effect of these genetic factors on the warfarin response in Chinese. Also there are many patients, such as rheumatic heart disease patients, undergoing mechanical heart valve replacement in China. These patients can also suffer from thromboembolism and haemorrhage due to inappropriate warfarin dose. Therefore, it is necessary to know whether this genetic factor plays a key role in Chinese patients and how much it can explain the interindividual variability in warfarin dose.

Taking the above-mentioned into consideration, the aim of our study was to investigate the effect of *CYP4F2* polymorphisms on the stable warfarin dose and to confirm the contribution of *VKORC1*, *CYP2C9* and *CYP4F2* genetic polymorphisms by multiple regression model analysis in Han Chinese patients with MHVR.

Methods

Study population and data collection

From March 2007 to November 2008, 222 Han Chinese MHVR patients were recruited from the Cardiovascular Clinic of the First Affiliated Hospital, Sun Yat-sen University Guangzhou, PR China. Patient demographics, medical history, medication used and daily warfarin dose were recorded. The routine INR values were also recorded to determine the stable dose, defined as a constant dose for at least 3 months with the INR measurements within the range of 1.5–3.0 for multiple time periods. Patients who were younger than 18 years, or who also received a platelet aggregation inhibitor, such as aspirin, clopidogrel or dipyridamole or other drugs known to interact with warfarin (according to the report of Holbrook *et al*, such as celecoxib [13]) with the exception of amiodarone, were excluded from this study.

Blood samples (2 ml) were collected in evacuated tubes containing ethylenediaminetetraacetic acid (EDTA) for DNA extraction. The study was approved by the Ethics

Committee of Sun Yat-sen University, Guangzhou, PR China. Written informed consent was obtained from all subjects.

Genotyping

Total DNA was extracted from peripheral leukocytes by the phenol-chloroform extraction method as described [14].

All PCRs were carried out in a 25 µl volume containing 50 ng genomic DNA, 2.5 mM dNTPs, 10 mM of primer F and primer R, 2.5 ml 10×Ex Taq buffer and 0.75 U Ex Taq DNA polymerase (Takara, Japan). Details on primer sequences, amplicon sizes and restriction enzymes for *VKORC1*, *CYP2C9* and *CYP4F2* are shown in Table 1.

VKORC1 3673G>A, 9041G>A (rs7294) and the newly discovered SNP 5417G>T for Asp36Tyr were detected.

A PCR restriction fragment length polymorphism (RFLP) genotyping test to detect the 3673G>A base pair change was performed as described by Obayashi *et al*. [15]. The cycling profile consisted of the first step being held at 95°C for 5 min, followed by 35 cycles at 95°C for 60 s, 60°C for 30 s, and 72°C for 2 min and a 10 min final extension at 72°C. The 636–base pair (bp) product was digested with BcnI at 37°C for 5 h and analyzed by 2.5% agarose gel electrophoresis.

For 5417G>T, the primers were designed by Primer premier 5.0. The cycling profile consisted of the first step being held at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 61°C for 30 s, and 72°C for 1 min and a 7 min final extension at 72°C. The product was digested with AfaI. 9041G>A genotype was sequenced by direct sequencing, using primers as described by Scott *et al*. [3].

Primers for *CYP2C9* *3 (rs1057910) were described by Sullivan *et al*. [16]. The cycling profile consisted of the first step being held at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 1 min and a 7 min final extension at 72°C. PCR products were digested with AvaIII and KpnI and separated on 3% agarose gel.

CYP4F2 rs2108622 genotyping was also performed by the PCR-RFLP method. The primers were designed by Primer premier 5.0. The cycling profile consisted of the first step being held at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 60°C for 30 s and 72°C for 1 min and a 7 min final extension at 72°C. The product was digested with PvuII at 37°C over night.

Statistical analysis

Deviations from the Hardy–Weinberg equilibrium were assessed by the goodness-of-fit χ^2 test. Allele and genotype frequencies of *CYP4F2* rs2108622 were compared with Hapmap reports using the χ^2 test. LD analyses of *VKORC1* polymorphisms were performed by using Haploview.

Because the distribution of stable warfarin dose was skewed, logarithmic transformation was performed to better meet normality. Each genotype was assigned into two groups (*VKORC1* AA group and AG/GG group; *CYP2C9* *1*3/*3*3 group and *1*1 group; *CYP4F2* CC group and CT/TT group). Differences in the daily stable dose of warfarin of different genotype groups were evaluated by a two-sample *t*-test. Multiple linear regression analysis was performed to analyze the contribution of demographic characteristics (age, weight, height, gender and combination use of amiodarone) and genetic polymorphisms to the variation of daily stable warfarin dose. Variables were selected in a stepwise manner. All analyses were performed with the Statistical Package for Social Science (ver. 17.0; SPSS, Chicago, IL). A P value <0.05 was considered to be statistically significant.

Results

Patient characteristics

Table 2 summarizes the demographics and clinical characteristics of the 222 MHVR patients (104 males and 118 females, with a mean age of 45 ± 12 years). The mean daily warfarin dose requirement was 3.0 ± 1.1 mg day^{-1} and ranged from 1.1 to 8.8 mg day^{-1}.

Genotypic analysis

The genotyping results showed that the polymorphism of *VKORC1* 5417G>T was absent in this study. The allelic fre-

Table 1

Polymerase chain reaction-restriction fragment length polymorphism genotyping

	Forward primer	Reverse primer	Amplicon size (bp)	Restriction enzyme	Reference
***CYP4F2* rs2108622**	5′-CGGAACTTGGACCATCTACA-3′	5′-CCTACTCTCCCACAGGCATTA-3′	439	PvuII	
***VKORC1* 3673G>A**	5′-ATCCCTCTGGGAAGTCAAGC-3′	5′-CACCTTCAACCTCTCCATCC-3′	636	BcnI	[15]
***VKORC1* 9041G>A**	5′-TTTGCTTTGGCATGTGAGCCTTGC-3′	5′-ACAGTCCATGGCAGACACATGGTT-3′	282		[3]
***VKORC1* 5417G>T**	5′-AACCTGGAGATAATGGGCAGCA-3′	5′-ACACCGATCCCAGACTCCAGAATA-3′	351	AfaI	
***CYP2C9**3**	F1 5′-AATAATAATATGCACGAGGTCCAGA GATGC-3′ F2 5′-AATAATAATATGCACGAGGTCCAGA GGTAC-3′	5′-GATACTATGAATTTGGGACTTC-3′	141 141	AvaIII KpnI	[16, 29]

Table 2

Demographic characteristics of MHVR patients

Demographic characteristic	Patients (222)
Age ± SD (range) (years)	45 ± 12 (17–77)
Gender [*n* (%)]	
Male	104 (47)
Female	118 (53)
Weight ± SD (kg)	57 ± 10
Height ± SD (cm)	162 ± 7.50
Combination use of amiodarone [*n* (%)]	12 (5)
Stable warfarin dose ± SD (mg day^{-1})	2.9 ± 1.4
Target INR ± SD	2.1 ± 0.3

Table 3

Allelic frequency and genotype frequency distribution of *VKORC1*, *CYP2C9* and *CYP4F2* in Chinese MHVR patients

Gene	SNP	Genotype	Frequency number (%)	Allele	Frequency number (%)
VKORC1	3673 G>A	GG	4 (2)	G	61 (14)
		AG	53 (24)	A	383 (86)
		AA	165 (74)		
	9041G>A	GG	162 (73)	G	380 (86)
		AG	56 (25)	A	64 (14)
		AA	4 (2)		
	5417G>T	GG	160	G	320
		GT	0	T	0
		TT	0		
CYP2C9	42613A>C	*1*1	203 (91)	*1	424 (96)
		*1*3	18 (8)	*3	20 (4)
		*3*3	1 (1)		
CYP4F2	rs2108622	CC	115 (52)	C	322 (73)
		CT	92 (41)	T	122 (27)
		TT	15 (7)		

quencies of other SNPs (*VKORC1* 3673G>A, 9041G>A, *CYP2C9* *3 and *CYP4F2* rs2108622) were accorded with Hardy–Weinberg equilibrium. Table 3 shows the allelic frequencies and genotype distributions in Han Chinese patients with MHVR. *VKORC1* 3673 G>A and 9041G>A SNPs were in strong linkage disequilibrium (LD) in this study (D' = 0.963, r^2 = 0.864).

The *CYP4F2* rs2108622 genotype frequency was not significantly different from Han Chinese (Beijing) HapMap populations [17]. There were only 15 patients carrying the TT genotype.

Impact of VKORC1, CYP2C9 *and* CYP4F2 *polymorphisms on stable warfarin dose*

The associations of stable warfarin dose and *VKORC1*, *CYP2C9* and *CYP4F2* genotypes are shown in Table 4.

For *VKORC1* 3673 G>A genotype, patients with the AA genotype required a lower dose of warfarin (2.6 mg day^{-1}), AG/GG carriers required a higher dose (4.0 mg day^{-1}) (95% CI 1.3, 1.6, $P < 0.001$). Conversely, a variant genotype of *VKORC1* 9041G>A (AG/AA groups) had a higher stable warfarin dose than that of the GG groups (95% CI 1.3, 1.6, $P < 0.001$).

Patients with the*CYP2C9* variant genotype *3*3/*1*3 required much less warfarin than wild type patients (2.1 mg day^{-1} *vs.* 2.9 mg day^{-1}; 95% CI 1.3, 1.7, $P < 0.001$). The dose of the one patient carrying *3*3 was 1.3 mg day^{-1}.

CYP4F2 rs2108622 genotype also had a significant correlation with stable warfarin dose. The CT/TT group (*CYP4F2* T carriers) took a significantly higher stable warfarin dose (3.2 mg day^{-1}) than the CC group (2.9 mg day^{-1}; 95% CI 0.2, 1.0, $P = 0.033$).

Multiple regression analysis was used to depict the impact of clinical characteristics and genotypes on stable warfarin dose. The result shows that the model including *VKORC1* 3673G>A, *CYP2C9**3, *CYP4F2* rs2108622, age, weight and combination use of amiodarone together could explain 56.1% of individual differences in stable warfarin dose (Table 5). In this analysis, *VKORC1* and *CYP2C9* contributed most to the interindividual variability in warfarin dose, accounting for 40.1% and 26.3%, respectively. *CYP4F2* was a minor but significant factor, which could explain approximately 4% of individual differences in stable warfarin dose.

Discussion

In our study, the association between *CYP4F2* and stable warfarin dose was investigated, and the allele frequency of *CYP4F2* rs2108622 between Chinese and other ethnic groups was also compared. To our knowledge, this is the first research to study the effect of *CYP4F2* variants on warfarin requirements in Han Chinese population. Until now no relevant research has been done in other ethnic groups besides Caucasians [12, 18–20]. The Chinese population is known to be warfarin sensitive, and thus needs a lower warfarin dose than Caucasians. These two populations have quite different genetic backgrounds [21, 22]and therefore it is very important to investigate role of *CYP4F2* in the Chinese population. In this study, only Han Chinese patients with mechanical heart valve replacement were included. The study confirmed the association of *CYP4F2* rs2108622 with warfarin dose in a Chinese population and this association could be explored to use in the clinic.

As we know, distribution of the *VKORC1* 3673G>A genotype is significantly different between Chinese and Caucasians, which could explain the different dosage between these two ethnic groups [1, 21]. However, in our study, there was no significant difference in the allele frequency of the *CYP4F2* rs2108622 between Han Chinese (28%) and Caucasians (23%). Thus, *CYP4F2* is definitely not the genetic factor for ethnic differences in warfarin dose.

However, *CYP4F2* still could be the genetic factor for interindividual variability in warfarin dose. We found in this

Table 4

Comparison of mean stable warfarin doses for the four studied SNPs

Gene	SNP	Genotype	Number of patients (%)	Mean stable warfarin dose (mg day^{-1} ± SD)	95% CI*	95% CI on the difference**	*P* value
VKORC1	3673 G>A	GG/AG	57 (26)	4.0 ± 1.3	3.7, 4.4	1.3, 1.6	<0.001
		AA	165 (74)	2.7 ± 0.8	2.6, 2.8		
	9041G>A	GG	162 (73)	2.7 ± 0.8	2.6, 2.8	1.3, 1.6	<0.001
		AG/AA	60 (27)	4.0 ± 1.3	3.7, 4.3		
CYP2C9	42613A>C	*1*1	203 (91)	3.1 ± 1.1	3.0, 3.3	1.3, 1.7	<0.001
		*1*3/*3*3	19 (9)	2.1 ± 0.6	1.8, 2.4		
CYP4F2	rs2108622	CC	115 (52)	2.9 ± 1.1	2.7, 3.1	0.2, 1.0	0.033
		CT/TT	107 (48)	3.2 ± 1.1	3.0, 3.4		

*95% confidence interval; **95% CI on the difference: on the difference between different genotype groups.

Table 5

Multiple linear regression model for stable warfarin dose based on age, weight, *CYP2C9*, *VKORC1* 3673G>A, *CYP4F2* and combination use of amiodarone

Variable	Beta-standardized coefficients	*P* value	Partial r^2
***VKORC1* 3673 G>A**	–0.551	<0.001	0.401
***CYP2C9**3**	–0.401	<0.001	0.263
***CYP4F2* rs2108622**	0.135	0.003	0.040
Age	–0.096	0.036	0.020
Weight	0.102	0.026	0.023
Amiodarone	–0.343	<0.001	0.211

General r^2 model: 56.1% = percentage of stable interindividual variability explained by this model. Partial-univariate r^2 values (%) are also shown.

study that *CYP4F2* rs2108622 T carriers required about 0.3 mg day^{-1} more warfarin dose than patients with C homozygous. This variability is minor but significant. It is smaller than that reported by Caldwell *et al*. [11] and Brgiani *et al*. [10], who demonstrated that patients who were T homozygous required more than 1 mg day^{-1} more warfarin than patients who were C homozygous. However, in our study *CYP4F2* rs2108622 explained about 4% of the intervariability in stable warfarin dose, which is more than that reported by Caldwell *et al*. [11] and Takeuchi *et al*. [12], who demonstrated that *CYP4F2* rs2108622 could explain 2% and 1.5% of intervariability in warfarin response, respectively. This may due to the ethnic differences. It is well known that the Chinese population is warfarin sensitive and most patients thus need a lower warfarin dose. It makes the overall interindividual variability in stable warfain dose in the Chinese smaller than that in Caucasians. Even though the variability between different *CYP4F2* rs2108622 genotype groups is smaller than other reports, this genetic factor contributes more to interindividual variability of warfarin response in Han Chinese population.

Most of the previous reports included various kinds of indications for warfarin use, while our study only included patients with mechanical heart valve replacement. Since INR is known to be a kind of sensitive index, which is easily affected by patients' conditions, such as combination diseases and medication [23–26], our study may be less affected by such confounding factors and can reflect the effect of *CYP4F2* more accurately.

In this study, we also confirmed the impact of *VKORC1* and *CYP2C9* on stable warfarin dose. We investigated the effects of *VKORC1* 3673G>A, 9041G>A, and a new SNP *VKORC1* 5417G>T. However, *VKORC1* 5417G>T did not exist in our studied population. Previous studies have shown that this mutation was common in Ethiopian (15%) and existed in Jewish populations [3, 4]. This polymorphism is relevant to warfarin resistance, but Han Chinese people are known to be warfarin sensitive. Thus, it is not surprising that we did not observe this polymorphism. Together with *VKORC1* 3673G>A, *CYP2C9*, *CYP4F2* genotypes and age, weight, combination use of amiodarone, the multiple linear regression model could explain about 56.1% of the variation in the stable warfarin doses.

Most Chinese patients are warfarin sensitive, and are at risk of suffering haemorrhages. Many attempts have been made to use the pharmacogenentic approach to predict warfarin dose [27, 28]. *VKORC1* and *CYP2C9* are definitely the most important genetic factors, which can explain about 40% of intervariability in stable warfarin dose. Our research has confirmed a new meaningful genetic predictor (*CYP4F2* rs2108622) of the warfarin dose required for Chinese patients, which could explain about 4% of the intervariability in the stable warfarin dose. In the future, *CYP4F2* rs2108622 together with other genetic factors could be applied to prospective studies to evaluate further its power and to guide personalized pharmacotherapy in Han Chinese patients with MHVR.

Competing interests

There are no competing interests to declare.

The work was supported by the National Major Projects for science and technology development from Science and Technology Ministry of China (Grant no. 2009ZX09304-003) and the Guangzhou Bureau of Science And Technology Fund (Grant no. 2007Z3-E0081).

REFERENCES

1 Yuan HY, Chen JJ, Lee MT, Wung JC, Chen YF, Charng MJ, Lu MJ, Hung CR, Wei CY, Chen CH, Wu JY, Chen YT. A novel functional VKORC1 promoter polymorphism is associated with inter-individual and inter-ethnic differences in warfarin sensitivity. Hum Mol Genet 2005; 14: 1745–51.

2 Loebstein R, Dvoskin I, Halkin H, Vecsler M, Lubetsky A, Rechavi G, Amariglio N, Cohen Y, Ken-Dror G, Almog S, Gak E. A coding VKORC1 Asp36Tyr polymorphism predisposes to warfarin resistance. Blood 2007; 109: 2477–80.

3 Scott SA, Edelmann L, Kornreich R, Desnick RJ. Warfarin pharmacogenetics: CYP2C9 and VKORC1 genotypes predict different sensitivity and resistance frequencies in the Ashkenazi and Sephardi Jewish populations. Am J Hum Genet 2008; 82: 495–500.

4 Aklillu E, Leong C, Loebstein R, Halkin H, Gak E. VKORC1 Asp36Tyr warfarin resistance marker is common in Ethiopian individuals. Blood 2008; 111: 3903–4.

5 Hillman MA, Wilke RA, Yale SH, Vidaillet HJ, Caldwell MD, Glurich I, Berg RL, Schmelzer J, Burmester JK. A prospective, randomized pilot trial of model-based warfarin dose initiation using CYP2C9 genotype and clinical data. Clin Med Res 2005; 3: 137–45.

6 Caraco Y, Blotnick S, Muszkat M. CYP2C9 genotype-guided warfarin prescribing enhances the efficacy and safety of anticoagulation: a prospective randomized controlled study. Clin Pharmacol Ther 2008; 83: 460–70.

7 Powell PK, Wolf I, Jin R, Lasker JM. Metabolism of arachidonic acid to 20-hydroxy-5,8,11, 14-eicosatetraenoic acid by P450 enzymes in human liver: involvement of CYP4F2 and CYP4A11. J Pharmacol Exp Ther 1998; 285: 1327–36.

8 Sontag TJ, Parker RS. Cytochrome P450 omega-hydroxylase pathway of tocopherol catabolism. Novel mechanism of regulation of vitamin E status. J Biol Chem 2002; 277: 25290–6.

9 McDonald MG, Rieder MJ, Nakano M, Hsia CH, Rettie AE. Cyp4f2 is a vitamin K1 oxidase: an explanation for altered warfarin dose in carriers of the V433m variant. Mol Pharmacol 2009; 75: 1337–46.

10 Borgiani P, Ciccacci C, Forte V, Sirianni E, Novelli L, Bramanti P, Novelli G. CYP4F2 genetic variant (rs2108622) significantly contributes to warfarin dosing variability in the Italian population. Pharmacogenomics 2009; 10: 261–6.

11 Caldwell MD, Awad T, Johnson JA, Gage BF, Falkowski M, Gardina P, Hubbard J, Turpaz Y, Langaee TY, Eby C, King CR, Brower A, Schmelzer JR, Glurich I, Vidaillet HJ, Yale SH, Qi Zhang K, Berg RL, Burmester JK. CYP4F2 genetic variant alters required warfarin dose. Blood 2008; 111: 4106–12.

12 Takeuchi F, McGinnis R, Bourgeois S, Barnes C, Eriksson N, Soranzo N, Whittaker P, Ranganath V, Kumanduri V, McLaren W, Holm L, Lindh J, Rane A, Wadelius M, Deloukas P. A genome-wide association study confirms VKORC1, CYP2C9, and CYP4F2 as principal genetic determinants of warfarin dose. PLoS Genet 2009; 5: e1000433.

13 Holbrook AM, Pereira JA, Labiris R, McDonald H, Douketis JD, Crowther M, Wells PS. Systematic overview of warfarin and its drug and food interactions. Arch Intern Med 2005; 165: 1095–106.

14 Blin N, Stafford DW. A general method for isolation of high molecular weight DNA from eukaryotes. Nucleic Acids Res 1976; 3: 2303–8.

15 Obayashi K, Nakamura K, Kawana J, Ogata H, Hanada K, Kurabayashi M, Hasegawa A, Yamamoto K, Horiuchi R. VKORC1 gene variations are the major contributors of variation in warfarin dose in Japanese patients. Clin Pharmacol Ther 2006; 80: 169–78.

16 Sullivan-Klose TH, Ghanayem BI, Bell DA, Zhang ZY, Kaminsky LS, Shenfield GM, Miners JO, Birkett DJ, Goldstein JA. The role of the CYP2C9-Leu359 allelic variant in the tolbutamide polymorphism. Pharmacogenetics 1996; 6: 341–9.

17 The International HapMap Consortium. The International HapMap Project. Nature 2003; 426: 789–96.

18 Cooper GM, Johnson JA, Langaee TY, Feng H, Stanaway IB, Schwarz UI, Ritchie MD, Stein CM, Roden DM, Smith JD, Veenstra DL, Rettie AE, Rieder MJ. A genome-wide scan for common genetic variants with a large influence on warfarin maintenance dose. Blood 2008; 112: 1022–7.

19 Borgiani P, Ciccacci C, Forte V, Romano S, Federici G, Novelli G. Allelic variants in the CYP2C9 and VKORC1 loci and interindividual variability in the anticoagulant dose effect of warfarin in Italians. Pharmacogenomics 2007; 8: 1545–50.

20 Caldwell MD, Berg RL, Zhang KQ, Glurich I, Schmelzer JR, Yale SH, Vidaillet HJ, Burmester JK. Evaluation of genetic factors for warfarin dose prediction. Clin Med Res 2007; 5: 8–16.

21 Larramendy-Gozalo C, Yang JQ, Verstuyft C, Bodin L, Dubert L, Zhang Y, Xu C, Fan L, Jaillon P, Becquemont L. Genetic polymorphism of vitamin K epoxide reductase (VKORC1) 1173C>T in a Chinese and a Caucasian population. Basic Clin Pharmacol Toxicol 2006; 98: 611–3.

22 Wiwanitkit V. Pharmacogenomic effect of cytochrome P450 2C9 polymorphisms in different populations. Clin Appl Thromb Hemost 2006; 12: 219–22.

23 Perini JA, Struchiner CJ, Silva-Assuncao E, Santana IS, Rangel F, Ojopi EB, Dias-Neto E, Suarez-Kurtz G. Pharmacogenetics of warfarin: development of a dosing algorithm for Brazilian patients. Clin Pharmacol Ther 2008; 84: 722–8.

24 Hillman MA, Wilke RA, Caldwell MD, Berg RL, Glurich I, Burmester JK. Relative impact of covariates in prescribing warfarin according to CYP2C9 genotype. Pharmacogenetics 2004; 14: 539–47.

25 Gage BF, Eby C, Milligan PE, Banet GA, Duncan JR, McLeod HL. Use of pharmacogenetics and clinical factors to predict the maintenance dose of warfarin. Thromb Haemost 2004; 91: 87–94.

26 Aquilante CL, Langaee TY, Lopez LM, Yarandi HN, Tromberg JS, Mohuczy D, Gaston KL, Waddell CD, Chirico MJ, Johnson JA. Influence of coagulation factor, vitamin K epoxide reductase complex subunit 1, and cytochrome P450 2C9 gene polymorphisms on warfarin dose requirements. Clin Pharmacol Ther 2006; 79: 291–302.

27 Wang TL, Li HL, Tjong WY, Chen QS, Wu GS, Zhu HT, Hou ZS, Xu S, Ma SJ, Wu M, Tai S. Genetic factors contribute to patient-specific warfarin dose for Han Chinese. Clin Chim Acta 2008; 396: 76–9.

28 Miao L, Yang J, Huang C, Shen Z. Contribution of age, body weight, and CYP2C9 and VKORC1 genotype to the anticoagulant response to warfarin: proposal for a new dosing regimen in Chinese patients. Eur J Clin Pharmacol 2007; 63: 1135–41.

29 Yasar U, Eliasson E, Dahl ML, Johansson I, Ingelman-Sundberg M, Sjoqvist F. Validation of methods for CYP2C9 genotyping: frequencies of mutant alleles in a Swedish population. Biochem Biophys Res Commun 1999; 254: 628–31.