Association of Genetic Polymorphisms with Warfarin Dose Requirements in Chinese Patients

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Objective: Warfarin is a commonly used anticoagulant with a narrow therapeutic range and large interindividual differences in dosing requirements. Previously, studies have identified that the interindividual variability was influenced by varieties of factors, including age, body size, vitamin K intake, interacting medications, as well as genetic variants. We aimed to investigate the effect of single-nucleotide polymorphisms (SNPs) on the interindividual variability of warfarin dose requirements in Chinese patients. **Methods:** The study population consisted of 300 patients with a stable maintenance dose of warfarin. We examined SNPs in eight genes involving in the biotransformation and mode of action of warfarin (i.e., *CYP4F2*, *CYP2C19*, *APOE*, *CALU*, *EPHX1*, *PROC*, *CYP2C9*, and *GGCX*) using the SNaPshot assay. **Results:** The mean daily warfarin dose in patients carrying *CYP2C19* rs3814637CC, *CYP2C9* rs1057910AA, and *GGCX* rs699664AA genotype was 3.39, 3.34, and 3.51 mg/day, respectively, which was higher than those carrying *CYP2C19* rs3814637TT, *CYP2C9* rs1057910CC, and rs699664GG genotype (2.00, 0.81, and 3.09 mg/day, respectively). **Conclusion:** These findings indicate that individuals carrying the *CYP2C19* rs3814637CC or *CYP2C9* rs1057910AA or *GGCX* rs699664AA genotype needed higher warfarin doses in the Chinese population.

Introduction

WARFARIN IS A commonly used anticoagulant prescribed for patients with chronic atrial fibrillation, deep vein thrombosis, pulmonary embolism, recurrent stroke, and prosthetic heart valves (Oldenburg *et al.*, 2007). However, there are numerous challenges in clinical practice because of interindividual differences in drug responses, a narrow therapeutic range, and the risk of bleeding (Landefeld and Beyth, 1993; Takahashi and Echizen, 2003). Incorrect dosage administration may cause a high risk of either potentially devastating bleeding or failure to prevent thrombosis (Landefeld and Beyth, 1993). The therapeutic response must be monitored by serial determinations of the prothrombin time/international normalized ratio (INR). Several factors have been reported to influence therapeutic warfarin dose requirements, including age, body size, vitamin K intake, interacting medications, as well as genetic variants (Gage *et al.*, 2004; Kamali *et al.*, 2004).

Cytochrome P450 oxidoreductase is a membrane-bound enzyme, which plays critical roles in the metabolism of many

drugs, including warfarin (Guengerich, 2008). Recently, pharmacogenomics studies have demonstrated that single-nucleotide polymorphisms (SNPs) in genes involving in warfarin metabolism and action may contribute to interindividual differences in patients' responses to warfarin (Lee *et al.*, 2009; Cen *et al.*, 2010; Pathare *et al.*, 2012; Valentin *et al.*, 2012; Wang *et al.*, 2012; Zhong *et al.*, 2012). Nevertheless, some of the results were not conclusive, even in the same ethnic group. Cen *et al.* (2010) reported that patients carrying *CYP4F2* rs2108622 CT/TT genotypes had a significantly higher stable warfarin dose than those carrying the rs2108622 CC genotype in a Chinese population, whereas Lee *et al.* (2009) did not find that the *CYP4F2* rs2108622 polymorphism influenced warfarin dosage in another Chinese population. Therefore, it is of great value to replicate the association between SNPs and warfarin responses.

In this study, we chose and genotyped SNPs in eight genes involving in the biotransformation and mode of action of warfarin (i.e., *CYP4F2*, *CYP2C19*, *APOE*, *CALU*, *EPHX1*, *PROC*, *CYP2C9*, and *GGCX*). We aimed to investigate the

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effect of these SNPs on the interindividual variability of warfarin dose requirements in Chinese patients.

Materials and Methods

Study population

The study population consisted of 300 unrelated patients who were enrolled from the Yan An Hospital, Kunming Medical University. The study protocol was approved by the University Ethics Committee, and all subjects signed the written informed consent. All the patients were Chinese Han individuals who lived in the Yunnan province of southwest China. The inclusion criteria were age ≥ 18 years and current treatment with a stable maintenance dose of warfarin. Patients with a history of liver dysfunction (serum transaminase levels greater than 1.5 times the upper limit of normal) or renal dysfunction (serum creatinine levels $>120 \mu\text{M}$) were excluded. After written informed consent was obtained, EDTA-anticoagulated peripheral blood was collected for genetic analysis and clinical information was recorded through patient interviews (Table 1).

Genotyping

Genomic DNA was extracted from peripheral blood using the phenol/chloroform method. The SNPs were genotyped using the SNaPshot assay. The resulting data were analyzed using an ABI Prism 3130 DNA analyzer (Applied Biosystems, Foster City, CA). To ensure quality control, DNA sequencing was performed to confirm the results, and they were 100% concordant.

Statistical analysis

Baseline demographic and clinical characteristics were analyzed in patients with warfarin treatment. Data are expressed as numbers for categorical variables and mean $\pm$ standard deviation for numerical variables. Associations between genotypes and warfarin dose requirements were examined by one-way analysis of variance (ANOVA). All the data were analyzed using the SPSS for windows software package version 15.0 (SPSS, Inc., Chicago, IL). A p -value less than 0.05 was considered as statistical significance.

Results

We analyzed the frequency of 19 SNPs in 300 patients under stable anticoagulation with warfarin. Demographic and clinical

characteristics of the patients are summarized in Table 2. Of the 19 SNPs, 3 polymorphisms (i.e., *CYP2C19* rs3814637, *CYP2C9* rs1057910, and *GGCX* rs699664) exhibited a significant association with warfarin dose requirements (Table 2). The mean warfarin dose in patients with the *CYP2C19* rs3814637CC genotype was 3.39 mg/day, which was higher than that in patients with the *CYP2C19* rs3814637TT genotype (2.00 mg/day). The mean warfarin dose in patients with the *CYP2C9* rs1057910AA genotype was 3.34 mg/day, which was higher than that in patients with the *CYP2C9* rs1057910CC genotype (0.81 mg/day). The mean warfarin dose in patients with the *GGCX* rs699664AA genotype was 3.51 mg/day, which was higher than that in patients with the *GGCX* rs699664GG genotype (3.09 mg/day).

Discussion

In this study, we genotyped the SNPs in eight genes involving in warfarin metabolism and action (i.e., *CYP4F2*, *CYP2C19*, *APOE*, *CALU*, *EPHX1*, *PROC*, *CYP2C9*, and *GGCX*), and examined the contribution of the SNPs to the warfarin maintenance dose required in Chinese patients. We found that individuals carrying the *CYP2C19* rs3814637CC or *CYP2C9* rs1057910AA or *GGCX* rs699664AA genotype needed higher warfarin doses when compared with those carrying the *CYP2C19* rs3814637TT or *CYP2C9* rs1057910CC or *GGCX* rs699664GG genotype. These findings indicate that personalized dosing guided by genotyping data from *CYP2C19* rs3814637 or *CYP2C9* rs1057910 or *GGCX* rs699664 may be a solution for the safety of warfarin therapy.

The cytochrome P450 superfamily is a group of enzymes that plays key roles in the metabolism and bioactivation of many drugs, such as warfarin. Warfarin is metabolized primarily by the liver, with more than one cytochrome P450 isoenzyme involved in the metabolism. Previous studies have identified that *CYP2C9* is responsible for the metabolism of 90% of S-warfarin and *CYP2C19* for the metabolism of R-warfarin (Shek *et al.*, 1999). At present, management of warfarin therapy in clinical practice is difficult due to large interindividual variability, and the interindividual variability may be explained by the genetic variants of the warfarin metabolizing enzymes (Kimura *et al.*, 2007).

The gene that encodes *CYP2C9* is located on chromosome 10q24 in humans (Gray *et al.*, 1995). Previously, several polymorphic variants of the *CYP2C9* gene have been identified. Among them, the *CYP2C9**2 (R144C, rs1799853) and *CYP2C9**3 (I359L, rs1057910) variants were known to influence warfarin dose requirements (Higashi *et al.*, 2002). In this study, we found that *CYP2C9* rs1057910CC genotype required lower doses of warfarin in Chinese patients. Our data were consistent with previous results, which reported that the *CYP2C9* rs1057910C allele was associated with diminished warfarin clearance and a lower average warfarin dose (Steward *et al.*, 1997; Sanderson *et al.*, 2005; Gan *et al.*, 2011; Eriksson and Wadelius, 2012). As for the *CYP2C9**2, Ross *et al.* (2010) reported that the variant leads to reduced warfarin metabolism and increased risk of bleeding in Caucasian subjects, but is very rare in Chinese. In this study, we failed to detect the *CYP2C9**2 in Chinese patients, which confirmed the result of Ross *et al.* (2010). These results suggested that the *CYP2C9* polymorphism exhibited ethnic specificity and association studies investigating the influence of *CYP2C9*

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF WARFARIN-TREATED PATIENTS

Characteristics	Patients (n = 300)
Age (years)	47.9 $\pm$ 12.5
Gender	
Male	138
Female	162
Goal INR range	1.5–3.0
Warfarin dose (mg/day)	3.28 $\pm$ 1.19

Age and warfarin dose are shown as mean $\pm$ SD.

INR, international normalized ratio; SD, standard deviation.

TABLE 2. ASSOCIATION OF GENETIC POLYMORPHISMS WITH WARFARIN DOSE REQUIREMENTS IN CHINESE PATIENTS

Genes	SNPs	Genotypes	n	Warfarin dose (mean $\pm$ SD, mg/day)	P- Value
CYP4F2	rs2108622	CC	172	3.26 $\pm$ 1.16	0.47
		CT	120	3.31 $\pm$ 1.31	
		TT	8	3.66 $\pm$ 1.28	
	rs2189784	GG	135	3.22 $\pm$ 1.05	0.44
		GA	124	3.35 $\pm$ 1.26	
		AA	41	3.34 $\pm$ 1.15	
CYP2C19	rs3814637	CC	251	3.39 $\pm$ 1.19	<0.001
		CT	43	2.84 $\pm$ 1.01	
		TT	5	2.00 $\pm$ 0.97	
	rs4417205	CC	154	3.37 $\pm$ 1.22	0.32
		CG	105	3.20 $\pm$ 1.19	
		GG	41	3.23 $\pm$ 1.08	
	rs17879456	GG	162	3.35 $\pm$ 1.30	0.30
		GA	118	3.24 $\pm$ 1.11	
		AA	20	3.12 $\pm$ 1.06	
APOE	rs7412	TT	6	2.95 $\pm$ 0.54	0.52
		TC	28	3.60 $\pm$ 1.60	
		CC	266	3.25 $\pm$ 1.15	
	rs429358	CC	6	2.84 $\pm$ 0.88	0.30
		CT	17	3.14 $\pm$ 1.00	
		TT	277	3.30 $\pm$ 1.21	
CALU	rs1006023	GG	4	3.03 $\pm$ 0.68	0.68
		GT	69	3.25 $\pm$ 1.33	
		TT	227	3.29 $\pm$ 1.16	
	rs2307040	TT	5	3.40 $\pm$ 0.76	0.91
		TC	49	3.27 $\pm$ 1.29	
		CC	246	3.28 $\pm$ 1.18	
	rs339054	GG	22	3.35 $\pm$ 1.34	0.23
		GT	138	3.14 $\pm$ 1.16	
		TT	140	3.40 $\pm$ 1.19	
EPHX1	rs2292566	AA	23	3.26 $\pm$ 1.36	0.35
		AG	129	3.17 $\pm$ 1.11	
		GG	148	3.36 $\pm$ 1.23	
	rs4653436	AA	6	3.03 $\pm$ 1.49	0.37
		AG	85	3.20 $\pm$ 1.00	
		GG	209	3.32 $\pm$ 1.26	
	rs1877724	TT	32	3.07 $\pm$ 0.98	0.40
		TC	117	3.49 $\pm$ 1.21	
		CC	151	3.16 $\pm$ 1.20	
PROC	rs1799809	AA	201	3.33 $\pm$ 1.22	0.73
		AG	79	3.16 $\pm$ 1.11	
		GG	20	3.32 $\pm$ 1.19	
	rs2069910	CC	14	2.98 $\pm$ 0.99	0.40
		CT	125	3.39 $\pm$ 1.29	
		TT	160	3.23 $\pm$ 1.13	
CYP2C9	rs1057910	AA	276	3.34 $\pm$ 1.17	<0.001
		AC	22	2.71 $\pm$ 1.03	
		CC	2	0.81 $\pm$ 0.26	
	rs9332127	GG	281	3.35 $\pm$ 1.35	0.63
		GC	19	3.39 $\pm$ 1.25	
GGCX	rs699664	AA	24	3.51 $\pm$ 1.44	0.01
		AG	137	3.46 $\pm$ 1.24	
		GG	119	3.09 $\pm$ 1.04	
	rs12714145	TT	33	3.35 $\pm$ 1.35	0.30
		TC	146	3.39 $\pm$ 1.25	
		CC	104	3.20 $\pm$ 1.02	

SNP, single-nucleotide polymorphism.

polymorphism on warfarin dose requirements in different ethnic groups were valuable.

In addition to the CYP2C9 polymorphism, several other genetic variants have been presented to affect interindividual variability in the response to warfarin therapy, such as CYP2C19. Lane *et al.* (2012) reported that the CYP2C19 rs3814637 genotype had a significant impact on R-warfarin clearance. Indeed, the effect of CYP2C19 on warfarin has been studied extensively (Scordo *et al.*, 2002; Obayashi *et al.*, 2006; Lee *et al.*, 2007; Uno *et al.*, 2008; Lane *et al.*, 2012). However, limited data are shown in the Chinese population, especially in patients living in the southwest region of China. In the current study, we found that CYP2C19 rs3814637 genotype influenced the between-subject variability, which may be used as a reference in developing dosing algorithms.

Gamma-glutamyl carboxylase (GGCX) is known as the vitamin K-dependent carboxylase, which activates a subset of proteins with calcium-binding properties. The GGCX gene is located on chromosome 2p12 in human, consisting of 15 exons (Kuo *et al.*, 1995; Wu *et al.*, 1997). Over the past decades, the association of GGCX polymorphisms with warfarin maintenance-dose requirements has been extensively investigated (Wadelius *et al.*, 2005; Herman *et al.*, 2006; Cha *et al.*, 2007; Kimura *et al.*, 2007; Rieder *et al.*, 2007). Conflicting results, however, was obtained. Wadelius *et al.* (2005) reported that a common nonsynonymous polymorphism Q325R (rs699664) was not associated with warfarin dose in a Swedish population. In contrast, Kimura *et al.* (2007) reported that the polymorphism explained 4.6% of the variation in warfarin dose in Japanese patients. In this study, we found that the GGCX rs699664 polymorphism was associated with increased sensitivity to warfarin in Chinese patients, suggesting that it may be used as a predictor of warfarin maintenance dose.

Even though we found that the CYP2C19 rs3814637, CYP2C9 rs1057910, and GGCX rs699664 genotypes were related to the dose of warfarin therapy, there are some limitations in the study. The power is not very strong due to the moderate sample size. Moreover, all the patients included in the study were Chinese, and thus, the results cannot be directly extended to other populations. Further studies with a larger sample size should be conducted to confirm the results.

In conclusion, this study provides evidence that patients with the CYP2C19 rs3814637CC or CYP2C9 rs1057910AA or GGCX rs699664AA genotype required higher warfarin doses in the Chinese population. The genotyping information might help to guide warfarin dosage for clinicians. However, replication studies with a larger sample size and a longer follow-up are needed to obtain an algorithm suitable for the Chinese population.

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Authors' Contributions

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Author Disclosure Statement

The authors of this article have no conflicts of interest with the information presented in any submitted manuscript, including consultancies, stock ownership, or other equity interests, patent licensing arrangements, and payments for conducting or publicizing a study described in the manuscript.

References

- Cen HJ, Zeng WT, Leng XY, *et al.* (2010) CYP4F2 rs2108622: a minor significant genetic factor of warfarin dose in Han Chinese patients with mechanical heart valve replacement. *Br J Clin Pharmacol* 70:234–240.
- Cha PC, Mushiroda T, Takahashi A, *et al.* (2007) High-resolution SNP and haplotype maps of the human gamma-glutamyl carboxylase gene (GGCX) and association study between polymorphisms in GGCX and the warfarin maintenance dose requirement of the Japanese population. *J Hum Genet* 52: 856–864.
- Eriksson N, Wadelius M (2012) Prediction of warfarin dose: why, when and how? *Pharmacogenomics* 13:429–440.
- Gage BF, Eby C, Milligan PE, *et al.* (2004) Use of pharmacogenetics and clinical factors to predict the maintenance dose of warfarin. *Thromb Haemost* 91:87–94.
- Gan GG, Phipps ME, Lee MM, *et al.* (2011) Contribution of VKORC1 and CYP2C9 polymorphisms in the interethnic variability of warfarin dose in Malaysian populations. *Ann Hematol* 90:635–641.
- Gray IC, Nobile C, Muresu R, *et al.* (1995) A 2.4-megabase physical map spanning the CYP2C gene cluster on chromosome 10q24. *Genomics* 28:328–332.
- Guengerich FP (2008) Cytochrome p450 and chemical toxicology. *Chem Res Toxicol* 21:70–83.
- Herman D, Peternel P, Stegnar M, *et al.* (2006) The influence of sequence variations in factor VII, gamma-glutamyl carboxylase and vitamin K epoxide reductase complex genes on warfarin dose requirement. *Thromb Haemost* 95:782–787.
- Higashi MK, Veenstra DL, Kondo LM, *et al.* (2002) Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. *JAMA* 287:1690–1698.
- Kamali F, Khan TI, King BP, *et al.* (2004) Contribution of age, body size, and CYP2C9 genotype to anticoagulant response to warfarin. *Clin Pharmacol Ther* 75:204–212.
- Kimura R, Miyashita K, Kokubo Y, *et al.* (2007) Genotypes of vitamin K epoxide reductase, gamma-glutamyl carboxylase, and cytochrome P450 2C9 as determinants of daily warfarin dose in Japanese patients. *Thromb Res* 120:181–186.
- Kuo WL, Stafford DW, Cruces J, *et al.* (1995) Chromosomal localization of the gamma-glutamyl carboxylase gene at 2p12. *Genomics* 25:746–748.
- Landefeld CS, Beyth RJ (1993) Anticoagulant-related bleeding: clinical epidemiology, prediction, and prevention. *Am J Med* 95:315–328.
- Lane S, Al-Zubiedi S, Hatch E, *et al.* (2012) The population pharmacokinetics of R- and S-warfarin: effect of genetic and clinical factors. *Br J Clin Pharmacol* 73:66–76.
- Lee MT, Chen CH, Chou CH, *et al.* (2009) Genetic determinants of warfarin dosing in the Han-Chinese population. *Pharmacogenomics* 10:1905–1913.
- Lee S, Hwang HJ, Kim JM, *et al.* (2007) CYP2C19 polymorphism in Korean patients on warfarin therapy. *Arch Pharm Res* 30:344–349.
- Obayashi K, Nakamura K, Kawana J, *et al.* (2006) VKORC1 gene variations are the major contributors of variation in warfarin dose in Japanese patients. *Clin Pharmacol Ther* 80:169–178.
- Oldenburg J, Bevans CG, Fregin A, *et al.* (2007) Current pharmacogenetic developments in oral anticoagulation therapy: the influence of variant VKORC1 and CYP2C9 alleles. *Thromb Haemost* 98:570–578.
- Pathare AV, Al Zadjali S, Misquith R, *et al.* (2012) Warfarin pharmacogenetics: polymorphisms of the CYP2C9, CYP4F2, and VKORC1 loci in a genetically admixed Omani population. *Hum Biol* 84:67–77.
- Rieder MJ, Reiner AP, Rettie AE (2007) Gamma-glutamyl carboxylase (GGCX) tagSNPs have limited utility for predicting warfarin maintenance dose. *J Thromb Haemost* 5:2227–2234.
- Ross KA, Bigham AW, Edwards M, *et al.* (2010) Worldwide allele frequency distribution of four polymorphisms associated with warfarin dose requirements. *J Hum Genet* 55: 582–589.
- Sanderson S, Emery J, Higgins J (2005) CYP2C9 gene variants, drug dose, and bleeding risk in warfarin-treated patients: a HuGEnet systematic review and meta-analysis. *Genet Med* 7:97–104.
- Scordo MG, Pengo V, Spina E, *et al.* (2002) Influence of CYP2C9 and CYP2C19 genetic polymorphisms on warfarin maintenance dose and metabolic clearance. *Clin Pharmacol Ther* 72:702–710.
- Shek KL, Chan LN, Nutescu E (1999) Warfarin-acetaminophen drug interaction revisited. *Pharmacotherapy* 19:1153–1158.
- Steward DJ, Haining RL, Henne KR, *et al.* (1997) Genetic association between sensitivity to warfarin and expression of CYP2C9*3. *Pharmacogenetics* 7:361–367.
- Takahashi H, Echizen H (2003) Pharmacogenetics of CYP2C9 and interindividual variability in anticoagulant response to warfarin. *Pharmacogenomics J* 3:202–214.
- Uno T, Sugimoto K, Sugawara K, *et al.* (2008) The effect of CYP2C19 genotypes on the pharmacokinetics of warfarin enantiomers. *J Clin Pharm Ther* 33:67–73.
- Valentin II, Vazquez J, Rivera-Miranda G, *et al.* (2012) Prediction of warfarin dose reductions in Puerto Rican patients, based on combinatorial CYP2C9 and VKORC1 genotypes. *Ann Pharmacother* 46:208–218.
- Wadelius M, Chen LY, Downes K, *et al.* (2005) Common VKORC1 and GGCX polymorphisms associated with warfarin dose. *Pharmacogenomics J* 5:262–270.

- Wang HJ, Ma C, Yang J, *et al.* (2012) CYP4F2 polymorphism as a genetic risk factor for major hemorrhagic complications in Chinese patients on warfarin therapy. *J Geriatr Cardiol* 9:209–210.
- Wu SM, Stafford DW, Frazier LD, *et al.* (1997) Genomic sequence and transcription start site for the human gamma-glutamyl carboxylase. *Blood* 89:4058–4062.
- Zhong SL, Yu XY, Liu Y, *et al.* (2012) Integrating interacting drugs and genetic variations to improve the predictability of warfarin maintenance dose in Chinese patients. *Pharmacogenet Genomics* 22:176–182.

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