RESEARCH ARTICLE

The Study of *CYP2C19* Genetic Polymorphisms in Thai Patients Taking Stable Doses of Warfarin

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Abstract

CYP2C19 is one of the metabolizing enzymes involved in the metabolism of both *R*- and *S*-warfarin. The aim of this study was to investigate the association of *CYP2C19* polymorphisms on the variability of stable warfarin doses in Thai patients. A total of 254 Thai patients with stable warfarin doses were enrolled in the study. Demographics and clinical data e.g. age, body mass index, and concomitant medications, were recorded. The single nucleotide polymorphisms in the *CYP2C19*2* and *CYP2C19*3* were detected from gDNA using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), while *CYP2C19*17* was detected by using TaqMan® allelic discrimination assay. The results showed that there were no significant differences in the mean stable warfarin doses after adjustment for confounding factors among those of four CYP2C19 metabolizer statuses including the extensive metabolizers (*CYP2C19*1/*1* or *CYP2C19*2/*17* or *CYP2C19*3/*17)*, and the intermediate metabolizers (*CYP2C19*1/*2* or *CYP2C19*1/*3* genotypes), the poor metabolizers (*CYP2C19*2/*2* or *CYP2C19*2/*3* genotypes), and the rapid metabolizers (*CYP2C19 *1/*17* genotype) ($p > 0.05$). In conclusion, the genetic polymorphisms of drug metabolizing enzyme *CYP2C19* were not associated with the stable warfarin doses in Thai patients. In addition, *CYP2C19* genotypes may not be a useful predictor of warfarin dose adjustments in clinical practice.

Keywords: *CYP2C19*, genetic polymorphisms, warfarin, Thai patients

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การศึกษาภาวะพหุสัณฐานทางพันธุกรรมของ *CYP2C19* ในผู้ป่วยชาวไทยที่ได้รับยาวาร์ฟารินขนาดคงที่

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บทคัดย่อ

เอนไซม์ไซโตโครม พี 450 2C19 (CYP2C19) เป็นหนึ่งในเอนไซม์ที่เกี่ยวข้องกับการเปลี่ยนแปลงยา warfarin ในรูป *R* และ *S* วัตถุประสงค์ของการศึกษานี้เพื่อศึกษาความสัมพันธ์ของภาวะพหุสัณฐานทางพันธุกรรมของยีน *CYP2C19* ที่มีต่อความแปรปรวนของขนาดยาวาร์ฟารินในผู้ป่วยชาวไทย ซึ่งการศึกษานี้ได้คัดเลือกผู้ป่วยซึ่งได้รับขนาดยาวาร์ฟารินในขนาดคงที่จำนวน 254 ราย โดยมีการบันทึกข้อมูลพื้นฐาน ข้อมูลทางคลินิกของผู้ป่วย เช่น อายุ ดัชนีมวลกาย และยาที่ใช้ร่วม การศึกษาภาวะพหุสัณฐานทางพันธุกรรมของ *CYP2C19*2* และ *CYP2C19*3* ใช้วิธี polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) ส่วน *CYP2C19*17* ใช้ TaqMan® allelic discrimination assay ซึ่งผลการทดลองไม่พบความแตกต่างของค่าเฉลี่ยขนาดคงที่ของยาวาร์ฟารินในผู้ป่วย 4 กลุ่มที่แบ่งตามความสามารถในการทำงานของเอนไซม์ CYP2C19 หลังจากปรับด้วยค่าตัวแปรกวน ได้แก่ กลุ่มเปลี่ยนแปลงยาได้ปกติ (จีโนไทป์แบบ *CYP2C19*1/*1* หรือ *CYP2C19*2/*17* หรือ*CYP2C19*3/*17*), กลุ่มเปลี่ยนแปลงยาได้ปานกลาง (จีโนไทป์แบบ *CYP2C19*1/*2* หรือ *CYP2C19*1/*3*), กลุ่มเปลี่ยนแปลงยาได้น้อย (จีโนไทป์แบบ *CYP2C19*2/*2* หรือ *CYP2C19*2/*3*) และกลุ่มเปลี่ยนแปลงยาได้เร็ว (จีโนไทป์แบบ *CYP2C19 *1/*17*) ($p > 0.05$) จึงสรุปได้ว่าไม่พบความสัมพันธ์ของภาวะพหุสัณฐานทางพันธุกรรมของยีน *CYP2C19* และขนาดยาวาร์ฟารินในผู้ป่วยชาวไทย ยิ่งกว่านั้นลักษณะจีโนไทป์ของยีน *CYP2C19* จึงอาจไม่ใช่ตัวทำนายที่มีประโยชน์ของการปรับขนาดยาวาร์ฟารินในเวชปฏิบัติ

คำสำคัญ: *CYP2C19*, ภาวะพหุสัณฐานทางพันธุกรรม, ยาวาร์ฟาริน, ผู้ป่วยชาวไทย

Introduction

Warfarin is one of the most widely used oral anticoagulants prescribed for patients with atrial fibrillation, deep vein thrombosis, pulmonary embolism, recurrent stroke, and prosthetic heart valves.[1] Warfarin is administered as a racemic mixture of *S*- and *R*-enantiomers. *S*-warfarin is 3-5 times more potent an inhibitor of vitamin K epoxide reductase complex subunit 1 (VKORC1) than *R*-warfarin.[2] *S*-warfarin is mainly metabolized by the polymorphic cytochrome P450 2C9 (CYP2C9), while *R*-warfarin is metabolized by multiple CYP enzymes including CYP1A2, CYP2C19, and CYP3A4.[3] Although highly efficacious, warfarin has several limitations including a narrow therapeutic index and the high inter-individual variability in dosing requirements.[4] Several non-genetic and genetic factors have been reported to influence warfarin dose requirements. Two major genes have been clearly associated with the variability of warfarin response including *CYP2C9* and *VKORC1*.[5] Taken together, non-genetic factors, *VKORC1* and *CYP2C9*, only explain approximately 50-58% of the variability in warfarin dosage.[6,7] Therefore, further studies are required to investigate the additional factors contributing to the unexplained variations of warfarin dose requirements.

The previous *in vitro* study reported that CYP2C19 metabolized both *R*- and *S*-warfarin to 6-, 7-, and 8-hydroxywarfarin.[8] Extensive metabolizers were subjects with the homozygous wild type allele *CYP2C19*1* whereas poor metabolizers were subjects who carried both loss-of-function or null alleles **2* or **3*.[9] The most common loss-of-function allele in a Thai population is *CYP2C19*2* (rs4244285), a 681G>A substitution, leading to a splice-defective site.[10,11] Moreover, *CYP2C19*3* (rs4986893), a 636G>A mutation, leads to a premature stop codon which *CYP2C19*2* and/or *CYP2C19*3* affects the pharmacokinetic variability of CYP2C19 substrate drugs.[9] Previous data showed that the area under the plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$) and the elimination half-life of *R*-warfarin with poor metabolizers of CYP2C19 were significantly higher than those in homozygous extensive metabolizers.[12] The higher *R*-warfarin $AUC_{0-\infty}$ seen in poor metabolizers of CYP2C19 has still not been interpreted into clinical relevance of warfarin therapy.

Pharmacogenetic studies, however, have reported the conflicting findings on the clinical relevance including the warfarin dose requirements of *CYP2C19* polymorphisms. The warfarin maintenance dose of extensive metabolizers with *CYP2C19*1/*1* was significantly higher than those patients with *CYP2C19*1/*2*, **1/*3*, **2/*2*, **2/*3*, and **3/*3* genotypes (all $P < 0.05$).[13] Moreover, the warfarin maintenance dose of the intermediate metabolizers with *CYP2C19*1/*2* and **1/*3* genotypes were significantly higher than those poor metabolizers with *CYP2C19*2/*2*, **2/*3*, and **3/*3* genotypes.[13] No statistically significant differences, however, in warfarin doses and/or *R*-warfarin clearances were found among patients with *CYP2C19*1/*1*, *CYP2C19*1/*2*, and *CYP2C19*2/*2* genotypes.[14,15] Apart from warfarin dose requirements, the previous study has reported a higher incidence of bleeding complications in patients who carried variant alleles *CYP2C19*2* and/or **3*.[16]

The gain-of-function variant allele *CYP2C19*17* (rs12248560) is associated with increased CYP2C19 activity as compared to *CYP2C19*1*.[17] A previous finding

showed that the clearance of *R*-warfarin was significantly higher in patients carrying *CYP2C19*17* allele as compared to *CYP2C19*2*.[18] Moreover, the warfarin INR/dose ratios were decreased by 37 and 42 % in patients carrying *CYP2C19*17* and patients with the *CYP2C19*2/*17* genotype, as compared to *CYP2C19*2* carriers.[18] In contrast, no statistically significant differences in warfarin doses were observed in patients carrying *CYP2C19*17* allele, as compared to those with *CYP2C19*1/*1* genotype.[14] The implications of *CYP2C19*17* on the clinical effects of warfarin require further study. Taken together, the influence of *CYP2C19* polymorphisms on clinical relevance of warfarin treatment (e.g. warfarin dose requirements) is still controversial. The aim of this study was to investigate the potential effects of *CYP2C19*2*, *CYP2C19*3*, and *CYP2C19*17* on stable warfarin dose variability.

Materials and methods

Patients

This study was approved by The Khon Kaen University Ethics Committee for Human Research (HE581068). Written informed consent was obtained from all patients. A total of 254 patients at The Queen Sirikit Heart Center of Northeast Thailand and Srinagarind Hospital of the Faculty of Medicine, Khon Kaen University who had stable doses of warfarin, were retrospectively recruited from the outpatient clinic during 2015-2016. Indications for warfarin in these patients were atrial fibrillation, heart valve replacement, deep vein thrombosis, pulmonary embolism, or chronic thromboembolic pulmonary hypertension. The inclusion criteria of study subjects included: (1) Thai patients were at least 18 years of age; (2) the international normalized ratio (INR) target range was 2.0-3.0; (3) The patients had received stable weekly doses (SWDs) or maintenance weekly doses of warfarin that were defined as the constant dose taken at two consecutive clinical visits for at least 1 month within a target INR range of 2-3 after their loading doses. Exclusion criteria included: (1) pregnant patients; (2) patients who had laboratory tests showing abnormal liver function tests (aspartate transaminase (AST), alanine aminotransferase (ALT) $\geq$ 3 fold the upper limit of normal); (3) patients suffering from thyroid disorders. These patients were from the same retrospective cohort study that has been published in our previous study.[7]

Data collection

Demographic and clinical data were assessed via interview, review of the medical records, and an electronic medical record. Demographic variables, i.e., age, gender, height, body weight, and body mass index (BMI), and clinical variables, i.e., SWDs of warfarin, INR values during SWDs, indications for warfarin, concurrent medications, comorbidity, alcohol drinking, and smoking status were recorded.

DNA extraction and genotyping

Genomic DNA (gDNA) of study patients were extracted from peripheral blood using QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

was carried out to detect *CYP2C19*2* (681G>A, rs4244285), *CYP2C19*3* (636G>A, rs4986893) which were as previously described with slight modifications.[11] The polymerase chain reaction (PCR) reaction mixture (25 μL) contained 10x PCR buffer 2.5 μL, deoxynucleotides (dNTPs) (2.5 mmol/L) 2 μL, each forward and reverse primers (10 pmol/μL) 2 μL, Taq enzyme (5 units/μL) (Invitrogen, Brazil) 0.3 μL, sterile water 13.2 μL, and gDNA 2 μL. The PCR process involved an initial denaturing step at 95˚C for 5 min which was followed by 35 cycles consisting of denaturation at 94˚C for 45 s, annealing at 53˚C for 45 s, and extension at 72˚C for 45 s. Subsequently, the final extension was performed at 72˚C for 7 min. The PCR products of *CYP2C19*2* and *CYP2C19*3* were digested with SmaI and BamH1 enzymes (New England Biolabs, MA, USA), respectively, and separated on a 3% agarose gel (Amresco, OH, USA). Genotyping of *CYP2C19*17* (-806C>T; rs12248560; assay ID C____469857_10) was performed by the Taqman® allelic discrimination assay using a 7500HT Applied Biosystems thermal cycler (Applied Biosystems, CA, USA).

The subjects were divided according to their *CYP2C19* genotypes into four groups. Patients with the *CYP2C19 *1/*1, *2/*17* and **3/*17* genotypes were classified as extensive metabolizers (n = 102). Patients with one allele loss-of-function (LOF) allele (*1/*2* or **1/*3*) were classified as intermediate metabolizers (n = 123). Patients with two LOF alleles (*2/*2, *2/*3* or **3/*3*) were classified as poor metabolizers (n = 25). While patients with one gain-of-function (GOF) allele (**1/*17*) were classified as rapid metabolizers (n = 4).

Statistical analyses

The Chi-square test was used to compare categorical clinical data including gender and concomitant drugs among those of different CYP2C19 metabolizer statuses. Kruskall-Wallis or Analysis of Variance (ANOVA) was used to compare continuous data such as patient ages, weights, heights, BMIs and, INRs among those of different CYP2C19 metabolizer statuses. Deviations from the Hardy-Weinberg equilibrium (HWE) were tested using a chi-square goodness-of-fit analysis. A *p* value of less than 0.05 was assumed to indicate a deviation from HWE. The Analysis of Covariance (ANCOVA) with the Bonferroni method was used to control for potential confounding variables which were reported from our previous study[7] e.g. *VKORC1* -1639G>A, *CYP2C9*3*, *CYP4F2*3*, age, amiodarone use, body mass index, antiplatelet drugs use including clopidogrel, prasugrel, ticagrelor, smoking status. Multiple linear regression analysis with enter method was used to investigate the factors that independently influenced the inter-individual variability of warfarin dose requirements. The enter method enters all independent predictors simultaneously into the model. The known predictors from our previous study[7] (e.g. *VKORC1* -1639G>A, *CYP2C9*3*, *CYP4F2*3*, age, amiodarone use, body mass index, antiplatelet drugs use including clopidogrel, prasugrel, ticagrelor, and current smoker) and the present variables (e.g. *CYP2C19* genotypes) were entered into the multiple linear regression model. A 5% two-tailed significance level was used in all tests, and the *p* value of < 0.05 was considered significantly different. The final R^2 of the model indicates the joint contribution of all predictors to the variability in warfarin dose requirements. All analyses were performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA).

Results

Patients' characteristics

A total of 254 patients were enrolled to participate in the study. The patients' demographics, clinical characteristics, indications for warfarin therapy, comorbidity, concomitant medication, and the frequencies of *CYP2C19*1*, *CYP2C19*2*, *CYP2C19*3*, and *CYP2C19*17* alleles are shown in Table 1.

Association between patients' demographics, clinical data, and four CYP2C19 metabolizer statuses

There were no associations between age, weight, height, BMI, and four different CYP2C19 metabolizer statuses (all $p > 0.05$) (Table 2). A Chi-square test indicated that four different metabolizer statuses showed no statistically significant differences of gender, concomitant drugs including amiodarone, aspirin, antiplatelet drugs, dual antiplatelet drugs or proton pump inhibitors (all $p > 0.05$) (Table 2).

Association between the stable warfarin doses and CYP2C19 metabolizer statuses

The *CYP2C19* genotype frequencies are summarized in Table 3. According to the χ^2 test for goodness of fit, all alleles were in Hardy–Weinberg equilibrium at $p < 0.05$ (data not shown). The comparisons of the mean stable warfarin doses among those of different CYP2C19 metabolizer statuses are shown in Table 3. The mean stable warfarin doses after adjustment for confounding factors as mentioned in the method were not significantly different among those of four CYP2C19 metabolizer statuses ($p = 0.348$). Moreover, there were no significant differences in INR values among those of four CYP2C19 metabolizer statuses ($p > 0.05$) (Table 3).

Association between the stable warfarin doses and clinical and genotyping data

The known predictors from our previous study[7] including *VKORC1* −1639G>A, *CYP2C9*3*, *CYP4F2**3, age, BMI, antiplatelet drug use, amiodarone use, and current smoking as well as *CYP2C19*2, CYP2C19*3,* and *CYP2C19*17* polymorphisms from the present study were used as the predictors for multiple linear regression model. All these predictors accounted for 53.5% of the total variance in warfarin dose requirement in the present study (Table 4 and Fig. 1). Among genotyping data, *VKORC1* (-1639G>A) GA genotype, *VKORC1* (-1639G>A) AA genotype, *CYP2C9*1/*3*, *CYP2C9*3/*3*, *CYP4F2*3/*3* were significantly associated with stable warfarin dose ($p < 0.05$) but not for *CYP4F2*1/*3*, *CYP2C19*1/*2*, *CYP2C19*2/*2*, *CYP2C19*1/*3*, *CYP2C19*2/*3*, *CYP2C19*1/*17*, *CYP2C19*2/*17*, *CYP2C19*3/*17* (Table 4). For clinical data, age, BMI, amiodarone use, and antiplatelet drugs use were significantly associated with stable warfarin dose ($p < 0.05$) but not for current smoker (Table 4).

Table 1. Baseline characteristics of study patients (n = 254).

Characteristics	
Age, mean ± SD, years	61 ± 13
Gender	
Women, n (%)	123 (48.4)
Men, n (%)	131 (51.6)
Weight, mean ± SD, kg	63.7 ± 13.6
Height, mean ± SD, cm	161.5 ± 8.3
BMI, mean ± SD, kg/m^2	24.4 ± 4.7
Stable warfarin dose, mean ± SD, mg/week	22.4 ± 9.2
Therapeutic INR, mean ± SD, units	2.43 ± 0.28
Indication	
Atrial fibrillation, n (%)	171 (67.3)
Valve replacement or valvular heart disease, n (%)	43 (17.0)
DVT or PE or CTEPH, n (%)	13 (5.1)
Others, n (%)	27 (10.6)
Comorbidity	
Hypertension, n (%)	57 (22.4)
Diabetes mellitus, n (%)	38 (15.0)
Dyslipidemia, n (%)	16 (6.3)
Congestive heart failure, n (%)	6 (2.4)
Prior stroke, n (%)	18 (7.1)
Concomitant medication	
Amiodarone, n (%)	16 (6.3)
Antiplatelet drugs*, n (%)	26 (10.2)
Aspirin, n (%)	38 (15.0)
Dual antiplatelet**, n (%)	14 (5.5)
Antihypertensive drugs***, n (%)	119 (46.9)
Beta blockers, n (%)	141 (55.5)
Digoxin, n (%)	70 (27.6)
Diuretics, n (%)	135 (53.1)
Proton pump inhibitors, n (%)	39 (15.4)
Statins, n (%)	110 (43.3)
Current smoker, n (%)	13 (5.1)
Current alcohol drinker, n (%)	20 (7.9)
CYP2C19*1, n (%)	327 (64.4)
CYP2C19*2, n (%)	156 (30.7)
CYP2C19*3, n (%)	19 (3.7)
CYP2C19*17, n (%)	6 (1.2)

INR, international normalized ratio; DVT or PE or CTEPH, deep vein thrombosis or pulmonary embolism or chronic thromboembolic pulmonary hypertension

*Antiplatelet drugs include clopidogrel or prasugrel or ticagrelor.

**Dual antiplatelet therapy is the combination between aspirin and clopidogrel.

***Antihypertensive drugs include angiotensin converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB) or calcium channel blockers.

Table 2. Basic characteristics of patients with different CYP2C19 metabolizer statuses.

Phenotype and genotype / Characteristics	Extensive metabolizer **1/*1, *2/*17, *3/*17* (n = 102)	Intermediate metabolizer **1/*2, *1/*3* (n = 123)	Poor metabolizer **2/*2, *2/*3* (n = 25)	Rapid metabolizer **1/*17* (n = 4)
Gender, male (%)	51 (50)	63 (51.2)	14 (56)	3 (75)
Age, years	61.1 ± 12.6	60.9 ± 12.6	59.8 ± 15.1	66.3 ± 5.6
Weight, kg	64.0 ± 14.1	63.6 ± 12.7	63.3 ± 15.7	60.8 ± 19.4
Height, cm	160.9 ± 9.1	161.5 ± 7.4	163.5 ± 9.2	162.3 ± 7.0
BMI, kg/m^2	24.6 ± 4.8	24.4 ± 4.5	23.5 ± 4.5	22.9 ± 6.2
Amiodarone	6 (5.9)	10 (8.1)	0 (0)	0 (0)
Aspirin	12 (11.8)	22 (17.9)	3 (12.0)	1 (25.0)
Antiplatelet drug*	9 (8.8)	16 (13.0)	1 (4.0)	0 (0)
Dual antiplatelet**	3 (2.9)	10 (8.1)	1 (4.0)	0 (0)
Proton pump inhibitors	12 (11.8)	23 (18.7)	3 (12)	1.0 (25.0)

*Antiplatelet drugs include clopidogrel or prasugrel or ticagrelor.
**Dual antiplatelet therapy is the combination between aspirin and clopidogrel.

Table 3. Differences in stable weekly doses of warfarin among CYP2C19 metabolizer statuses.

Phenotype/ genotype	Number (%)	INR value Mean ± SD	Stable weekly dose (mg)* Mean ± SE	*p* value
Extensive metabolizer		2.43 ± 0.29	22.6 ± 0.6	0.348
**1/*1*	100 (39.4)			
**2/*17*	1 (0.39)			
**3/*17*	1 (0.39)			
Intermediate metabolizer		2.43 ± 0.27	22.6 ± 0.6	-
**1/*2*	106 (41.7)			
**1/*3*	17 (6.69)			
Poor metabolizer		2.42 ± 0.29	20.5 ± 1.3	-
**2/*2*	24 (9.45)			
**2/*3*	1 (0.39)			
Rapid metabolizer		2.35 ± 0.34	18.9 ± 3.3	-
**1/*17*	4 (1.57)			

*Mean stable weekly doses of warfarin were adjusted for known predictors from our study including *VKORC1* -1639G>A, *CYP2C9*3, CYP4F2*3*, age, amiodarone use, body mass index, antiplatelet drugs use (e.g. clopidogrel, prasugrel, ticagrelor), smoking status.
SD is standard deviation; SE is standard error.
p values are used to compare the mean stable weekly doses among those four CYP2C19 metabolizer statuses.

Table 4. Multiple linear regression analysis for the predictors responsible for stable weekly warfarin doses.

Predictors	Unstandardized β Coefficients	*p* value	95% Confidence interval for β	
			Lower bound	Upper bound
Constant	44.508	< 0.001	36.314	52.701
Age (in years)♣	-0.248	< 0.001	-0.314	-0.183
Body mass index♣	0.330	< 0.001	0.147	0.513
Amiodarone use♣	-7.722	< 0.001	-11.191	-4.253
Antiplatelet drugs use♣	-3.550	0.011	-6.274	-0.826
Current smoker♣	3.645	0.057	-0.111	7.400
VKORC1 (-1639G>A) GA genotype♣	-9.249	< 0.001	-14.065	-4.432
VKORC1 (-1639G>A) AA genotype♣	-17.527	< 0.001	-22.252	-12.803
*CYP2C9*1/*3*♣	-7.832	< 0.001	-11.819	-3.845
*CYP2C9*3/*3*♣	-25.546	< 0.001	-38.633	-12.458
*CYP4F2*1/*3*♣	0.744	0.415	-1.050	2.538
*CYP4F2*3/*3*♣	5.628	0.002	2.130	9.127
*CYP2C19*1/*2*	0.180	0.845	-1.633	1.994
*CYP2C19*2/*2*	-1.850	0.222	-4.827	1.128
*CYP2C19*1/*3*	1.032	0.555	-2.406	4.470
*CYP2C19*2/*3*	-3.728	0.586	-17.188	9.731
*CYP2C19*1/*17*	-3.164	0.344	-9.733	3.406
*CYP2C19*2/*17*	10.399	0.158	-4.079	24.876
*CYP2C19*3/*17*	8.300	0.288	-7.054	23.654

Variables are defined as follows:
VKORC1-1639G > A genotype: Input 1 for GA or AA, 0 for GG; *CYP2C9* genotype: Input 1 for *CYP2C9*1/*3* or *CYP2C9*3/*3*, 0 for *CYP2C9*1/*1*; CYP2C19 genotype: Input 1 for *CYP2C19*1/*2* or *CYP2C19*2/*2* or *CYP2C19*1/*3* or *CYP2C19*2/*3* or *CYP2C19*1/*17* or *CYP2C19*2/*17* or *CYP2C19*3/*17*, otherwise zero; Age: Input age in years; Amiodarone use: Input 1 for patient taking amiodarone, otherwise zero; Body mass index: Input BMI in kg/m^2 ; CYP4F2 genotype: Input 1 for *CYP4F2*1/*3 or CYP4F2*3/*3*, otherwise zero; Antiplatelet drugs use: Input 1 for patient taking antiplatelet drugs including clopidoglel, prasugrel, ticagrelor, otherwise zero; Current smoker: Input 1 for current smoker, otherwise zero

♣ The known predictors from our previous study[7] including *VKORC1* -1639G>A, *CYP2C9*3*, *CYP4F2*3*, age, amiodarone use, body mass index, antiplatelet drugs use (e.g. clopidogrel, prasugrel, ticagrelor), and smoking status.

Discussion

CYP2C19 is an enzyme responsible for metabolizing several drugs including *R*-warfarin.[8,19] Moreover, the previous *in vitro* study has reported that CYP2C19 metabolized *S*-warfarin.[8] A question that needs to be answered is whether CYP2C19 genotyping is clinically useful in identifying patients with poor warfarin metabolism. In the present study, *CYP2C19*1*, *CYP2C19*2*, *CYP2C19*3*,

and *CYP2C19*17* allele frequencies were similar to the previous studies in the Thai population.[10,11] Results from the present study suggest that *CYP2C19* polymorphisms do not affect the stable warfarin dose in Thai patients. In the present study, however, the poor metabolizers e.g. *CYP2C19*2/*2* and **2/*3*, tend to have a slightly lower stable warfarin dose as compared with the extensive metabolizers e.g. *CYP2C19*1/*1*, *CYP2C19*2/*17*, *CYP2C19*3/*17*, but the difference is not statistically significant (Table 3). Similarly, the previous study has reported that the warfarin maintenance doses of patients with a poor metabolizer e.g. *CYP2C19*2/*2*, were not statistically significantly different from those with extensive metabolizers, e.g. *CYP2C19*1/*1, CYP2C19*2/*17* ($p = 0.66$).[14]

The previous study has reported that the clearance of *R*-warfarin was approximately 30% higher in Italian warfarin-treated patients carrying *CYP2C19*17* compared to *CYP2C19*2*.[18] In this current study, four rapid metabolizers (e.g. *CYP2C19*1/*17*) received slightly lower stable warfarin doses as compared with the extensive metabolizers, but the differences were not statistically significant (Table 3). Similarly, there was no difference in stable warfarin dose between these two groups, *CYP2C19*1/*1* vs. *CYP2C19*1/*17*.[14] The previous study has reported that *CYP2C19**2 and *CYP2C19*17* are in complete linkage disequilibrium with *CYP2C9*1*.[14] Herein, it was noticed that the mean stable warfarin doses for the extensive metabolizers, intermediate metabolizers, poor metabolizers, and rapid metabolizers of CYP2C19 were not different from the mean stable warfarin doses of the patients with *CYP2C9*1/*1* genotype (22.6±9.2 mg/week) from the previous study by the present authors.[7] The present results may support that *CYP2C19*2* and *CYP2C19*17* are in complete linkage disequilibrium with *CYP2C9*1*.

Multiple linear regression analysis showed that the known predictors from our previous study[7], *VKORC1* −1639G>A and *CYP2C9*3* were still the major genetic determinants of warfarin dose requirements, with *CYP4F2*3* having a smaller effect in Thai patients (Table 4). *CYP2C19*2*, *CYP2C19*3*, and *CYP2C19*17* polymorphisms, however, were not associated with stable warfarin doses in the present study (Table 4). In contrast, the previous study has shown that the warfarin maintenance doses of Chinese patients with intermediate metabolizers (e.g. *CYP2C19*1/*2* and *CYP2C19*1/*3*) and poor metabolizers (e.g. *CYP2C19*2/*2*, *CYP2C19*2/*3*, and *CYP2C19*3/*3*) were significantly lower than those with the extensive metabolizer (*CYP2C19*1/*1*) (both $p < 0.05$).[13] It is possible that lower frequencies of the *CYP2C19*2* (30.7% vs. 47.2%) and *CYP2C19*3* (3.7% vs. 19.0%) were found in Thais as compared with Chinese patients in the previous study[13] which could explain the no contribution of the *CYP2C19*2* and *CYP2C19*3* alleles in this study. Moreover, lower frequencies of the *CYP2C19*17* (1.2% vs. 20.2%) were found in Thais as compared with Caucasians in the previous study[14] which could explain the lack of effect of the *CYP2C19*17* on the stable warfarin doses in the present study. For known non-genetic factors from our previous study[7], age, BMI, co-medications including amiodarone, antiplatelet drugs (e.g. clopidogrel, prasugrel, ticagrelor) were also associated with the stable warfarin doses in the present study but not for current smokers. Similarly, there was no difference in the stable warfarin dose between smoking and non-smoking statuses.[20] It is possible that the minor role of the smoking–induced CYP1A2 in warfarin dosage variability was found.

Conclusion

Taken together, the present results therefore suggest that altered metabolism of *R*-warfarin may only play a minor role in stable warfarin doses in Thai patients. Moreover, these results suggest that role of CYP2C19 in the metabolism of *S*-warfarin is less important role of than CYP2C9. In conclusion, *CYP2C19* genetic polymorphisms were not associated with stable warfarin doses in Thai patients. Moreover, *CYP2C19* genotypes may not be used as a predictor for adjusting warfarin doses in clinical practice.

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