The Influence of *VKORC1* Polymorphisms on Warfarin Doses in Thai Patients with Deep Vein Thrombosis

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Background: *Polymorphisms in cytochrome P450 2C9 and vitamin K epoxide reductase complex, subunit 1 genes (CYP2C9 and VKORC1, respectively) were previously shown to affect the warfarin dose required in anticoagulant therapy of deep vein thrombosis (DVT). However, little is known about the role of these genetic variants in the Thai population.*
Objective: *To identify the effect of CYP2C9 and VKORC1 genetic variants on warfarin dosage in the Thai population with DVT.*
Material and Method: *Genotyping of CYP2C9 (*2 and *3) and VKORC1 promoter (-1639G>A) variants were carried out in 97 Thai DVT patients receiving constant warfarin therapy and with a stable international normalized ratio using real-time PCR assays.*
Results: *VKORC1 AA, GA, and GG genotype frequencies were found to be 49.5%, 46.4%, and 4.1%, respectively, while those of CYP2C9 genotypes were 88.7% for *1/*1 and 11.3% for *1/*3. The CYP2C9*2 variant was not present in the patients studied. The mean daily warfarin dose required to maintain a therapeutic INR differed significantly according to VKORC1 genotype, with 3.6 mg/day required for AA, 4.7 mg/day for GA, and 7.4 mg/day for GG (p-value <0.001). The CYP2C9 genotype did not significantly affect the warfarin dosage requirement (p-value = 0.29).*
Conclusion: *These findings underline the impact of VKORC1 genotypes on the wide variation in warfarin maintenance dosing in Thai patients with DVT.*

Keywords: *Warfarin, VKORC1, CYP2C9, Deep vein thrombosis (DVT), Single nucleotide polymorphism*

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Warfarin is the most commonly used anticoagulant therapy worldwide, with demonstrated effectiveness for the treatment of deep vein thrombosis. The level of blood coagulation achieved by anticoagulant was monitored and standardized by the international normalized ratio (INR), and warfarin has been shown to have a narrow therapeutic INR range[1]. Maintaining this therapeutic range is extremely important because a subtherapeutic INR causes thrombosis and a supratherapeutic INR leaded to an increased risk of bleeding. Clinical factors including body weight, body surface area, and age were poor predictors of the warfarin dose requirement, so genetic influences have been studied instead[1,2].

Warfarin is metabolized primarily in the liver by cytochrome P450 2C9 (*CYP2C9*), and exhibits its anticoagulant effect by inhibiting the protein vitamin K epoxide reductase complex, subunit 1 (*VKORC1*). Single nucleotide polymorphisms (SNPs) in *CYP2C9* and *VKORC1* have previously been identified to be key players in determining the effect of warfarin on anticoagulation[2]. The normal (wild-type) *CYP2C9* variant was referred to as *1 and the two important polymorphisms are *2, and *3[3,4]. Individuals could carried any two polymorphisms. Homozygous normal patients with two normal variants were *1/*1, while those carrying polymorphisms could be *1/*2, *1/*3, or *2/*3[5,6]. While *CYP2C9**1 metabolized warfarin normally, *CYP2C9**2 reduces warfarin metabolism by 30%, and *CYP2C9**3 by 90%, such that warfarin persists in the body for longer, necessitating a reduced dose[7].

VKORC1 polymorphisms explain 30% of warfarin dose variations between patients. Individuals homozygous for wild-type alleles of the *VKORC1* promoter at position -1639 have the GG genotype. Heterozygous or homozygous substitutions of -1639G>A (GA and AA genotypes) leaded to reduced

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enzyme levels[5], and patients who had harbored one or more A alleles required a decreased dose of warfarin treatment because they are sensitive to inhibition by anticoagulants[8].

Data on the impact of these genetic variations on warfarin dosage requirements in the Thai population were limited[9-12], so the present study aimed to identify the effect of *CYP2C9**2, *CYP2C9**3, and *VKORC1* -1639G>A genetic variations on warfarin dosage in Thai deep vein thrombosis (DVT) patients with stable INRs.

Material and Method

Data collection

The present study recruited patients receiving warfarin therapy for DVT at the Division of Vascular Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand between February 2012 and February 2014. DVT patients were included if they were aged 18 to 70 years, were being administered a stable warfarin dose that had remained constant for the three previous visits over a minimum of three months, and had an INR of 2.0 and 3.0. Patients with liver disease, and those taking non-steroidal anti-inflammatory drugs or anti-platelet therapy were excluded. Demographic and clinical data including age, gender, weight, mean daily warfarin dosage, mean INR, platelet count, and serum creatinine and albumin levels were recorded for each patient. The study was approved by the Siriraj Institutional Review Board and informed consent was obtained from each participant. This study had been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Genotyping of CYP2C9 and VKORC1

Whole blood samples were collected in collection tubes containing anticoagulant. Genomic DNA was extracted from blood using the QIAamp DNA Blood Mini Kit (Qiagen). Determination of *CYP2C9**2 (rs1799853), *CYP2C9**3 (rs1057910), and *VKORC1* -1639G>A (rs9923231) genotypes were performed using commercially available real-time PCR assays (LightMix® Kit, TIB Molbiol) according to the manufacturer's protocol.

Statistical analysis

The distribution of alleles for their accordance with the Hardy-Weinberg equilibrium (HWE) was calculated using Pearson's Chi-squared test. Comparisons of mean daily warfarin dosage between different genotypes were calculated by the Mann-Whitney test. The effects of patient age, weight, serum albumin levels, and *CYP2C9* and *VKORC1* genotypes on the warfarin dosage were calculated by multiple regression analysis. Statistical analysis was performed using Predictive Analytics Software Statistics 18.0 software (SPSS) and p-value <0.05 were considered to be statistically significant.

Results

Ninety-seven patients (52 males and 45 females) taking warfarin therapy for DVT and with a stable INR (2.0-3.0) for at least three months participated in our study. The mean age was 54±15 years, and the mean weight was 68.7±13.6 kg. DVT etiologies were unprovoked (64.0%), provoked (20.6%), and cancer (15.5%) (Table 1).

The frequency of *VKORC1* homozygous wild-type (GG genotype) individuals was 4.1%, while homozygous (AA genotype) and heterozygous variants (GA genotype) were seen in 49.5% and 46.4% of patients, respectively. *CYP2C9* homozygous wild-type (*CYP2C9* *1/*1) patients comprised 88.7% of the total, whereas the heterozygous variant *CYP2C9* *1/*3 was found in 11.3% of participants. The frequency of the *VKORC1* variant allele was found to be 73% and its wild-type allele 27%, while the *CYP2C9* wild-type allele *1 had a frequency of 94%, and that of the variant allele *CYP2C9**3 was 6%. The variant allele *CYP2C9**2 was not found in our study sample. All variations were found to be in accordance with HWE (Table 2). The most common combined *VKORC1*/*CYP2C9* genotype was *VKORC1* AA/*CYP2C9* *1/*1 (43.3%), followed by *VKORC1* GA/*CYP2C9* *1/*1 (41.2%), *VKORC1*

Table 1. Baseline characteristics of DVT patients with stable INR

Characteristic	n (%), total = 97 cases
Age (years), mean ± SD	54.0±15.0
Weight (kg), mean ± SD	68.7±13.6
Gender	
Male	52 (53.6)
Female	45 (47.4)
Cause of DVT	
Provoked DVT	20 (20.6)
Unprovoked DVT	62 (64.0)
Cancer	15 (15.5)

DVT = deep vein thrombosis; INR = international normalized ratio

AA/*CYP2C9* *1/*3 (6.2%), *VKORC1* GG/*CYP2C9* *1/*1 (5.2%), and *VKORC1* GA/*CYP2C9* *1/*3 (4.1%) (Table 3).

The effect of various parameters, including age, weight, albumin levels, and *CYP2C9* and *VKORC1* genotypes, on warfarin dose were evaluated by multiple regression analysis. However, only *VKORC1* genotypes significantly affected warfarin dosage (R^2 = 20.5%, p<0.001; see Table 4). *CYP2C9* variants, age, weight and albumin level were not found to have significant effects on the variability in warfarin maintenance dose. The mean daily warfarin dosage among different genotypes is shown in Fig. 1. Warfarin dose requirements in patients with the *CYP2C9* *1/*3 variant were not significantly different from those of *CYP2C9* wild-type (*CYP2C9* *1/*1) patients (p = 0.19). However, daily warfarin dosages in participants carrying the *VKORC1* -1639A allele were significantly lower than those with wild-type alleles (GG genotype)

Table 2. Genotype and allele frequencies of *CYP2C9**2, *CYP2C9**3, and *VKORC1* polymorphisms (n = 97)

Gene	Genotype	Frequency (%)
VKORC1	GG	4.00 (4.1)
	GA	45.00 (46.4)
	AA	48.00 (49.5)
	Wild type allele (G)	0.27 (27.0)
	Variant type allele (A)	0.73 (73.0)
CYP2C9	*1/*1	86.00 (88.7)
	*1/*3	11.00 (11.3)
	Wild type allele (*1)	0.94 (94.0)
	Variant type allele (*3)	0.06 (6.0)

Table 3. Warfarin dose according to genotype for Thai deep vein thrombosis patients

Genotypes *VKORC1*/*CYP2C9*	Frequency number (%) (n = 97 cases)	Warfarin dosage mean ± SD (mg/day)
AA/*1/*1	42 (43.3%)	3.6±1.0
GA/*1/*1	40 (41.2%)	4.7±1.7
AA/*1/*3	6 (6.2%)	2.5±0.4
GA/*1/*3	5 (5.2%)	5.1±2.5
GG/*1/*1	4 (4.1%)	7.4±1.0

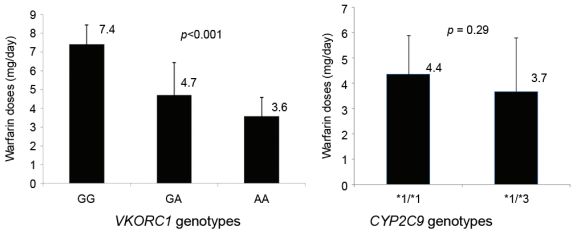

Fig. 1 Mean warfarin dosage according to *VKORC1* and *CYP2C9* genotypes.

(p<0.001), requiring dose reductions of 51.4% (3.8 mg/day) and 36.5% (2.7 mg/day) in *VKORC1* AA and GA genotypes, respectively, compared with wild-type dosages.

The mean warfarin dose required by patients with *CYP2C9* *1/*1/*VKORC1* AA genotypes (3.6 mg/day) was around half that of those with *CYP2C9* *1/*1/*VKORC1* GG genotypes (7.4 mg/day), while that required by *CYP2C9* *1/*1/*VKORC1* GA patients (4.7 mg/day) was about 40% lower than the dosage of *CYP2C9* *1/*1/*VKORC1* GG patients. The lowest mean warfarin dose was required by patients with *CYP2C9* *1/*3/*VKORC1* AA genotypes (2.5 mg/day).

Discussion

Polymorphisms in *CYP2C9* and *VKORC1* have previously been shown to have an impact on warfarin metabolism. Our genotyping results were consistent with earlier studies that found *VKORC1* polymorphisms to be more frequent than *CYP2C9* polymorphisms in the Asian population. Although only 20% of Caucasians were reported to carry *VKORC1* variations, our results support the previous finding that 90% of individuals from East and South East Asia carry *VKORC1* variant alleles[13,14]. Our results are also comparable to other studies in Thai patients that showed 95% of the Thai population has a *VKORC1* variant allele[9,11].

The prevalence of *VKORC1* GG, GA, and AA genotypes in the present study showed the result slightly differences from previously published Thai studies, probably because of the diversity of the recruited population, which included patients with

Table 4. Multiple stepwise regression analysis of factors affecting warfarin daily maintenance dose

Factor	Unstandardized coefficients β	Standardized coefficients β	t	*p*-value
Constant	2.116	-	4.167	<0.001
VKORC1	1.303	0.430	4.207	<0.001

valvular heart disease, venous thromboembolism, and atrial fibrillation who were administered warfarin treatment[9,11]. However, our results were consistent with earlier findings that patients with *VKORC1* variant alleles (both heterozygous and homozygous) require lower warfarin dosage than standard warfarin doses to maintain their INR within a therapeutic range. Even the presence of a single variant allele of *VKORC1* (heterozygous variant) made the patients sensitive to warfarin therapy[15].

In Caucasians, *CYP2C9**2 and *CYP2C9**3 variants are observed in 16% and 6.5% of the population, respectively[6,16], but these variants are extremely rare in the Asian population[14]. We found *CYP2C9**3 variation to be present in 11.3% of Thai patients in the present study, and did not observe any *CYP2C9**2 variant alleles. Because of the low frequency of the *CYP2C9**2 variant allele, however, this may have been undetected due to small sample size.

Several studies have characterized the importance of *CYP2C9**2,*3 and *VKORC1* -1639G>A polymorphisms on the variation in warfarin dosage requirement[17,18]. Our data support the role of the *VKORC1* -1639G>A polymorphism in lowering the required dose of warfarin. Our patients with the *VKORC1* AA genotype required the lowest warfarin dose, while those with the *VKORC1* GG genotype required the highest dose, which is consistent with previous studies in Asian and Caucasian populations[4,19,20]. *CYP2C9* variants were shown to have very little or no effect on the warfarin dosage in the present study, which was in agreement with earlier findings that the reduced warfarin dosage in Asians is mainly caused by *VKORC1* variations because *CYP2C9* variants was quite rare[13,15].

Patient ethnicity is related to variation in the warfarin maintenance dose, which has been reported to be approximately 40% lowering dosage of warfarin treatment in Asian populations compared with Caucasians. These differences are attributable to genetic differences in *CYP2C9* and *VKORC1*[21]. This demonstrates the importance of genotype-based warfarin dosing in different countries to determine a standard dosage according to genotype distribution[14].

In summary, our study underlines the importance of the *VKORC1* -1639G>A polymorphism on the variation of the warfarin maintenance dose in Thai patients with DVT. *VKORC1* variant genotyping will therefore help the clinician to decision making to adjust on the appropriate warfarin dose for individual patients. Lowering this dose and frequent laboratory monitoring are recommended in those patients who are high responders to warfarin to reduce potential adverse bleeding events during warfarin treatment.

What is already known on this topic?

Polymorphisms in vitamin K epoxide reductase complex, subunit 1 genes (*VKORC1*) were previously shown to affect the warfarin dose required in anticoagulant therapy.

What this study adds?

VKORC1 variant genotyping will help the clinician decide on the appropriate warfarin dose for individual Thai patients with DVT. Lowering warfarin dosage and frequent laboratory monitoring are recommended in patients who are high responders to warfarin to reduce potential adverse bleeding events.

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Potential conflicts of interest

None.

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อิทธิพลของการเปลี่ยนแปลงทางพันธุกรรมจีน VKORC1 ต่อขนาดยาวาร์ฟาริน ในผู้ป่วยไทยที่เป็นโรคหลอดเลือดดำชั้นลึกของขาอุดตัน

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ภูมิหลัง: มีรายงานว่า การเปลี่ยนแปลงทางพันธุกรรมจีน CYP2C9 และ VKORC1 มีผลต่อขนาดยาวาร์ฟาริน ที่ผู้ป่วยโรคหลอดเลือดดำชั้นลึกของขาอุดตันควรได้รับ เพื่อได้ระดับยาที่เหมาะสมในการรักษา

วัตถุประสงค์: ศึกษาอิทธิพลของการเปลี่ยนแปลงทางพันธุกรรมจีน CYP2C9 และ VKORC1 มีผลต่อขนาดยาวาร์ฟาริน ในผู้ป่วยไทยที่เป็นโรคหลอดเลือดดำชั้นลึกของขาอุดตัน

วัสดุและวิธีการ: ผู้ป่วยไทยที่เป็นโรคหลอดเลือดดำชั้นลึกของขาอุดตัน 97 ราย ที่ได้รับยาวาร์ฟารินในการรักษาที่มีระดับของ INR อยู่ระหว่าง 2 ถึง 3 คงที่ติดต่อกันอย่างน้อยสามเดือน จะได้รับการเจาะเลือดเพื่อตรวจหาการเปลี่ยนแปลงทางพันธุกรรมจีน CYP2C9 และ VKORC1 โดยเทคนิคเรียลไทม์พีซีอาร์ เพื่อเปรียบเทียบกับขนาดยาที่ผู้ป่วยได้รับ

ผลการศึกษา: การเปลี่ยนแปลงทางพันธุกรรมจีน VKORC1 ชนิด AA, GA และ GG genotype พบได้ร้อยละ 49.5, 46.4 และ 4.1 ตามลำดับ ในขณะที่การเปลี่ยนแปลงทางพันธุกรรมจีน CYP2C9 ชนิด *1/*1 พบได้ร้อยละ 88.7 และ ชนิด *1/*3 พบได้ร้อยละ 11.3 ในการศึกษานี้ไม่พบการเปลี่ยนแปลงทางพันธุกรรมจีนชนิด CYP2C9*2 ค่าเฉลี่ยของขนาดของยาวาร์ฟารินต่อวันที่เหมาะสมในการรักษาโรคหลอดเลือดดำชั้นลึกของขาอุดตัน ในผู้ป่วยที่มีการเปลี่ยนแปลงทางพันธุกรรมจีน VKORC1 ชนิด AA, GA และ GG คือ 3.6 มิลลิกรัม, 4.7 มิลลิกรัม และ 7.4 มิลลิกรัม ตามลำดับ (p-value <0.001) การเปลี่ยนแปลงทางพันธุกรรมจีน CYP2C9 ไม่มีผลต่อขนาดของยาวาร์ฟารินในผู้ป่วยกลุ่มนี้ (p-value = 0.29)

สรุป: การเปลี่ยนแปลงทางพันธุกรรมจีน VKORC1 มีผลต่อขนาดยาวาร์ฟาริน ในผู้ป่วยไทยที่เป็นโรคหลอดเลือดดำชั้นลึกของขาอุดตัน