Original Article

# Prevalence of VKORC1 Single Nucleotide Polymorphism (SNP) – 1639 in Thai Adult Patients Who Have INR More Than 4 from Warfarin

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***Abstract :*** ***Background :*** *Previous studies showed that VKORC1 -1639 SNP genotype AA was more common in Asian compared with Caucasian population and associated with lower warfarin dose requirement. We hypothesized that this genetically-determined warfarin sensitivity may predispose the patients with this genotype to warfarin overdosage.* ***Method :*** *A descriptive study was conducted in adult patients taking warfarin with INR more than 4 at King Chulalongkorn Memorial Hospital. The VKORC1 -1639 genotyping in these cases was performed.* ***Results :*** *There were a total of 44 cases of warfarin overdose. The median age was 66 yr, range 21-90 yr, and 15 patients (34%) were male. The numbers (%) of VKORC1 -1639 genotype AA, AG and GG were 26 (59.1%), 16 (36.4%) and 2 (4.5%), respectively. This prevalence was similar to general Thai population and thromboembolic patients receiving warfarin.* ***Conclusion :*** *Our result implies that VKORC1 -1639 polymorphism is not a strong risk factor for warfarin overdose.*

***Key Words :*** ● *Warfarin* ● *Overdosage* ● *VKORC1* ● *Single nucleotide polymorphism*

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## Introduction

Since 1954, warfain has been used worldwide as an anticoagulant for treatment or prophylaxis of venous thromboembolism.[1] Warfarin has good oral bioavailability. Almost 100% is absorbed and approximately 99% is bound with albumin. It comprises a racemic mixture of S-enantiomer (free form) and R-enantiomer (bound form) showing the ratio of 1 to 1. Notably, S-form is 2.5 times more potent than R-form. Warfarin is metabolized by cytochrome P450 (CYP) enzymes. Different CYPs eliminate different forms of warfarin as shown in figure 1. S-enantiomer is metabolized by CYP2C9. The target enzyme of warfarin is vitamin K oxide reductase (VKORC1). Vitamin K is the cofactor of hepatic gamma carboxylation. After the reaction, vitamin K is transformed into an oxidized from that is inactive. VKORC1 can reduce the vitamin to be active again. Upon inhibition of VKORC1 by warfarin, post-translational gamma-carboxylation of glutamic acid residues on vitamin K dependent coagulation factors (factors II, VII, IX, X) is diminished and, thus the active forms of coagulation factors are depressed. The pharmacodynamics of warfarin is shown in figure 2.

Due to the highly variable responses and narrow therapeutic index of the drug,[2] serious complications such as intracerebral bleeding can occur leading to extensive investigations for factors that interfere with warfarin effects.

Currently, several clinical and genetic factors have been identified. Both clinical factors, such as several co-morbid diseases, age, body mass index, food and drug interactions, and genetic factors, such as CYP450 2C9

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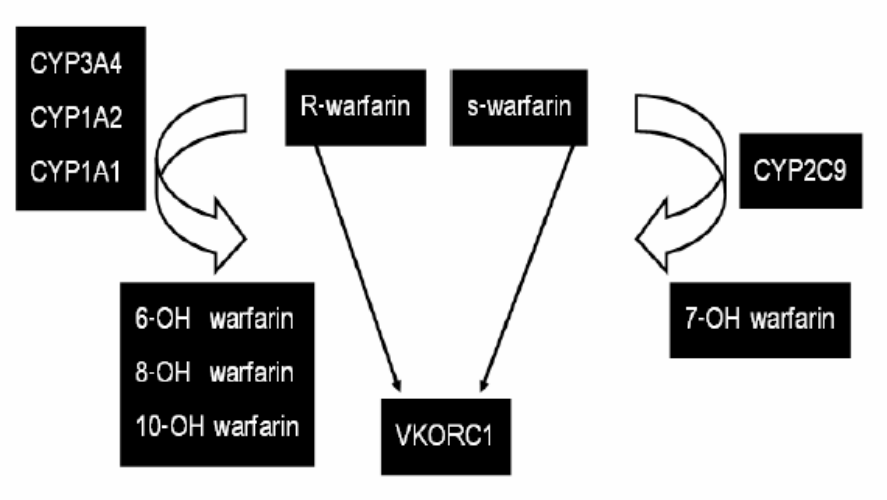


**Figure 1** Pharmacokinetics of Warfarin

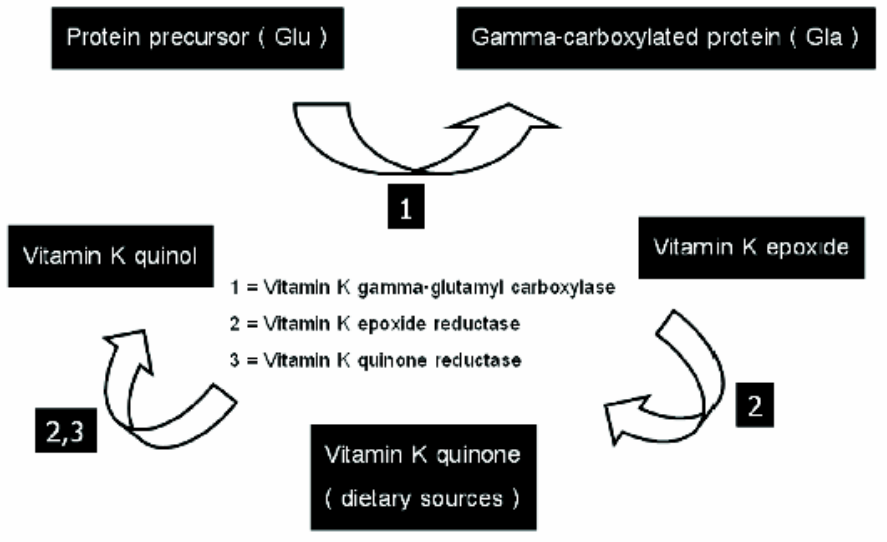


**Figure 2** Pharmacodynamics of Warfarin

and VKORC1[3,4,5] have impacts on warfarin dosage both for initiation and for maintenance. Some studies used multivariate regression analysis to formulate equations combining clinical and genetic variables to calculate the maintenance dose of warfarin[6]

Vitamin K epoxide reductase enzyme (VKOR) single nucleotide polymorphisms (SNPs) control the expression of messenger ribonucleic acid (mRNA) of VKOR. In sensitive alleles, the expression of mRNA of VKOR is low, hence, requiring low doses of warfarin maintenance. The most important VKORC1 SNPs determining warfarin sensitivity were at the -1639 and 1173 sites.[7] Both alleles showed strong linkage disequilibrium. The data were consistent in both Europcan-American and Asian studies. Obayashi et al,[8] demonstrated the linkage disequilibrium of VKORC1-1639, 1173 and 1542. Genotype AA of VKORC1 -1639 usually accompany genotype TT of VKORC1 1173 and genotype CC of VKORC1 1542. Therefore, a warfarin sensitive allele was defined as haplotye A (allele A, T, C) and a warfarin insensitive allele as haplotype non A (allele G, C, G)

Prevalence of VKORC1 and CYP450 2C9 polymorphism is different among various racial populations, VKORC1 haplotype A is more common in Asians resulting in higher warfarin sensitivity. We propose that this susceptible genotype may prone to warfarin overdosage. Therefore, our study aims to investigate the frequency of genetic variations in a group of Thai warfain overdose patients

and compare with general Thai population derived from previous reports. Due to the low prevalence of warfarin-sensitive CYP450 2C9 *2 and *3 in Asians,[9,10] our study focused on the more common VKORC1 -1639 SNP.

## Materials and Methods

*Patients and study design*

A descriptive cross-sectional study to determine the prevalence of VKORC1 -1639 SNPs was conducted. The subjects were recruited from February 2008 to January 2009 at department of medicine, King Chulalongkorn Memorial Hospital.

The inclusion criteria were patients who were at least 15 years of age, using warfarin and had INR level more than 4.0. Pregnant women and patients who are unwilling to participate in study were excluded.

The project was performed in accordance with the Declaration of Helsinki. The study protocol was reviewed and approved by the ethical committee of the Faculty of Medicine, Chulalongkorn University. All subjects gave written informed consent before entry the study.

*VKORC1 -1639 SNP genotyping*[11]

Peripheral blood samples were collected in 3-ml EDTA tubes and subjected to DNA extraction using JETQUICK kit (GENOMED GmbH, lohne, Germany).

The VKORC1 -1639 region was subjected to polymerase chain reaction (PCR) technique for DNA amplification. The PCR cocktail final volume was 20 ul containing 10 ul of green master mix, 1.5 ul of forward VKORC1 -1639 primer (5' GCC AGC AGG AGA GGG AAA TA 3'), 1.5 ul of reverse VKORC1 -1639 primer (5' AGT TTG GAC TAC AGG TGC CT 3') and 3 ul of template DNA. The PCR was performed for 38 cycles (94 °C denaturation for 1 min, 57 °C annealing for 1 min and 72 °C extension for 1 min in each cycle). The final products were separated on acrylamide gel electrophoresis showing 290 base pairs.

Genotypes of VKORC1 -1639 SNP were identified by digestion the PCR products with the *MspI* restriction enzyme that could cut a G allele, not an A allele as shown in figure 3.

Genotype AA was not digested and showed 290 base pair product on 10% acrylamide gel electrophoresis, while genotype GG was digested into 122 and 165 base pair bands. Genotype AG demonstrated 122, 165 and 290 base pair products.

*Statistics*

The primary result of the study was the prevalence of VKORC1 – 1639 single nucleotide polymorphism AA genotype in patients with warfarin overdose reported as percent.

## Results

A total of 44 patients were enrolled in the study. The baseline characteristics were shown in Table 1.

The ratio of female to male in this study was 2 to 1 (15 : 29). The median age was 66 years (range

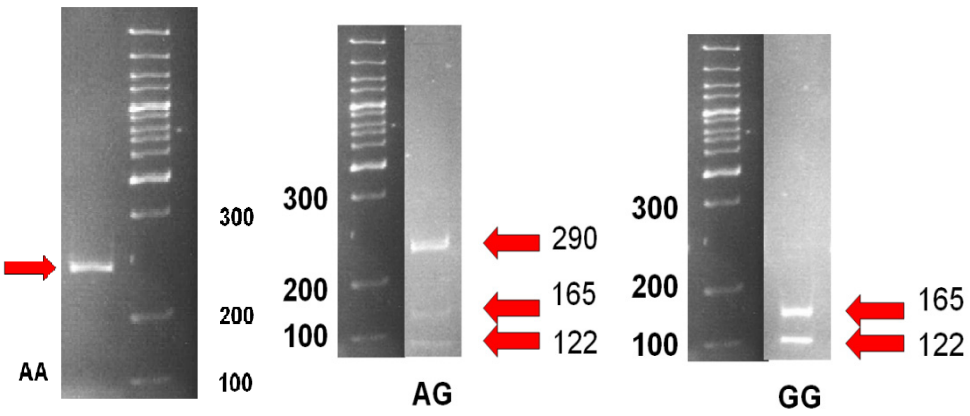


**Figure 3** Polymerase chain reaction (PCR) products after digestion with *MspI* enzyme demonstrate genotype AA, AG and GG.

**Table 1.** Clinical Characteristics of the study group (n=44)

| Parameters | Values |
|---|---|
| VKORC1 – 1639 genotype AA/AG/GG (percent) | 59.1 % / 36.4 % / 4.5 % |
| Indications for warfarin (percent) | |
| - Atrial fibrillation | 45.5% ( 20/44 ) |
| - Deep vein thrombosis | 40.9% ( 18/44 ) |
| - Valvular heart disease | 9.1% ( 4/44 ) |
| - Arterial occlusion | 2.3% ( 1/44 ) |
| - Venous sinus thrombosis | 2.3% ( 1/44 ) |
| Concomitant medication (percent) | |
| - No effect | 84.1% ( 37/44 ) |
| - Increase anticoagulation effect | 15.9% ( 7/44 ) |
| Underlying diseases (percent) | |
| - Cardiovascular disease | 65.9% ( 29/44 ) |
| - Malignancy | 18.2% ( 8/44 ) |
| - Neurology | 4.5% ( 2/44 ) |
| - No underlying | 4.5% ( 2/44 ) |
| - Other diseases | 6.8% ( 3/44 ) |

21-90 years). Atrial fibrillation was the most common indication for warfarin uses. The second most common indication for warfarin was deep venous thrombosis (DVT) without pulmonary embolism. The causes of DVT were idiopathic and malignancy associated thrombosis in 22.7% and 3.5% respectively. There was one case of arterial occlusion and one case of cerebral venous sinus thrombosis. Mean INR of patients was 6.73±2.99 (4-17.7 )

Seventy percent of patients had cardiovascular diseases including ischemic heart disease and/or atrial fibrillation. There were 7 malignant cases including 2 cervical, 2 breast, 1 colon and 1 ovarian cancers, as well as 1 diffuse large B cell lymphoma.

The frequencies of VKORC1 – 1639 promoter region single nucleotide polymorphism genotype AA, AG and GG were 26 (59.1%), 16 (36.4%) and 2 (4.5%), respectively

Clinical factors that could interfere with warfarin effects were analyzed.

All of the patients had normal liver and kidney functions, baseline albumin was more than 3 g/dL and they consumed normal kinds of food.

All patients take concomitant drugs. The medications used could be divided in to two groups, The first group, such as statin, acetaminophen, potentially increased warfarin effects, while the second group had neutral effect on warfarin dosage, such as vitamin B, enalapril, furosemide. There were 7 patients who used statin regularly and acetaminophen at the time of INR more than 4.

## Discussion

Warfarin, the most widely used oral anticoagulant, showed marked inter-ethnic and inter-individual differences in dose requirements. Among other factors, genetics is an important part. In 2007, US Food and Drug Administration (FDA) approved phamacogenomic tests (VKORC1 and

CYP450 2C9 genotyping) for adjustment of warfarin doses.

Polymorphisms of 3' non coding promoter region 3673 (-1639 G>A) strongly determine the pharmacodynamic aspects of warfarin. The G allele showed 40% higher VKOR enzyme when compared with the A allele.[12]

Klumcheun et al studied the prevalence of VKORC1 and CYP450 2C9 in normal Thai healthy volunteers and patients who took warfarin and found the frequencies of VKORC1-1639 AA, AG and GG alleles to be 61.1%, 33.6% and 5.3% in healthy volunteer and 63.2%, 31.1% and 5.7% in patients taking warfarin, respectively.[14] In our study, we found the prevalence rates of these genotypes in patients with warfarin overdose were the same as previous study. The frequencies of genotype AA, AG and GG were 59.1%, 36.4%, and 4.5%, respectively. Therefore, the association of VKORC1 -1639 polymorphisms and overanticoagulation cannot be demonstrated. However, we found that VKORC1 genotype AA required significantly lower maintenance doses of warfarin to keep INR between 2.0-3.0 compared with the other genotypes.

In contrast to this report, Schwarz et al found that VKORC1 and CYP2C9 could predict the time to first INR more than 4 and also discovered significant effects on the required warfarin dose after the first 2 weeks of therapy.[13] The sensitive haplotype A of VKORC1 -1639 showed a shorter median time to INR over 4 compared with the non-A haplotype (17 days versus 23 days, respectively). Furthermore, it could predict time to first INR in a therapeutic range. The median time was 7 days versus 15 days in haplotype A versus haplotype non AA, respectively. Notably, the subjects in our study usually had been taking warfarin for a much longer time than those in this report. It is possible that VKORC1 SNPs do not predict risks of warfarin overdosage after patients have received the drug for a certain period of time. Future studies in newly prescribed Thai cases are required.

CYP450 2C9 was discovered in 1990 and later found to play an important role in elimination of the active S-form of warfarin. The *2 and *3 variant alleles were more warfarin sensitive due to the reduced CYP450 2C9 function compared with the wild-type *1 allele. Aithal et al found that patients with CYP450 2C9 *2 and *3 allele need lower maintenance dose of warfarin.[15] Our study did not investigate the prevalence of CYP450 2C9 due to low prevalence of *2 and *3 allele in Asian population and the small sample size of the study. CYP2C9 genotyping will be performed in the future study. In addition, more patients will be included to improve statistical power.

In conclusion, VKORC1 -1639 SNP prevalence in warfarin overdose group is not different from normal Thai population suggesting that it is not a strong factor associated with over-anticoagulation among Thais.

**Acknowledgments**

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# ความชุกของการเปลี่ยนแปลงทางพันธุกรรมยีนวีคอซีวันตำแหน่ง – 1639 ในผู้ป่วยผู้ใหญ่ไทยที่รับประทานยาวอร์ฟารินและมีระดับไอเอ็นอาร์มากกว่าสี่

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***บทคัดย่อ : ที่มา :*** *การศึกษาก่อนหน้านี้พบว่าการเปลี่ยนแปลงทางพันธุกรรมยีนวีคอซีวันตำแหน่ง – 1639 ชนิด เอเอ พบได้ในชาวเอเชียได้บ่อยกว่าชาวตะวันตก และ ทำให้ต้องการขนาดยาวอร์ฟารินในขนาดน้อยกว่าจึงเป็นที่มาของสมมุติฐานว่าการเปลี่ยนแปลงทางพันธุกรรมแบบนี้อาจเป็นปัจจัยเสี่ยงของการเกิดภาวะวอร์ฟารินเกินขนาด* ***วิธีการ :*** *เป็นการศึกษาแบบพรรณนาหาความชุกของการเปลี่ยนแปลงทางพันธุกรรมยีนวีคอซีวัน -1639 ในผู้ป่วยที่รับประทานวอร์ฟารินและมีระดับ INR มากกว่าสี่ ที่โรงพยาบาลจุฬาลงกรณ์* ***ผลการศึกษา :*** *มีผู้ป่วยทั้งสิ้น 44 รายที่มีระดับ INR มากกว่าสี่ ค่ามัธยฐานของอายุ คือ 66 ปี (พิสัย 21-90 ปี) ผู้ป่วย 15 ราย (ร้อยละ 34) เป็นเพศชาย ตรวจพบการเปลี่ยนแปลงทางพันธุกรรมวีคอซีวัน -1639 ชนิด เอเอ เอจี และ จีจี เป็น 26 ราย (ร้อยละ 59.1) 16 ราย (ร้อยละ 36.4) และ 2 ราย (ร้อยละ4,5) ตามลำดับ ความชุกนี้ใกล้เคียงกับความชุกในประชากรไทย และ ผู้ป่วยไทยที่ได้รับยาวอร์ฟาริน* ***สรุป :*** *การเปลี่ยนแปลงทางพันธุกรรมยีนวีคอซีวัน -1639 ชนิดที่ไวต่อวอร์ฟารินไม่สัมพันธ์กับการเกิดระดับ INR ที่มากกว่าสี่*

***คำสำคัญ :*** ● *วอร์ฟาริน* ● *เกินขนาด* ● *วีคอซีวัน* ● *การเปลี่ยนแปลงทางพันธุกรรม*

***วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2553;20:113-8.***