Original Article

Frequencies of Polymorphism Associated with Cytochrome P450 2C9 in Thais

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Abstract : Background: *The use of warfarin for prevention and treatment of thromboembolic disorder is complicated by unpredictable dose response, partly attributed to polymorphisms of the CYP2C9 which can alter catalytic properties and increase anticoagulant effect. This study aims to determine the frequencies of CYP2C9 polymorphisms in Thai people and functional effects of CYP2C9 genotype in responsible for warfarin dosage.* ***Methods:*** *Patients requiring warfarin therapy and healthy blood donors were recruited. Warfarin dosage and clinical informations were collected. The CYP2C9 genotype was determined by standard PCR and RFLP.* ***Results:*** *The genotype of 326 blood donors and 67 patients were CYP2C9*1/CYP2C9*1; 91.4%, 91.1% and CYP2C9*1/CYP2C9*3; 8.6%, 8.9%, respectively. There were no gender difference ($p = 0.781$). The mean warfarin dose (mean$\pm$SD) for patients who had INR 2.0-3.0 was 27.8$\pm$12.7 mg/week. Patients with CYP2C9*1/CYP2C9*3 required a lower warfarin doses (20.1$\pm$5.9 mg/week) compared to CYP2C9*1/CYP2C9*1 (28.7$\pm$13.0 mg/week) but there was not statistically significant ($p = 0.081$). However, the mean warfarin dose was lower in elderly of more than 60 years compared to those less than 60 years ($p < 0.01$).* ***Conclusion:*** *Frequencies of CYP2C9 polymorphisms in Thais are mainly CYP2C9*1/CYP2C9*1 91.4% and CYP2C9*1/CYP2C9*3 8.6%, similar to previous studies in Asian people. The patients with CYP2C9*1/CYP2C9*3 tended to receive lesser dose of warfarin than CYP2C9*1/CYP2C9*1 genotype.*

Key Words : ● *Warfarin* ● *Cytochrome P450 2C9*

Thai J Hematol Transf Med 2006;16:213-20.

Received January 17, 2006. Accepted February 3, 2003.

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Warfarin is an oral anticoagulant used for the prevention and treatment of thromboembolic disorder, warfarin produce its anticoagulant effect by directly inhibits two enzymes responsible for regeneration of vitamin KH_2, vitamin K epoxide reductase and vitamin K reductase which prevent activation of vitamin K-dependent coagulation factors. Warfarin therapy is problematic due to the narrow therapeutic index and frequent bleeding complications. The estimated average annual frequency of fatal, major and minor bleedings are 0.6%, 3.0%, and 9.6%, respectively. The frequency of major bleeding in a prospectively follow-up population after beginning therapy was 3.0% during the first month, 0.8% per month during the remaining of the first year, and 0.3% per month after the first year[1].

Warfarin is available as a racemic mixture of two R-and S-enantiomers. The clinical effect of warfarin relies mainly on S-warfarin, which is pharmacologically more active than R-warfarin. S-warfarin is mainly hydroxylated by the cytochrome P450 2C9 (CYP2C9) enzyme to 7-hydroxywarfarin[1,2]. Recent pharmacogenetic studies indicated that interindividual sensitivity to warfarin may be partly attributed to polymorphisms of CYP2C9.

CYP2C9 has six variants alleles but the most frequent studies include Arg144 Ile359 (CYP2 C9*1), Cys144Ile359 (CYP2C9*2) and Arg144Ieu 359 (CYP2C9*3). CYP2C9*1 is predominant or wild type allele, whereas CYP2C9*2 and CYP2C 9*3 are less common and have approximately 12% and 5% of wild-type enzymatic activity, respectively[14]. The polymorphisms of CYP2C9 can alter catalytic properties by decrease hydroxylation and clearance of S-warfarin which cause increase in warfarin level and the risk of bleeding complication[57].

The frequency of CYP2C9 allelic variants has been reported to differ in individual of distinct ethnicity. Among Caucasian and Turkish populations, two thirds of individuals express the wild-type genotype, one thirds have either CYP2C9*1/CYP2C9*2 or CYP2C9*1/CYP2C9*3 genotype and less than 2.5% of individual have CYP2C9*2/CYP2C9*2, CYP2C9*2/CYP2C9*3 and CYP2C9*3/ CYP2C9*3 genotypes[2]. The CYP2C9*2 allelic variant has not found in East Asian including Chinese, Japanese and Korean population[8-10]. Therefore, this study aims to determine the frequencies of CYP2C9 polymor-

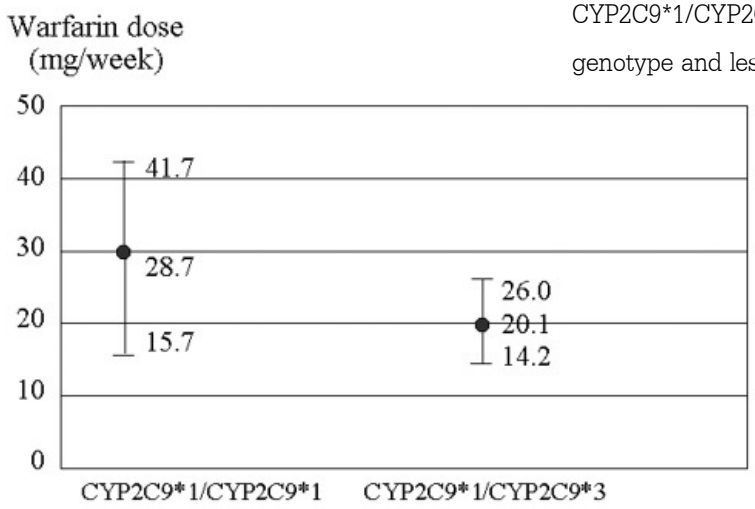

Fig 1 Correlation of CYP2C9 genotype and warfarin doses

Table 1. Indication for warfarin therapy in studied patients.

Diagnosis	Number (%)
Deep venous thrombosis with pulmonary embolism.	9 (13.4)
Deep venous thrombosis without pulmonary embolism.	42 (62.6)
Atrial fibrillation.	3 (4.5)
Antiphospholipid syndrome.	3 (4.5)
Valvular heart disease.	3 (4.5)
Branch retinal vein occlusion.	2 (3)
Transverse sinus thrombosis.	2 (3)
Superior mesenteric vein/superior mesenteric artery thrombosis.	2 (3)
Portal vein/splenic vein thrombosis	1 (1.5)

phisms in Thai people and functional effects of CYP2C9 genotype in responsible for warfarin dosage.

Material and Method

Subjects

A total of 326 normal healthy donors (223 males and 103 females), age 20-62 years (mean 39±15 years) and 67 individuals with warfarin therapy (23 males, 44 females), age 17-83 years (mean 49±14 years) attended in the Division of Hematology, Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University over 9-months period (September 2004 through May 2005). The indications for warfarin therapy were listed in Table 1. We excluded patients with significant end-organ dysfunction or who were taking medication known to significant interact with warfarin therapy and defined the stable maintenance warfarin dose as a dose that did not vary by more than 10% in the previous 4 weeks and INR vary less than 15%. Following informed consent, a sample of blood (10 mL) was obtained from individual after taking warfarin for 16 hours. In addition, demographic and clinical information were collected: gender, ethnicity, weight, indication for anticoagulation, dose of warfarin, PT/INR value and other medication.

Genotyping

Genomic DNA was isolated from whole blood using phenol-chloroform technique, the polymerase chain reaction (PCR) to detect CYP2C9*1 (Arg144⟶Cys144), and CYP2C9*3 (Ile359⟶Leu 359) were performed as described by Stubbin[11] and Gill[12]. Briefly PCR was performed in 25 μL volume, 1.5 mM $MgCl_2$, 1 x PCR buffer (Invitrogen) 200 μM dNTP, 0.5 U of Taq DNA polymerase (Invitrogen) and 100 ng of forward and reverse primers. Thrity-five PCR cycles were used and the annealing temperature was 61°C, each PCR products were digested with restriction enzymes (AvaII for CYP2C9*1, NsiI and KpnI for CYP2C

Table 2. Distribution of CYP2C9 genotypes in Thai population and in patients receiving warfarin therapy

Genotype	Normal Population (%)	Patients (%)
CYP2C9*1/CYP2C9*1	91.4 (298/326)	91.1 (61/67)
CYP2C9*1/CYP2C9*2	0	0
CYP2C9*1/CYP2C9*3	8.6 (28/326)	8.9(6/67)
CYP2C9*2/CYP2C9*2	0	0
CYP2C9*2/CYP2C9*3	0	0
CYP2C9*3/CYP2C9*3	0	0

9*3). The DNA fragments were separated in 8% acrylamide gel electrophoresis and detected by ethidium bromide staining[11,13].

Statistical analysis

Chi-square or Fisher's exact test was used for discrete data, where appropriate. Mann-Whitney U or Wilcoxon Signed Rank tests were used for continuous data.

Results

The frequencies of CYP2C9 polymorphisms in healthy Thai peoples and patients received warfarin therapy were shown in Table 2. CYP2 C9*1/CYP2C9*1 was found in 298 out of 326 blood donors (91.4%) including 205 males (68.8%) and 93 females (31.2%), in 61 out of 67 patients (91.1%) including 19 males (31.1%) and 42 females (68.8%); and CYP2C9*1/CYP2C9*3 in 28 out of 326 blood donors (8.6%) including 18 males (64.3%) and 10 females (35.7%), in 6 out of 67 patients (8.9%) including 2 males (33.3%) and 4 females (66.7%). There were no gender differences in polymorphism frequency ($p = 0.893$) and the distribution of the genotypes were similar between blood donors and patients ($p = 0.577$).

Fifty-seven patients with INR 2.0-3.0 were selected for analyzing the association of CYP2CP variants with individual sensitivity to warfarin therapy. Among 52 patients with CYP2C9*1/CYP2C9*1 with a mean prothrombin time of 29.1±3.7 seconds and a mean INR of 2.45±0.3 had a mean weekly warfarin dose of 28.7±13.0 mg/week whereas 6 patients with CYP2C9*1/CYP2C9*3 with a mean prothrombin time of 31.1±4.5 seconds and a mean INR of 2.5±0.3 had a mean weekly warfarin dose of 20.1±5.9 mg/week. However, there was not statistically significant ($p = 0.081$). Sensitivity to warfarin therapy influenced by other factors including gender, age and bodyweight was also analyzed. The mean weekly warfarin dose was 27.8±12.7 mg/week which was not influenced by gender (25.7±10.0 mg/week, 0.48±0.2 mg/kg/week in female; 31.4±13.2 mg/week, 0.42±0.15 mg/kg/week in male, $p = 0.124$). On the contrary, the mean weekly warfarin dose among patients whose ages >60 years (18.0±6.3 mg/wk, 0.2±0.13 mg/kg) was

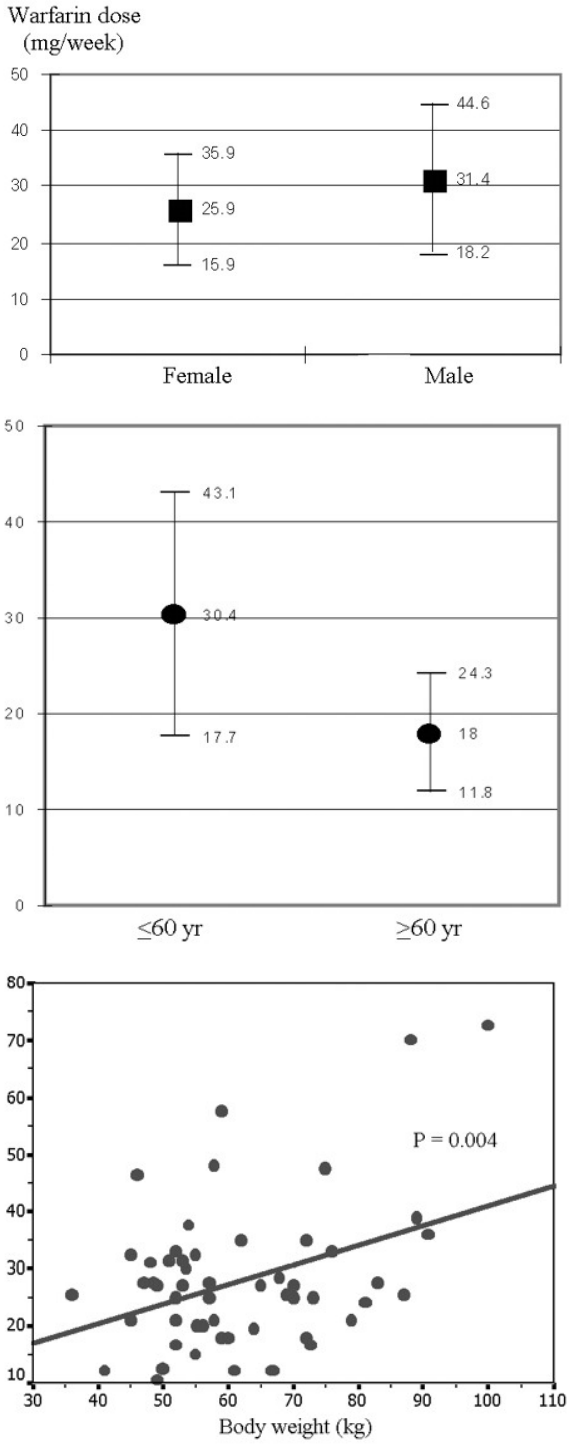

Fig 2 Association of gender, age and body weights and the warfarin requirement.

significantly lower than those with age <60 years (30.4±12.7 mg/wk, 0.5±0.18 mg/kg/week, $p < 0.001$). Moreover, patients with higher body weight significantly required a higher weekly warfarin dose compared to those with less body weight ($p = 0.004$).

Discussion

Genetic polymorphism of cytochrome P450 (CYP)2C9 is one of the major factor responsible for the metabolism of warfarin. Several reports have documented the difference in prevalence of the allelic variants of CYP2C9 among different ethnic group involving Caucasian, African and Asian populations. The allele frequencies of CYP2C9*2 and CYP2C9*3 tend to be greater in Caucasian and African-Americans populations but CYP2C9*2 allelic variant has not been found in Asian population[13,14]. Frequency of CYP2C9 polymorphisms in Thais is mainly CYP2C9*1/CYP2C9*1 91.4% and CYP2C9*1/CYP2C9*3 8.6%, but no individual expressing the CYP2C9*2 allele and homozygous CYP2C9*3, which was similar to several previous studies among Japanese[8], Chinese, Taiwanese[9], Korean[10] and Malaysian[14].

The allelic variants CYP2C9*2 and CYP2C9*3 have been reported to have decreased enzymatic activity in the metabolism of warfarin with 16-20% and 5% catalytic efficiency of CYP2C9*1[15-17] respectively.

Currently, warfarin is initiated with the standard "loading" doses and the appreciable fraction of patients became excessively or inad-

equately anticoagulated which will lead to an increased risk of both hemorrhagic and thrombotic complication. A more accurately warfarin dose may potential extend the safety and efficacy of this medication. In previously reports found that age and bodyweight accounted for approximately 25% and 14% to the interpatient variability in warfarin dosing[18]. And a number of studies examine the functional effects of CYP2C9 genotypes in patients receiving warfarin have been conducted. Significantly lower warfarin dose requirements have been reported for patients carrying either the CYP2C9*2 or CYP2C9*3 allele. The result of our study found that the weekly maintenance dose of warfarin in patients having CYP2C9*1*CYP2C9*3 tended to receive lesser dose of warfarin than patients with CYP2C9*1/CYP2C9*1 genotype (20.1±5.9 mg/week versus 28.7±13.0 mg/week) but it was not statistically significant ($p = 0.081$). However, the number of patients in our study is rather small and also other factors may influence the metabolism of warfarin, for instance, dietary intake and the polymorphism of VKORC1 gene which can alter the vitamin K epoxide reductase activity in vitamin K metabolism[19]. As well as the CYP2C9 variant in Thai population is heterozygous CYP2C9*1/CYP2C9*3 which may effect the catalytic activity of the enzyme less than those with homozygous CYP2C9*2 or CYP2C9*3 variants.

We also found that age and weight were influenced the warfarin dose especially in elderly (age >60 years). Since aging is associated with a decrease in plasma albumin concentrations but warfarin is highly plasma protein-bound, the elderly have less circulating albumin, which results in a decrease in bound warfarin and an increase in unbound warfarin, the active form of warfarin. Other factors that may contribute to the development of problems associated with anticoagulant therapy in the elderly include a decrease in haemostatic response, resulting from a 33 to 50% decrease in the synthesis of clotting factors in the elderly as opposed to the young due to age-related reductions in receptor sensitivity to vitamin K[20].

On the basis of small number of individuals in our study, we were unable to establish the significant relationship between CYP2C9 polymorphisms and warfarin dose, however, we can conclude that the individual with CYP2C9 variant has trended to receive lower dose of warfarin compare to wild type genotype. Further study in a large number of individuals is warranted.

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ความถี่ของยีนไซโตโครม P450 2C9 ในคนไทย

สรัญญา บุษกรเรืองรัตน์, อำไพวรรณ จวนสัมฤทธิ์, พันธุ์เทพ อังชัยสุขศิริ*, วีระศักดิ์ ศาสนกุล และ ประกายวรรณ เกษเกษม

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บทคัดย่อ : *ผู้ป่วยที่ได้รับยาวาร์ฟารินเพื่อการรักษาและป้องกันภาวะลิ่มเลือดอุดตันมีโอกาสเกิดข้อแทรกซ้อนจากยาวาร์ฟาริน เนื่องจากไม่สามารถคาดคะเนการตอบสนองต่อยาในผู้ป่วยได้ล่วงหน้า ยีนไซโตโครม P450 (CYP) 2C9 ซึ่งเกี่ยวข้องกับการออกฤทธิ์ของยาวาร์ฟาริน อาจเพิ่มฤทธิ์ต้านการแข็งตัวของเลือดทำให้เกิดข้อแทรกซ้อนได้ คณะผู้วิจัยจึงได้ศึกษาความถี่ของยีน CYP 2C9 ในผู้บริจาคเลือดจำนวน 326 ราย และในผู้ป่วยที่ได้รับยาวาร์ฟารินจำนวน 67 ราย ผลการศึกษาพบว่า ความถี่ของยีน CYP 2C9 ชนิด *1/*1 ซึ่งเป็น wild type เท่ากับร้อยละ 91.4 ในผู้บริจาคเลือดและร้อยละ 91.1 ในผู้ป่วยและชนิด *1/*3 เท่ากับร้อยละ 8.6 ในผู้บริจาคเลือด และร้อยละ 8.9 ในผู้ป่วย ซึ่งไม่มีความแตกต่างระหว่างเพศหญิงและเพศชาย ส่วนค่า mean±SD ของปริมาณยาวาร์ฟารินที่ผู้ป่วยที่มีระดับ prothrombin time (INR เท่ากับ 2.0-3.0) เท่ากับ 27.8±12.7 มก./สัปดาห์ และผู้ป่วยที่มียีนไซโตโครมชนิด *1/*3 ใช้ยาวาร์ฟาริน 20.1±5.9 มก./สัปดาห์ ซึ่งต่ำกว่าผู้ป่วยที่มียีนไซโตโครมชนิด *1/*1 (28.7±13.0 มก./สัปดาห์) แต่ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ (p = 0.081) นอกจากนี้ ผู้ป่วยที่มีอายุมากกว่า 60 ปี มีความต้องการใช้ยาวาร์ฟารินต่ำกว่าผู้ป่วยที่มีอายุน้อยกว่า 60 ปี*

Key Words : ● *Warfarin* ● *Cytochrome P450 2C9*

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2549;16:213-20.