Tzu Chi Medical Journal 25 (2013) 213–217

Contents lists available at ScienceDirect

Tzu Chi Medical Journal

journal homepage: www.tzuchimedjnl.com

Original Article

Sex differences in the effective warfarin dosage in Han and aboriginal Taiwanese patients with the *VKORC1-1639AA* genotype

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ARTICLE INFO

Article history:
Received 24 April 2013
Received in revised form
9 May 2013
Accepted 21 May 2013

Keywords:
Aborigines
CYP2C9
Genetic polymorphism
VKORC1
Warfarin

ABSTRACT

Objective: This study aims to investigate whether single-nucleotide polymorphisms (SNPs) *CYP2C9* and *VKORC1* can be used to adjust effective warfarin treatment for aboriginal Taiwanese population.
Materials and Methods: This study investigates the association of SNPs *CYP2C9* and *VKORC1* and clinical factors [sex, age, and body mass index (BMI)] with variable responses to warfarin treatment in 42 aboriginal and 63 Han Taiwanese people.
Results: The incidence of the *VKORC1-1639AA* genotype and the effective warfarin dosage were similar in the populations studied. However, the required dosage of warfarin for women with *VKORC1-1639AA* polymorphism was significantly lower than for their male counterparts.
Conclusion: This result provides guidance for prescribing an effective warfarin dosage for aboriginal and Han Taiwanese patients with genetic polymorphisms.
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1. Introduction

Warfarin is the most common oral anticoagulant for patients with cardioembolic stroke and for secondary prevention of venous thromboembolism, acute myocardial infarction, embolic stroke, and chronic atrial fibrillation [1–3]. However, the dosage required for a therapeutic effect varies widely among different people and ethnic groups [4–7]. The therapeutic dosage range of warfarin is narrow, and there is a risk of serious bleeding with overdosing [8]. Complex factors including age, body mass index (BMI), sex, intake of vitamin K, concomitant diseases, and drugs influence the effective warfarin dosage [8–10]. In addition to the potential influence of these clinical variables, inherited differences in drug metabolism and drug targets can also affect the efficacy and toxicity of warfarin.

Twenty-nine genes are suspected to mediate the effects of warfarin, mainly contributing to the pharmacokinetics and pharmacodynamics of the drug [11]. Most pharmacogenomic research on warfarin has been based on association studies examining polymorphisms in the cytochrome p450 2C9 (*CYP2C9*) and vitamin K epoxide reductase complex 1 (*VKORC1*) genes. Common variants in the *CYP2C9* and *VKORC1* genes, combined with a subset of environmental determinants, account for approximately 50–60% of the variability in the required warfarin dosage [11,12].

The *CYP2C9* gene, which encodes for the key enzyme that metabolizes warfarin, has a number of functional variants associated with lower warfarin requirements. For example, *CYP2C9*2* and *CYP2C9*3* differ in prevalence among different ethnic groups [13–16]. However, these two *CYP2C9* genetic polymorphisms cannot account for lower warfarin requirements among Chinese patients because they rarely occur in Asian populations [17–19]. Mutation in the coding region of the *VKORC1* gene has been shown to cause multiple coagulation factor deficiency type 2 and warfarin resistance [20,21]. Several single-nucleotide polymorphisms (SNPs) of the *VKORC1* gene also correlate with variable warfarin dosages and adverse bleeding events [22,23]. Yuan et al reported that the *VKORC1* promoter polymorphism (-1639G>A) was present in all warfarin-sensitive patients. They showed that the frequency of the *AA* genotype was significantly higher in Han Chinese than in a Caucasian population receiving warfarin. Later, several other studies also demonstrated that the *VKORC1* promoter polymorphism (-1639G>A) is a major determinant of warfarin response in anticoagulated patients of various ethnic groups [18,24–28].

The Taiwanese population is comprised of 98% Han Chinese and 2% aboriginal. The Han Chinese people have been immigrating to Taiwan since the 17th century, whereas aborigines have been

Conflict of interest: none.

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http://dx.doi.org/10.1016/j.tcmj.2013.06.005

living in Taiwan for approximately 5500 years. The genetic and linguistic backgrounds of Taiwanese aborigines differ from Han Chinese people, but are closely related to other Austronesian ethnic groups found across the islands of Southeast Asia [29]. Warfarin is not widely considered in treating Taiwanese aborigines because the effective dosage range and pharmacogenomic associations have not been investigated; therefore, the risk of adverse bleeding is high. Elucidating the association of *CYP2C9* and *VKORC1* SNPs with the effective warfarin dosage for Taiwanese aborigines will ensure precise prescription and treatment. This study investigates the frequency of *CYP2C9* and *VKORC1* SNPs in aboriginal and Han Taiwanese patients and analyzes the association of these SNPs with warfarin responses and adverse bleeding effects at different dosages. Our results demonstrated that the effective warfarin dosage ranges and incidence of the *VKORC1-1639* variant are similar for the two populations studied. More than 80% of recruited patients carried the *VKORC1-1639AA* genotype, which is associated with a lower warfarin maintenance dosage. The *CYP2C9*3* allele, which contributes to warfarin-sensitive responses in the Caucasian population, was not found among the recruited patients. Among *VKORC1-1639AA* carriers, the required warfarin dosage for women was significantly lower than for men. Our results can provide guidance for clinicians in prescribing an effective warfarin dosage to aboriginal Taiwanese and genetically related Austronesians.

2. Materials and methods

2.1. Ethics statement

This research was approved by the Institutional Review Board of Buddhist Tzu Chi Medical Center (IRB096-58). All patients who participated in this study provided written informed consent before blood sampling for genetic analysis as well as for publication of the clinical data.

2.2. Recruited patients

From 2006 to 2009, researchers recruited patients receiving maintenance warfarin therapy for secondary stroke prevention who had a stable international normalized ratio (INR) between 1.5 and 3 for 3 months. Anticoagulation of all patients was stably controlled with a target INR between 1.5 and 3, according to different indications, for the prevention of secondary stroke [2,30].

Indicators for warfarin treatment included different stroke subtypes including cardioembolic infarction, embolic infarction of unknown origin, basilar artery occlusion with brain-stem infarction, and stroke in evolution. The stroke subtype of the recruited patients was diagnosed and clarified by magnetic resonance imaging of the brain. Cardioembolic stroke was verified by examining the thrombus in the left atrium of the heart through a transesophageal endoscopic examination. Stroke in evolution was defined clinically as neurological deficit progression within 48 hours after a stroke.

Concomitant liver function tests, including evaluating levels of aspartate transaminase (AST) and alanine transaminase (ALT) [31], were done, and patients with moderate to severe liver function impairment were excluded. A questionnaire based on principles of reference [32] was completed by recruited patients or their families; patients with poor compliance to warfarin therapy were also excluded based on the answers provided by the patients on the questionnaire. The ethnicity of patients was included in the questionnaire. A participant was considered Han Taiwanese or aboriginal Taiwanese if his or her parents were both of that same ethnic group.

Clinical data collection consisted of a review of inpatient and outpatient medical records. Retrospectively, researchers collected clinical factors such as age, sex, body weight, height, concurrent medications, and concomitant diseases, which could influence the response to warfarin. The average warfarin maintenance dosage was the mean dosage between two consecutive INR values collected from the medical records. Any adverse bleeding events during anticoagulation were recorded in the medical records. Venous blood was sampled from participants for genomic DNA extraction and genetic analysis.

2.3. Genomic DNA extraction

Approximately 10 mL of venous blood was drawn from each patient using ethylenediaminetetraacetic acid (EDTA) tubes and sent to a laboratory for genomic DNA extraction. Leukocyte DNA was isolated using a commercial DNA purification kit (QIAamp DNA Mini Kit; QIAGEN, Hamburg, Germany) from 10 mL of venous blood, according to the manufacturer's instructions. After purification, genomic DNA integrity was verified through electrophoresis on 1% Tris–acetate–EDTA agarose gel. The concentration of genomic DNA was measured spectrophotometrically at 260 nm.

2.4. DNA sequencing

DNA sequences of *CYP2C9* and *VKORC1* were analyzed according to the National Center for Biotechnology Information database. Primers were used to detect *CYP2C9*3* allelic variants in exon 7 of the *CYP2C9* gene and -1639G>A variants in the *VKORC1* promoter regions as listed in Table 1. Primers were designed using the primer 3 polymerase chain reaction primer program. Genotypes of *CYP2C9*3* and *VKORC1-1639G>A* were extracted using the direct DNA-sequencing method (3700 DNA analyzer; Applied Biosystems, Grand Island, NY) at the National Yang-Ming University Genome Research Center. Sequence variants were directly analyzed by DNA sequence viewer software (Chromas Lite v2.01). This research did not generate new gene-sequencing data.

2.5. Statistical analysis

The warfarin maintenance dose was compared across *VKORC1-1639* genotypes and ethnic groups using Student unpaired *t* test. The Chi-square test was used to analyze sex across the *VKORC1* genotype. A simple regression test correlated the warfarin dose with variables including age, sex, and BMI. The results are reported as mean ± standard deviation. A p value < 0.05 was considered to be significant.

3. Results

3.1. Patient characteristics

The frequencies of *CYP2C9*3* and *VKORC1-1639G>A* sequence variants were analyzed in 105 stroke patients regularly receiving warfarin with stable INR monitoring for 3 months. The mean age of these patients was 65.6 ± 2.2 years (range: 46–84 years). The sex

Table 1
Primers used for amplification and sequencing of polymorphisms of the *CYP2C9* and *VKORC1* genes.

Gene	Forward primer	Reverse primer
*CYP2C9*3*	5′-CCCCTGAATTGCT ACAACAAA-3′	5′-GGGACTTCGAAAA CATGGAG-3′
VKLRC1 (−1639G>A)	5′-CAGAAGGGTAGG TGCAACAGTAA-3′	5′-CACTGCAACTTGTT TCTCTTTCC-3′

ratio (male to female) was 0.70 ($n = 74$) to 0.30 ($n = 31$). The mean BMI was 25.8 ± 2.7 (range: 20.6–30.1). Liver function tests, including measurement of AST and ALT levels, were within the normal ranges (mean AST: 27.9 ± 2.5 IU/L, range: 12–66 IU/L; and mean ALT: 22.8 ± 2.7 IU/L, range: 8–77 IU/L) in all patients but one who had mildly elevated liver function results. Recruited patients were 60% ($n = 63$) Han and 40% ($n = 42$) aboriginal Taiwanese (Table 2). All participants in this study met strict recruiting criteria, and all aborigine patients had a pure genetic background. Ethnicity was determined after the patients signed informed consents. Participants were required to have parents who were both Han Taiwanese or both aboriginal Taiwanese to be eligible for this study.

The medical indicators for warfarin therapy were cardioembolic stroke (83.8%), basilar artery occlusion with brain-stem infarction (11.4%), and stroke in evolution (4.8%). The average stable target INR in the recruited patients was 2.19 ± 0.05 (range: 1.60–2.69) and was calculated according to standard guidelines [2,3,30,33]. The average maintenance dosage of warfarin was the mean dosage during the period when two consecutive INR values were collected from the medical records. The mean daily maintenance dose of warfarin was 3.15 ± 0.3 mg (range: 1–6 mg) to achieve an INR value 2.19 ± 0.05 (range: 1.60–2.69).

Researchers also recorded and analyzed concomitant chronic illnesses and concurrent administration of drugs in recruited patients, which could influence the warfarin maintenance dosage [9,34]. There was an average of 2.5 ± 0.2 concomitant chronic disorders per patient (range: 1–4 disorders). The most common concomitant disorders were hypertension (63.8%), hyperlipidemia (56.2%), chronic atrial fibrillation (9.5%), diabetes mellitus (11.4%), and coronary artery disease (12.4%), which are all risk factors for cerebrovascular disorders. The mean number of concurrent medications taken by the recruited patients was 3.4 ± 0.3 (range: 1–6).

Table 2
Demographic data of recruited patients.

Characteristic	Patients ($n = 105$)
Age [y; mean (range)]	65.6 (46–84)
Sex (n, %)	
Male	$n = 74$, 70.5%
Female	$n = 31$, 29.5%
BMI [mean ± SD (range)]	25.8 ± 2.7 (20.6–30.1)
Liver function test	
AST [IU/L; mean ± SD (range)]	27.9 ± 2.5 (12–66)
ALT [IU/L; mean ± SD (range)]	22.8 ± 2.7 (8–77)
Concomitant diseases [type, IU/L; mean ± SD (range)]	2.5 ± 0.2 (1–4)
Hypertension (n, %)	$n = 67$, 63.8%
Hyperlipidemia (n, %)	$n = 59$, 56.2%
Atrial fibrillation (n, %)	$n = 10$, 9.5%
Diabetes mellitus (n, %)	$n = 12$, 11.4%
Coronary artery diseases (n, %)	$n = 13$, 12.4%
Concurrent medications [no.; mean n ± SD (range)]	3.4 ± 0.3 (1–6)
Indication for warfarin (n, %)	
Cardioembolic stroke	$n = 88$, 83.8%
BAO with brain-stem infarction	$n = 12$, 11.4%
Stroke in evolution	$n = 5$, 4.8 %
INR [mean n ± SD (range)]	2.19 ± 0.05 (1.60–2.69)
Warfarin dosage [mg/day; mean n ± SD (range)]	3.1 ± 0.3 (1–6)
Ethnic group (n, %)	
Han Taiwanese	$n = 63$, 60%
Aboriginal Taiwanese	$n = 42$, 40%
CYP2C9 genotype (n, %)	
*1/*1	105, 100%
VKORC1 genotype (n, %)	
AA	89, 84.8%
AG	16, 15.2%
GG	0

ALT = alanine transaminase; AST = aspartate transaminase; BAO = basilar artery occlusion; BMI = body mass index; INR = international normalized ratio; SD = standard deviation; y = years.

Interactions with concurrent medications with warfarin were analyzed according to a review lecture by Holbrook et al, and included atorvastatin ($n = 13$), phenytoin ($n = 5$), omeprazole ($n = 7$), diltiazem ($n = 6$), allopurinol ($n = 5$), fenofibrate ($n = 1$), and nonsteroidal anti-inflammatory drugs ($n = 3$) [9].

3.2. Required warfarin dosage for Han and aboriginal Taiwanese patients is similar

The mean daily warfarin dose required to maintain a stable INR between 1.60 and 2.69 among patients was 3.15 ± 0.3 mg (range: 1–6 mg). Student unpaired t test showed that the mean warfarin dosage was similar for Han and aboriginal Taiwanese patients (3.0 ± 0.4 mg/day for Hans and 3.3 ± 0.4 mg/day for aborigines, $p = 0.51$) to achieve a stable INR.

3.3. Frequency of CYP2C9 and VKORC1-1639G>A variants in warfarin-treated Han and aboriginal Taiwanese patients is similar

The *CYP2C9*3* and *VKORC1-1639G>A* SNPs were selected for analysis because these two genetic polymorphisms have been reported to be the main contributors to variable warfarin responses in different populations [25,27]. All Taiwanese Han and aboriginal patients treated with warfarin carried the *CYP2C9*1* variant; no *CYP2C9*3* variant was recorded. The *VKORC1-1639AA* genotype was found in the majority of patients (89/105, 84.8%). Among them, 82.5% (52/63) of Han and 88% (37/42) of aboriginal Taiwanese patients were carriers (Table 3). The remaining patients had the *VKORC1-1639AG* genotype (17.5% for Han Taiwanese and 12% for aboriginal Taiwanese patients). The *VKORC1-1639GG* genotype was not detected.

3.4. Association analysis of the VKORC1-1639G>A genotype with warfarin dosage

To evaluate the association of the *VKORC1* genotype with the daily warfarin dosage, the mean warfarin dosages were analyzed and compared against different *VKORC1* genotypes using Student unpaired t test. Patients with *VKORC1-1639AA* had significantly lower dosage requirements (mean: 2.51 ± 0.2 mg/day; range: 1–5 mg/day, $p < 0.001$) than those with the heterozygous *VKORC1-1639AG* genotype (mean: 4.28 ± 0.2 mg/day, range: 4–6 mg/day) (Table 3). The mean required warfarin dosage was similar for both Han (2.33 mg/day) and aboriginal Taiwanese patients (3.19 mg/day) with the *AA* genotype ($p = 0.08$).

3.5. Association of age, sex, and BMI with effective warfarin dosage

Consistent with the results of previous studies, data from this study showed that the warfarin dosage requirement correlated negatively with age, decreasing by 1.1 mg per decade within the age

Table 3
Clinical characteristics of patients against the *VKORC1* genotype.

Clinical characteristic	*−1639AA* ($n = 89$)	*−1639AG* ($n = 16$)	p
Men:women (n)	$n = 58:31$	$n = 16:0$	—
Hans:aborigines	$n = 52:37$	$n = 11:5$	—
Mean age ± SD (y)	66 ± 2.3	65 ± 6.2	0.72
Mean BMI ± SD (y)	25 ± 2.3	24 ± 0.8	0.31
Mean maintenance INR ± SD (y)	2.18 ± 0.05	2.18 ± 0.21	0.29
Required dosage of warfarin (mg/day)	2.51 ± 0.2	4.28 ± 0.2	0.0009

BMI = body mass index, calculated as body weight (kg)/body height (m^2); INR = international normalized ratio = (patient prothrombin time/mean normal prothrombin time)^{ISI}; SD = standard deviation; y = years.

range of 45–85 years, irrespective of body weight, ethnicity, or sex differences (Fig. 1). To examine the correlation between sex and warfarin dosage, the dosage was compared between men and women with the same *VKORC1-1639AA* genotype. The warfarin dosage required to achieve an INR between 1.60 and 2.69 ($p < 0.01$) in women was significantly lower ($n = 31$, 1.96 mg/day) than that required to achieve the same INR in men ($n = 58$, 3.07 mg/day) (Fig. 2). This difference had no relation to age and could not be explained by differences in the BMI between men and women ($p = 0.51$). No woman had the *VKORC1-1639AG* genotype among our recruited patients.

4. Discussion

This study demonstrated that the frequency of the *CYP2C9* and *VKORC1-1639G>A* polymorphisms in aboriginal Taiwanese patients is similar to those in Han Taiwanese patients, and verified that *VKORC1-1639AA* can also indicate a need for a lower warfarin maintenance dosage to prevent secondary stroke in Taiwanese aborigines. The warfarin maintenance dosage was negatively correlated with age. In addition, researchers discovered that the maintenance dosage was lower for women than men carrying the same *VOKRC-1639AA* polymorphism.

Consistent with previous studies for the Asian populations [16], the *CYP2C9*3* polymorphism was not present in the Han Taiwanese patients recruited for this study. The data also demonstrated that the aboriginal Taiwanese people, who have a different genetic background from Hans, do not carry this polymorphism either. All patients had the *CYP2C9*1* wild-type genotype. Thus, the *CYP2C9* genetic polymorphism cannot explain variable warfarin responses observed in the Taiwanese population.

The majority of patients, both in the Han (82.5%) and in the aboriginal (88%) population, had the *VKORC1-1639AA* genotype. No significant difference in the frequency of *VKORC1-1639AA* was observed between the two populations studied. Patients with the *VKORC1-1639AA* genotype required a significantly lower daily warfarin dose (2.51 ± 0.2 mg/day) than patients with the *VKORC1-1639AG* genotype (4.28 ± 0.2 mg/day, $p < 0.001$). The mean warfarin dosage requirement was similar between Han (2.33 ± 0.6 mg/day) and aboriginal Taiwanese patients (3.19 ± 1.2 mg/day) who had the same *VKORC1-1639AA* genotype ($p = 0.08$). The differences in warfarin sensitivity between patients with the *VKORC1-1639AA* and *AG* genotypes could be explained by changes in *VKORC1* promoter activity. Yuan et al demonstrated that

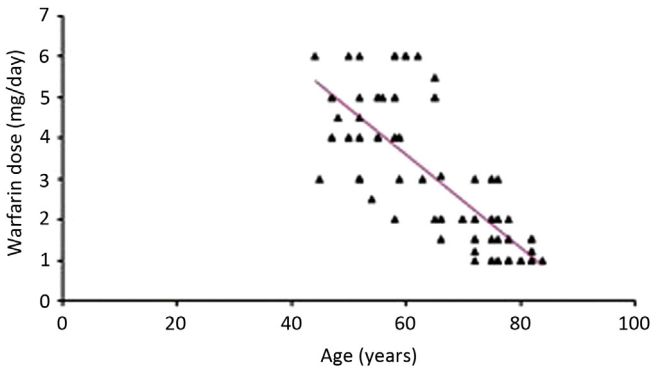

Fig. 1. Correlation of age with warfarin dosage. The correlation between age and warfarin dosage is analyzed using simple linear regression analysis. The regression line represents the regression equation $y = -0.1136x + 10.404$, $R^2 = 0.6149$. Dosage requirements for warfarin fall with age, decreasing by approximately 1.1 mg per decade between the age of 45 years and 85 years, irrespective of genotype, body weight, ethnic group, or sex.

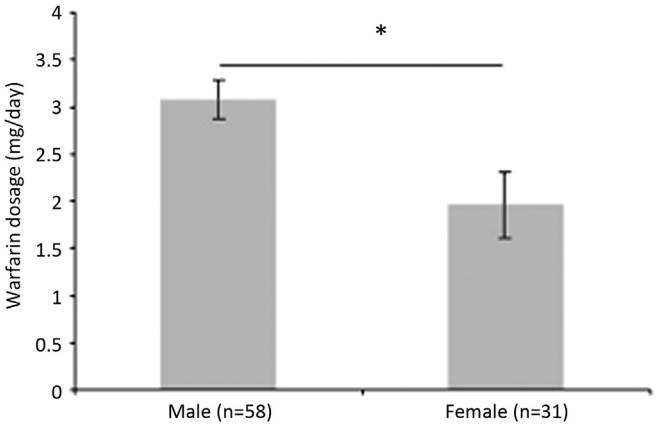

Fig. 2. Correlation of sex with warfarin dosage requirement. The histogram shows the mean warfarin dosage by sex. Student unpaired *t* test was used to compare the mean warfarin dosage used in men and women with the same genotype (*VKORC1-1639AA*). The difference had no relation to age or body mass index. *: $p < 0.05$.

VKORC1 promoter activity is decreased by the *VKORC1-1639AA* polymorphism. Decreased levels of *VKORC1* messenger RNA can lead to lower *VKORC1* activity, and ultimately result in a lower required warfarin dosage for appropriate anticoagulation [35].

Previous studies have shown that the warfarin dosage requirement diminishes with age. Older patients show increased sensitivity to the anticoagulant effects of this drug [36]. The cause of this phenomenon is unknown, and may be due to a combination of pharmacokinetic and pharmacodynamic factors. This study also found that the warfarin dosage requirement was reduced by approximately 1.1 mg per decade within the age range of 45–85 years. This finding confirms those of previous studies and suggests that physicians prescribe a lower warfarin dosage for elderly Taiwanese patients because of a higher risk of bleeding [10,34,36].

Whether sex is a factor contributing to variability in patient responses to warfarin remains controversial. Regardless of genetic polymorphisms, some previous studies reported no gender differences for the warfarin dosage in atrial fibrillation patients [37] and Han Chinese patients [38], whereas others indicated that women were more sensitive to warfarin than men [39–41]. This study found significantly different warfarin dosage requirements between men (3.07 mg/day) and women (1.96 mg/day, $p < 0.01$) with the same *VKORC1-1639AA* genotype. The gender difference for *VKORC1-1639AA* carriers could not be explained by differences in BMI ($p = 0.51$). Several factors have been studied to explain gender differences in the pharmacokinetics of drugs, including the rate of gastric emptying, intestinal transit time, expression of gut enzymes, total body water space, muscle mass, organ blood flow, organ function, body fat, and hepatic metabolism [42]. Further research is necessary to elucidate the cause of gender differences in the warfarin dosage for the *VKORC1-1639AA* population.

Two patients demonstrated adverse bleeding during the course of warfarin therapy, although they had stable INR monitoring. One patient presented with upper gastrointestinal bleeding, and the other demonstrated spontaneous retroperitoneal hematoma. Warfarin treatment was withdrawn from these two patients immediately when bleeding was noted. Two other patients showed variable warfarin responses (INR between 1.12 and 4.02) with a steady warfarin maintenance dosage (approximately 1.5–1.75 mg/day). However, no hemorrhage was detected, even in patients with unstable INRs. These four patients had the *VKORC1-1639AA* genotype; therefore, other genetic factors in addition to *VKORC1-1639AA* polymorphism may contribute to the variable warfarin responses observed in these cases.

This study concludes that the *VKORC1-1639G>A* genotype was the primary polymorphism in the *VKORC1* gene contributing to the need for a reduced warfarin dosage in the Taiwanese population, including the genetically different aboriginal population. The frequency of the *VKORC1-1639G>A* genotype is similar between Han and aboriginal Taiwanese patients, and the *VKORC1-1639AA* genotype correlates strongly with a lower daily warfarin dose in both Han and aboriginal Taiwanese patients. Women require a lower warfarin dosage than men in the *VKORC1-1639AA* genotype group. Combining genetic information of *VKORC1-1639G>A* polymorphism with the age and sex of patients may enable physicians to choose an optimal warfarin maintenance dosage for members of the Taiwanese population. The results will not only benefit Taiwanese aborigines, but will also be valuable for clinicians prescribing warfarin to other patients with Austronesian genetic backgrounds.

Acknowledgments

We are grateful to all the study participants. This study was supported by the Ministry of Economic Affairs, Taiwan (grant no. 98-EC-17-A-19-S2-0110 to I.Y.L).

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