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CYP2C9 gene polymorphisms and warfarin dose requirement: a single-center experience in Turkey

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Aim: In this study, we aimed to evaluate the relationship between genetic structure and daily dose requirement in patients under warfarin therapy presenting at our hospital.

Materials and methods: A total of 80 patients (45 female and 35 male) aged between 29 and 80 years (mean age: 59.86 ± 14.46 years), using warfarin for at least 1 month and presenting at Atatürk University Medical Faculty Outpatient Clinic between May 2009 and May 2010, were included in the study. Analysis of CYP2C9 1075 A>C and CYP2C9 430 C>T polymorphisms were performed using polymerase chain reaction (PCR) and the reverse-hybridization method.

Results: Patients with CYP2C9 genotype variants *2/*2, *2/*3, and *3/*3 required 3.40 mg/day, which was 1.57 mg less warfarin per day than patients with the wild-type genotype (*1/*1) (4.97 mg/day), and this difference was statistically significant (P = 0.014). Additionally, the patients with *1/*2 and *1/*3 genotypes required 3.93 mg/day, which was 1.04 mg less warfarin per day than those with the wild-type genotype (P = 0.01).

Conclusion: Because patients with *2 and *3 variants of the CYP2C9 gene are at a high risk for bleeding while taking an oral anticoagulant, clinicians should start at lower doses of warfarin in these patients and should make strict dose adjustments.

Key words: CYP2C9 1075 A>C, CYP2C9 430 C>T, warfarin, polymorphism

Introduction

Oral anticoagulants are commonly used drugs in the treatment and prophylaxis of arterial and venous thromboembolic disorders, such as deep vein thrombosis due to immobilization, surgery, postpartum care, or malignancy in clinical practice (1-3). Warfarin is the most commonly used oral anticoagulant (4). Individual response to warfarin is very variable. Because the therapeutic index of warfarin, which can be prescribed in daily doses of up to 40 mg, is low, patients should be closely monitored with an international normalized ratio (INR). However, despite this close monitoring, bleeding complications occur at an estimated rate of 7.6%-16.5% each year. Bleeding complications occur most commonly at the beginning of warfarin therapy (2,5-7).

Cytochrome P450 2C9 (CYP2C9) is a liver enzyme necessary for the oxidative metabolism of many major drugs like warfarin. A series of genetic polymorphisms has been defined in the area of CYP2C9 (8,9). One of these genetic polymorphisms is CYP2C9*2 (Arg144Cys), where arginine changes place with cysteine in exon 3 position 144; the other is CYP2C9*3 (Ile359Leu), where isoleucine changes place with leucine in exon 7 position 359. These polymorphisms show in vitro disruption of warfarin

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hydroxylation (10,11). One of the CYP2C9 alleles, the CYP2C9*1 allele wild type, is the most common type and is known as the natural genotype.

Earlier studies revealed that enzyme activity in patients carrying the CYP2C9*2 allele is 12% of the wild-type enzyme activity and 5% of the enzyme activity in patients carrying the CYP2C9*3 allele (10,12). Both variant alleles have been shown to cause difficulty in metabolizing steady doses and have been associated with a decrease in warfarin dose requirement and a higher risk of bleeding in the early stage of drug use (13-16).

During warfarin therapy, the dose of warfarin should be adjusted according to the diet of the patient, any accompanying disease conditions, body weight, and other drugs used, as well as other genetic factors. An efficient and safe dose adjustment is difficult. Previous studies have shown that knowing the genotype of patients allows for the determination of the optimal warfarin dose and rapid access to target INR values (17-19). In this study, we aimed to evaluate the relationship between genetic structure and daily dose requirement in patients under warfarin therapy presenting at our hospital.

Materials and methods

A total of 80 patients (45 female and 35 male) aged between 29 and 80 years (mean age: 59.86 ± 14.46 years), using warfarin for at least 1 month and presenting at Atatürk University Medical Faculty Internal Medicine Clinic between May 2009 and May 2010, were included in the study. Written informed consent was obtained from all subjects before participation. The study protocol was approved by the local institutional ethics board.

Detailed history was obtained from all participants and physical examinations were performed. The presence of bleeding was recorded during examination. In addition to investigating for genotypes, blood was drawn for renal, liver, and thyroid function tests, erythrocyte sedimentation rate (ESH), prothrombin time, activated partial thromboplastin time, and INR levels. Body mass index (BMI) was determined. Exclusion criteria were malignancy, hepatic and renal impairment, use of drugs metabolized by CYP2C9, impaired thyroid functions, use of warfarin for less than 1 month, or patient noncompliance.

Polymorphism analyses were carried out at the Molecular Polymerase Chain Reaction (PCR) Laboratory of the Department of Medical Biochemistry, Atatürk University. Genomic DNA was extracted from 100 µL of venous blood sample using the GEN-X-Tract Resin (ViennaLab Diagnostics, Austria). Molecular analysis of CYP2C9 1075 A>C and CYP2C9 430 C>T polymorphisms were performed using PCR and the reverse-hybridization method (Thrombo StripAssay kit, ViennaLab Diagnostics). CYP2C9 alleles *1 (the wild type), *2, and *3 and the resulting heterozygous and homozygous genotypes (*1/*2, *1/*3, *2/*2, etc.) were classified as follows in Table 1.

SPSS 11 for Windows was used for statistical analyses. Results are given as mean ± standard deviation (SD). For warfarin doses, the Mann-Whitney U test was used to compare 2 groups. $P < 0.05$ was accepted as statistically significant.

Table 1. Classification of CYP2C9 genotypes.

	CYP2C9 genotypes					
	***1/*1**	***1/*2**	***1/*3**	***2/*2**	***2/*3**	***3/*3**
1075 A>C mutant	-	-	+	-	+	+
430 C>T mutant	-	+	-	+	+	-
1075 A>C wild-type	+	+	+	+	+	-
430 C>T wild-type	+	+	+	-	+	+

Results

The mean age of the 80 patients included in the prospective study was 59.86 ± 14.46 years. Thirty-five patients (43.75%) were male and 45 (56.25%) were female. The mean age for male patients was 61.71 ± 2.59 years, and for female patients it was 56.82 ± 2.59 years. Causes of anticoagulation in patients were atrial fibrillation in 26.25% (n = 21), cardiac valvular disease in 25% (n = 20), deep vein thrombosis in 7.50% (n = 6), pulmonary embolism in 2.50% (n = 2), and thrombophilia and other conditions in 38.75% (n = 31). At the time of inclusion in the study, there was bleeding in 27 patients (33.75%) in the form of epistaxis (n = 5), gastrointestinal (n = 11), subcutaneous (n = 5), urinary system (n = 3), or intracranial (n = 3) bleeding. The mean INR value was 5.15 ± 0.56. Characteristics of the patients are given in Table 2.

Two of 80 patients were homozygous (TT) for CYP2C9 430 C>T and 20 subjects were heterozygous (CT). Mutant allele frequency for CYP2C9 430 C>T gene polymorphism was found to be 15% (Table 3). For the CYP2C9 1075 A>C gene, mutant allele frequency was found to be 12.5%; 14 of 80 patients were heterozygous (AC) and 3 patients were homozygous (CC) (Table 4).

Table 2. Patient characteristics.

	X ± SD	(Max-Min)
Mean age (years)		
Male (n = 35)	61.71 ± 2.59	(30-35)
Female (n = 45)	56.82 ± 2.59	(17-81)
Mean daily warfarin dose (mg)	4.51 ± 1.62	(1.60-10.0)
BMI	25.44 ± 4.81	(17-38)
Mean duration of warfarin therapy (months)	32.29 ± 52.80	(1-324)
Causes of anticoagulation	n (%)	
Deep vein thrombosis	6 (7.5%)	
Pulmonary embolism	2 (2.5%)	
Cardiac valvular disease	20 (25%)	
Atrial fibrillation	21 (26.3%)	
Others	31 (38.3%)	

Table 3. CYP2C9 430 C>T (CYP2C9*2) frequencies in study subjects.

Genotypes	n	Frequency
CC (wild-type)	58	0.725
CT (heterozygous variant)	20	0.250
CC (homozygous variant)	2	0.025
Alleles	n	Frequency
C	136	0.850
T	24	0.150

Table 4. CYP2C9 1075 A>C (CYP2C9*3) frequencies in study subjects.

Genotypes	n	Frequency
AA (wild-type)	63	0.787
AC (heterozygous variant)	14	0.175
CC (homozygous variant)	3	0.037
Alleles	n	Frequency
A	140	0.875
C	20	0.125

The association of mean warfarin dose and genotypes are shown in the Figure.

Patients with CYP2C9 genotype variants *2/*2, *2/*3, and *3/*3 required 3.40 mg/day, which was 1.57 mg less warfarin per day than patients with the wild-type genotype (*1/*1) (4.97 mg/day). This difference was statistically significant (P = 0.014). Additionally, the patients with *1/*2 and *1/*3 genotypes required 3.93 mg/day, which was 1.04 mg less warfarin per day than those with the wild-type genotype (P = 0.01).

Discussion

Genetic polymorphisms have an important place in the response of individuals to pharmacologic agents. Pharmacogenetic information is provided by studies identifying gene polymorphisms, encoding proteins affecting drug signal pathways, receptors and carriers, and drug metabolizing enzymes. Pharmacogenetic information allows for an effective treatment dose without exposure to toxic effects of the drug. In daily practice, warfarin is one of the drugs on which pharmacogenomic tests are used (20).

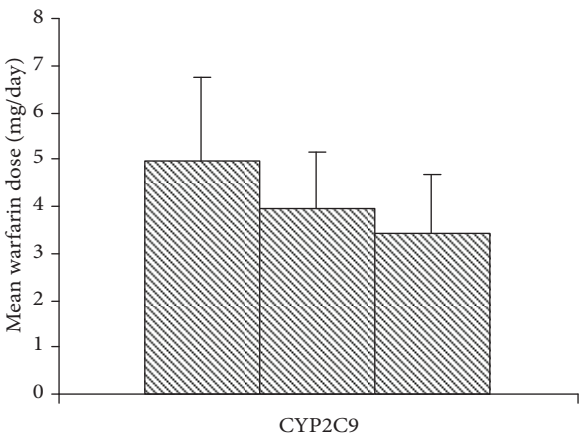

Figure. Genetic polymorphisms in CYP2C9 genes

There are 2 noteworthy steps in the use of warfarin. The first step is to determine an efficient, safe, and steady dose during treatment, especially in the first month of treatment when bleeding is frequent (21-23). The second step is to maintain this determined dose according to the diet, weight, and disease state of the patient, and any additional drugs that the patient might be taking. Recent studies have shown that genetic factors should also be evaluated as additional factors for warfarin dose maintenance (15).

One study was largely responsible for establishing warfarin sensitivity associated with CYP2C9 variants in a European American population (24-26). In another study in Malaysia, it was revealed that the prevalence of CYP2C9*2 and *3 variants was low in healthy and warfarin-treated Malays and Chinese at 2.6% and 4.6%, respectively (27). Similarly, in a Han Chinese study, it was found that CYP2C9 DNA sequence variants were present in only 5.4%-7.3% of the Chinese population (28). In our population, the prevalence of *2 and *3 variants was 15% and 12.5%, respectively. Similarly, it was found that the CYP2C9*1/*2 variant was higher (20%) in the Caucasian population. However, in this study high INR levels were also reported in the heterozygous group, rather than in the homozygous group as determined in previous studies (22).

In the present study, we determined the genetic predictors of warfarin dose requirements in 80 warfarin patients from eastern Turkey. The mean warfarin dose was shown to be significantly lower in CYP2C9 variant carriers compared with CYP2C9 wild-type homozygous subjects.

In another study conducted in Turkey, the influence of CYP2C9 genotypes, age, and body surface area on warfarin dose requirements was investigated in an adult Turkish population. This study revealed that the mean warfarin daily dose requirement was higher in CYP2C9 homozygous wild-type patients compared to those with the variant *3 and *2 alleles, as in our study. Additionally, the time to reach therapeutic INR was longer in CYP2C9 homozygous wild-type patients compared with those with the variant *2 and *3 alleles (29). The time to therapeutic INR was not evaluated in our population.

A study reported by Yıldırım et al. investigated the role of CYP2C9 gene polymorphisms after heart valve replacement in a group of patients on warfarin therapy. The data showed that patients with CYP2C9*1/*3 and CYP2C9*2/*3 genotypes needed a lower sustained dose of warfarin than patients with the CYP2C9*1/*1 wild-type genotype, as seen in our study (30).

Oner Ozgon et al. reported in 2008 that patients who carried CYP2C9 variants needed a 40% lower mean weekly warfarin dose compared to wild-type variants (31). In our study, this rate was found to be 31.5% for homozygous variants and 21% for heterozygous variants.

In a study in southeastern Turkey, the population was analyzed for the CYP2C9 genotype and for serum carcinoembryonic antigen, alpha-fetoprotein, CA 19-9, CA 15-3, ferritin, IL-6, and IL-8 concentrations. CYP2C9*1 was found to be the most prevalent allele and CYP2C9*1/*1 was found to be the most frequent genotype, represented in 64% of the population in southeastern Turkey (Gaziantep). Although slight differences in serum tumor marker and cytokine concentrations were observed for CYP2C9 genotypes, the differences were statistically insignificant (32). Similarly, CYP2C9*1/*1 was the most frequently represented genotype in the present study, seen in 58% of our study subjects.

Because patients with *2 and *3 variants are at a high risk for bleeding while using oral anticoagulants, clinicians should start at lower doses of warfarin in these patients and should make strict dose adjustments.

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