The Influence of CYP2C9 and VKORC1 Gene Polymorphisms on Optimal Warfarin Doses After Heart Valve Replacement

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Key words: *warfarin; gene polymorphism; CYP2C9; VKORC1.*

Summary. *A clinical effect of warfarin depends on highly polymorphic drug-metabolizing (CYP2C9) and drug-target (VKORC1) enzymes. The objective of this study was to investigate the impact of CYP2C9*2, CYP2C9*3, and VKORC1 (G-1639A) polymorphisms on the variability of warfarin dosage requirements in Lithuanian patients after heart valve replacement.*

Materials and Methods. The study included 83 patients with a mean age of 65.2 years (SD, 13.31) after heart valve replacement with an achieved stable international normalized ratio of 2–3.5. The restriction fragment length polymorphism method was used to identify polymorphisms of VKORC1 and CYP2C9.

*Results. Daily warfarin dosage significantly correlated with weight (r=0.4087) and height (r=0.3883) of the patients. Patients younger than 60 years required significantly higher daily warfarin dosages than older patients. Two-thirds (66.3%) of the patients had the wild-type (WT) CYP2C9*1/*1 genotype; 38.6% and 54.2% of the patients had WT VKORC1 (G/G) and VKORC1 (G/A) genotypes, respectively. WT CYP2C9*1/*1 genotype was associated with a higher daily warfarin dosage (5.84 mg [SD, 2.84]) as compared to other CYP2C9 genotypes. Carriers of WT VKORC1 (G/G) required a higher warfarin dose as compared to (A/A) carriers (6.20±2.78 mg and 3.75±1.40 mg, respectively; P=0.04). Patients having CYP2C9*1/*1 or 1/*2 in combination with VKORC1 (G/G) or (G/A) genotypes required the highest daily warfarin dosage in comparison to other combinations of genotypes.*

Conclusions. The Lithuanian study sample is characterized by high a frequency (92.8%) of VKORC1 G/G and G/A genotypes that determines a higher warfarin-loading dose. Analysis of combined CYP2C9 and VKORC1 gene variants allows the prediction of warfarin dosage. These results can be used to individualize treatment with warfarin in the field of heart surgery in Lithuania.

Introduction

Warfarin is one of the most widely prescribed oral anticoagulants worldwide; it is used to prevent and treat venous or arterial thrombi and emboli associated with atrial fibrillation or cardiac valve replacement (1). Warfarin is administered as a racemic mixture of S- and R-warfarin. S-warfarin is 3–5 times more active and metabolized primarily by cytochrome P450 2C9 (CYP2C9). CYP2C9 is a highly polymorphic hepatic enzyme of the cytochrome P450 family that metabolizes a variety of drugs. Currently 6 defective alleles of CYP2C9 are of clinical importance in different populations (2). Among Caucasians, two alleles – CYP2C9*2 and CYP2C9*3 – reduce the rate of hydroxylation of warfarin in vitro resulting in an approximately 12% (CYP2C9*2) and 5% (CYP2C9*3) decrease in enzymatic activity when compared with the wild-type genotype CYP2C9*1. R-warfarin is metabolized by other polymorphic enzymes. S-warfarin has a higher clearance and a shorter half-life for elimination as compared with R-warfarin. These pharmacokinetic differences between the two enantiomers determine the higher circulating plasma levels of R-warfarin as compared with S-warfarin during prolonged treatment (3). Both S- and R-warfarin block VKORC1 (vitamin K epoxide reductase), the main enzyme in the reduction pathway of vitamin K, which participates in the biotransformation of nonactive blood clotting factors into their active forms. The post-translational (gamma) carboxylation of the vitamin K-dependent coagulation factors is then terminated. In 2004, genetic variations within the gene encoding for a subunit of the vitamin K epoxide reductase complex, namely the VKORC1 gene, were found to correlate strongly with sensitivity to warfarin

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therapy. VKORC1 (G-1639A) polymorphism was identified to be the main polymorphism responsible for a clinically effective warfarin dose (4, 5). The polymorphisms in CYP2C9 and VKORC1 mentioned above resulted in excessive anticoagulation, diminished warfarin dosage, and a sustained, stable drug dose (6). In 2007, the Food and Drug Administration (FDA, USA) approved genetic testing for determining warfarin dosage and initiated the update of warfarin labeling with genetic information. Two main polymorphic enzymes, CYP2C9 and VKORC1, that enabled a reduction in the warfarin dose, were selected for analysis and labeling (7). Until now, no genetic testing related to the effects of warfarin dosage has been carried out in Lithuania.

Several complications of valvular heart disease can be more devastating than systemic thromboembolism. Unfortunately, antithrombotic therapy carries a substantial risk of bleeding (8). Studies have shown that patients starting oral anticoagulation after heart valve replacement (HVR) are significantly more sensitive to warfarin than nonsurgical patients due to their sedentary state and lower metabolism (9). Anticoagulant treatment with warfarin is usually achieved by a trial and error method and is regularly monitored according to the international normalized ratio (INR) value. In most cases, the INR is kept within the range of 2 to 3.5. High interindividual variability in warfarin dosage is a result of narrow therapeutic window of a drug, ethnic differences, clinical state of the patient, polymorphic receptors, and enzymes involved in its pharmacodynamics and pharmacokinetics (10).

The present study investigated the impact of CYP2C9*2, CYP2C9*3, and VKORC1 (G-1639A) polymorphisms on the variability of warfarin dosage requirements in Lithuanian patients after HVR.

Materials and Methods

The study cohort consisted of patients who underwent HVR at the Department of Cardiac, Thoracic, and Vascular Surgery, Lithuanian University of Health Sciences. All patients received anticoagulation treatment with warfarin to achieve an INR in the target range of 2 to 3.5. Written informed consent was obtained from all the patients included in this study. Only those patients (n=83) with a stable INR range of 2–3.5 during discharge from the hospital were included. Patient data on height and weight were obtained from case histories. Patients were grouped according to the age as those younger than 60 years (n=23) and those older than 60 years (n=60). Anthropometric data of the study population are presented in Table 1.

Blood samples for DNA extraction were collected in 3-mL tubes containing potassium EDTA. Whole blood DNA was extracted by using a salting-out method and precipitated in 96% ethanol. DNA fragments of interest from each patient were amplified by a using polymerase chain reaction (PCR) in the Laboratory of Molecular Cardiology, Institute of Cardiology, Medical Academy, Lithuanian University of Health Sciences. PCR reactions were done in a final volume of 20 μL, containing 1X Hot Start PCR buffer (Fermentas, Lithuania), 2 mM $MgCl_2$, 0.1 mM deoxynucleoside triphosphate (dNTP), forward and reverse primers each 0.2 μM, 0.5 U Maxima^{TM} Hot Start Taq DNA polymerase (Fermentas, Lithuania), and 10 ng to 30 ng of genomic DNA. Amplification of DNA fragments containing polymorphic regions of CYP2C9*2 and CYP2C9*3 was done according to Schalekamp et al. (11). The region containing VKORC1 (G-1639A) was amplified by using forward 5′-GCCAGCAGGAGAGGGAAATA-3′ and reverse 5′-AGTTTGGACTACAGGTGCCT-3′ primers according to Sconce et al. (12). The restriction fragment length polymorphism method was used to identify the polymorphisms mentioned above. Fractionation of RFLP products on a 2% agarose gel stained with ethidium bromide was performed after digestion at 37°C overnight with 10 U of AvaII for CYP2C9*2, 10 U of NsiI and 10U of KpnI for CYP2C9*3, and 10 U of MspI for VKORC1 (G-1639A).

Table 1. Anthropometric Data of Studied Population

Gender	n (%)	Body Weight Mean (SD), kg	Height Mean (SD), cm
Men	50 (60.2)	86.08 (18.78)	175.08 (7.79)
Women	33 (39.8)	77.70 (15.09)*	161.91 (7.62)**
Total	83 (100)	82.75 (17.80)	169.84 (10.05)

*P=0.03 and **P=0.001, women versus men.

Statistical analysis. All the data are presented as mean and standard deviation (SD). Correlation between the daily dosage of warfarin and patient's anthropometric factors (body weight and height) was assessed by using the Pearson correlation coefficient. The genotype frequencies for each polymorphism were determined. Statistical analysis was done using STATISTICA and SPSS statistical software. A P value $\leqslant$0.05 was considered statistically significant.

Results

Daily warfarin dosage correlated with weight (r=0.4087, P<0.001) and height (r=0.3883, P<0.001) of the patients (Fig.). Men required higher daily dosages of warfarin than women (6.24 mg [SD, 2.84] and 4.88 mg [SD, 2.40], respectively; P=0.027). In addition, men were significantly taller and had higher weight as compared with women (Table 1).

Patients younger than 60 years required higher daily warfarin dosages than older patients (6.67 mg [2.84] versus 5.30 mg [2.62], P=0.038).

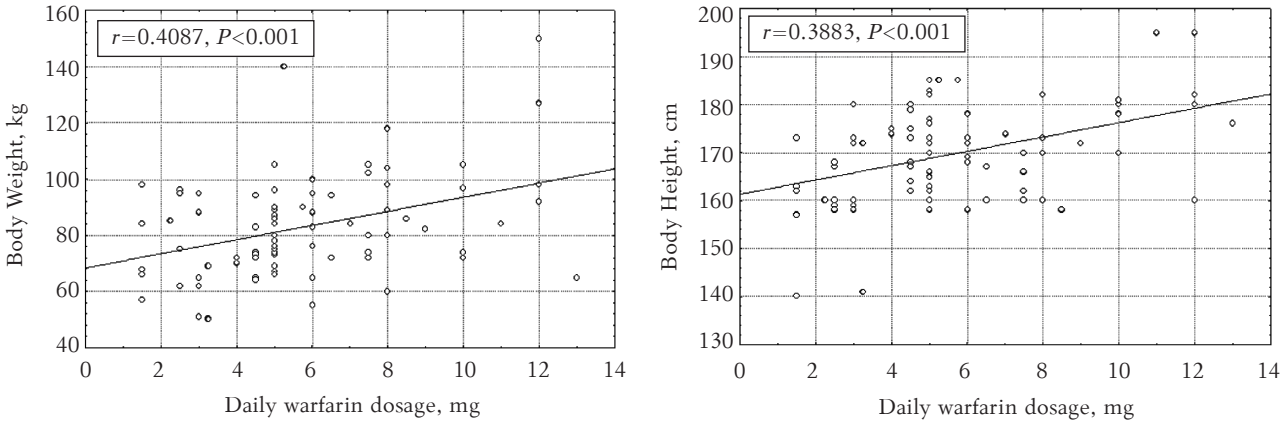

Fig. Correlation between daily warfarin dosage and body weight (left side) and body height (right side)

The prevalence of the CYP2C9 and VKORC1 genotypes and mean daily warfarin dosage is presented in Table 2. According to the CYP2C9 genotypes, the highest daily warfarin dosages were administered for carriers of the wild type (*1/*1) genotype, while carriers of CYP2C9*2/*2 were treated with lower daily warfarin dosages (5.84 mg [SD, 2.84] versus 2.83 mg [SD, 0.28], P=0.07). The carriers of the wild-type VKORC1 (G/G) genotype were treated with significantly higher daily warfarin dosages than homozygous (A/A) carriers (6.20 mg [2.78] and 3.75 mg [1.40], respectively; P=0.04).

According to the combined CYP2C9 and VKORC1 genotypes, patients having the wild type (G/G) or (G/A) heterozygous VKORC1 genotype in combination with the CYP2C9 wild type (*1/*1) or *1/*2 alleles required the highest mean daily warfarin dosage (Table 3). Such patients comprised 80.8% of the study subjects. The lowest mean daily warfarin dosage (less than 3 mg) to achieve the required clinical effect was documented for carriers of CYP2C9*2/*2 or 2/*3 alleles combined with wild type of VKORC1 or CYP2C9*1/*1 combined with VKORC1 (A/A) genotype and accounted for 8.4% of the study subjects.

Table 2. The Prevalence of CYP2C9 and VKORC1 Genotypes and Mean Daily Warfarin Dosage

Genotype	Prevalence n (%)	Daily Warfarin Dosage Mean (SD), mg
CYP2C9		
*1/*1	55 (66.3)	5.84 (2.84)
*1/*2	17 (20.5)	5.68 (2.79)
*2/*2	3 (3.6)	2.83 (0.28)
*1/*3	7 (8.4)	4.28 (1.92)
*2/*3	1 (1.2)	2.25
Total	83 (100)	5.70 (2.74)
VKORC1		
G/G	32 (38.6)	6.20 (2.78)
G/A	45 (54.2)	5.60 (2.77)
A/A	6 (7.2)	3.75 (1.40)
Total	67 (100)	5.70 (2.74)

Table 3. The Mean Daily Warfarin Dosage According to Combined CYP2C9 and VKORC1 Gene Variants

Gene Variant		N (%)	Daily Warfarin Dosage Mean (SD), mg
CYP2C9	VKORC1		
*1/*1	G/G	21 (25.3)	6.76 (2.62)
*1/*2	G/G	6 (7.2)	6.95 (2.73)
*1/*1	G/A	31 (37.3)	5.80 (2.82)
*1/*2	G/A	9 (10.8)	5.47 (2.96)
Total		67 (80.7)	6.47 (2.74)
*1/*2	A/A	2 (2.4)	4.5 (0)
*1/*3	A/A	1 (1.2)	5
*1/*3	G/G	1 (1.2)	4
*1/*3	G/A	5 (6.0)	4.60 (2.74)
Total		9 (10.8)	4.95 (2.33)
*2/*2	G/G	3 (3.6)	2.83 (0.28)
*2/*3	G/G	1 (1.2)	2.25
*1/*1	A/A	3 (3.6)	2.83 (1.52)
Total		7 (8.4)	2.75 (0.92)
Total		83 (100)	5.70 (2.74)

Discussion

This study was designed to analyze the influence of CYP2C9*1,*2,*3 allele variant and VKORC1 (G-1639A) gene polymorphism on warfarin dosage in Lithuanian patients after cardiac valve surgery. The impact of patients' demographic and anthropometric characteristic on warfarin dosage was taken into account as well. Higher daily warfarin dosages were prescribed to heavier and taller patients of the study population. Men required higher daily dosages of warfarin than women. So, the latter phenomenon can be explained by anthropometric differences in men and women, as men were significantly taller and had higher weight in comparison with women. Significant correlations between warfarin dosage and body weight and height have been demonstrated by other studies as well (12, 13). Patient's body weight and height indirectly reflect the warfarin distribution level within the human body, which theoretically is the volume of fluid in which drug must be dissolved to produce the required

plasma concentration. Warfarin is an acidic drug, so its characteristic feature is binding especially to plasma albumin, with concomitant low absorption by tissues (14, 15).

Patients younger than 60 years required higher daily warfarin dosages than older patients, presumably because of an impaired metabolism of drugs in the elderly (16), a slower renal elimination rate of warfarin alcohols (warfarin metabolites, which have anticoagulant activity) and altered synthesis of clotting factors. Impaired vitamin K absorption and a slower rate of oxidized vitamin K reduction in the elderly lead to a reduced synthesis of coagulation factors (17).

Over the past years, CYP2C9 polymorphisms have been extensively studied in various populations (Table 4). The frequency of CYP2C9*2 and *3 allele in the Lithuanian study sample was similar to that from other Caucasian populations and was most similar to the frequency obtained in a Spanish study sample. African-Americans and Asians differ from Caucasians by the low frequency of the CYP2C9*2 allele among carriers (3). According to the CYP2C9 genotypes, in our study population, the highest daily warfarin dosage was administered for carriers of the wild type (*1/*1) genotype, and carriers of CYP2C9*2/*2 were treated with lower warfarin doses. The daily warfarin dosage for carriers of CYP2C9*1/*2 did not differ from the dosage for carriers of the wild type genotype. Thus, according to the CYP2C9 genotype, more than 85% of our study subjects required the mean daily warfarin dosages of more than 5 mg, and less than 5% of study subjects required daily warfarin dosages of 3 mg and less.

As mentioned before, the main target of warfarin is VKORC1. Polymorphism of the promoter of this gene (G-1639A) determines the patient's sensitivity to warfarin treatment by lowering the rate of synthesis of this enzyme in patients having the A allele. Patients with the (A/A) allele require the lowest warfarin doses as compared to heterozygous carriers (G/A) and homozygous carriers (G/G) (12). Therefore, the carriers of the wild-type VKORC1 (G/G) of our study population were treated with significantly higher warfarin doses than homozygous (A/A) carriers.

The frequency of the VKORC1 (A/A) genotype in our cohort was 7.2%. It was less frequent than in other Caucasians (14%) and detected much more rarely than in Asians (83%) (6). This allele variant is very rare (<1%) in Africans (18). This means that Asians have higher allelic frequencies than either Caucasians or Africans, determining a lower warfarin dosage (16). Thus according to our data, the Lithuanian study sample is characterized by a high allelic frequency (92.8%) determining a higher warfarin dose. It is necessary to point out that CYP2C9 and VKORC1 genotypes in various populations results in different warfarin-dosing patterns in these populations. The mean warfarin dosage in Chinese patients having CYP2C9*1/*1 was 2.06±0.82 mg/day (n=162) in comparison to 1.60±1.29 mg/day (n=16) for CYP2C9*1/*3 carriers (P<0.001). Chinese patients having the VKORC1 (A/A) genotype were treated with 1.76±0.57 mg/day (n=149) as compared to 3.32±1.02 mg/day (n=29) for VKORC1 (G/G) carriers (P<0.001) (18). Such low warfarin doses in Chinese patients can only be explained in part by different anthropometric characteristics. The mean height and weight of Chinese patients were 161.9±7.3 cm and 58.2±10.2 kg, respectively (19).

According to the combined effect of the CYP2C9 and VKORC1 genotype, the lowest mean daily warfarin dosage (less than 3 mg) to achieve the required clinical effect was documented for carriers of CYP2C9*2/*2 or *2/*3 alleles combined with wild type of VKORC1 or CYP2C9*1/*1 combined with the VKORC1 (A/A) genotype and accounted for 8.4% of the study population. This suggests that the standard administration of 5 mg of warfarin to initiate treatment for such patients could lead to hypocoagulation complications.

Table 4. The Frequency of CYP2C9 Alleles in Study Samples From Different Populations

Population	Study Sample for CYP2C9	CYP2C9*2, %	CYP2C9*3, %	Authors (Reference)
American-Caucasian	200	8	6	Limdi et al. (2)
British	1188	10.6	5.3	Taube et al. (20)
Russian	290	11.4	0.3	Gaikovitch et al. (21)
Dutch	60	14.2	9.2	Weide et al. (22)
Croatian	200	16.5	9.5	Bozina et al. (23)
Spanish	200	22	12.5	Mas et al. (24)
Lithuanian	83	25.3	9.6	Present study
Chinese	147	0	7	Lee et al. (25)
Indian	43	4	18	Lee et al. (25)
African-American	36	1	8	Takahashi et al. (26)

Conclusions

This study is the first to our knowledge in Lithuania that provides evidence on the impact of CYP2C9*1,*2,*3 and VKORC1 (G-1639A) gene variants on clinically warranted warfarin dosing. The Lithuanian study sample is characterized by a high frequency (92.8%) of VKORC1 (G/G) and (G/A)that determines a higher warfarin-loading dose. Analysis of combined CYP2C9 and VKORC1 gene variant allows the prediction of warfarin dosage. These results can be used to individualize treatment with warfarin in the field of heart surgery in Lithuania.

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CYP2C9 ir VKORC1 genų polimorfizmo įtaka optimaliam varfarino dozavimui po širdies vožtuvų operacijos

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Raktažodžiai: varfarinas, geno polimorfizmas, CYP2C9, VKORC1.

Santrauka. Varfarino terapinis poveikis priklauso nuo vaistą metabolizuojančio CYP2C9 ir šio vaisto taikinio, VKORC1 fermento aktyvumo.

Tyrimo tikslas. Įvertinti CYP2C9*2, CYP2C9*3 ir VKORC1 (G-1639A) geno polimorfizmo, lemiančio varfarino dozavimą ligoniams po širdies vožtuvų operacijos, įtaką.

Tirtųjų kontingentas tyrimo metodai. Į tyrimą įtraukti 83 pacientai (amžiaus vidurkis – 65,20±13,31 metų) po širdies vožtuvų operacijos, kuriems išrašymo iš ligoninės metu buvo užtikrintas tarptautinis normalizuotas santykis – 2–3,5. Ligonių genotipas ištirtas restrikcijos fragmento ilgio polimorfizmo nustatymo metodu.

Rezultatai. Nustatyta tiesioginė dienos varfarino dozės ir svorio (r=0,4087, p<0,001) bei ūgio (r=0,3883, p<0,001) priklausomybė. Jaunesniems nei 60 metų ligoniams reikėjo skirti daugiau varfarino nei vyresniems. Iš viso 66,3 proc. ligonių turėjo CYP2C9*1/*1 (laukinio tipo) genotipą. VKORC1 (G/G) nustatytas 38,6 proc., VKORC1 (G/A) – 54,2 proc. ligonių. Ligoniams, kurių genotipas CYP2C9*1/*1 (laukinio tipo), skirtos didžiausios dienos varfarino dozės (5,84±2,84 mg), palyginus su ligoniais, turinčiais kitokį genotipą. Ligoniai, turintys VKORC1 (G/G) genotipą, buvo gydomi didesnėmis varfarino paros dozėmis, palyginus su VKORC1 (A/A) – (atitinkamai – 6,20±2,78 mg ir 3,75±1,40 mg, p=0,04). Didžiausios varfarino dozės buvo skiriamos tiems ligoniams, kurie turėjo CYP2C9*1/*1 arba *1/*2 kartu su VKORC1 (G/G), arba (G/A) genotipu.

Išvada. Tirtųjų lietuvių imtyje dažniau pasitaikė didelę varfarino dozę lemiantis VKORC1 (G/G) ir (G/A) genotipas (iš viso – 92,8 proc.). Prieš skiriant gydymą varfarinu, ištyrus CYP2C9 ir VKORC1 geno polimorfizmą, vaistą galima dozuoti tiksliau. Šio tyrimo duomenys gali būti naudingi gydant ligonius varfarinu po širdies vožtuvų operacijos.

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