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ORIGINAL ARTICLE

Proposal of pharmacogenetics-based warfarin dosing algorithm in Korean patients

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Warfarin is a commonly prescribed anticoagulant drug for the prevention of thromboembolic disorders. We investigated the contribution of genetic variations of four genes and clinical factors to warfarin dose requirement and provided a warfarin-dosing algorithm based on genetic and clinical variables in Korean patients. We recruited 564 Korean patients on stable anticoagulation. Single nucleotide polymorphisms (SNPs) for the VKORC1, CYP2C9, CYP4F2 and GGCX were analyzed. Using multiple regression analysis, we developed a model to predict the warfarin requirement. The SNPs of VKORC1, CYP2C9, CYP4F2 and GGCX showed significant correlation with warfarin dose. Patients with the 3730AA genotype received significantly higher doses of warfarin than those with the 3730GG (P=0.0001). For CYP2C9, the highest maintenance dose was observed in the patients with wild-type genotype compared with the variant allele carriers ($P<0.0001$). The multiple regression model including age, gender, body surface area (BSA), international normalized ratio (INR) and four genetic polymorphisms accounted for 35% of total variations in warfarin dose (R^2=0.3499; $P<0.0001$). This study shows that age, gender, BSA, INR and VKORC1, CYP2C9 and CYP4F2 polymorphism affect warfarin dose requirements in Koreans. Translation of this knowledge into clinical guidelines for warfarin prescription may contribute to improve the efficacy and safety of warfarin treatment for Korean patients.
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INTRODUCTION

Warfarin is a commonly prescribed oral anticoagulant for the prevention and treatment of atrial fibrillation, deep vein thrombosis, prosthetic heart valve replacement, recurrent stroke and pulmonary embolism.[1,2] The effectiveness and safety of warfarin is dependent on maintaining the prothrombin time, expressed as the international normalized ratio (INR), within the therapeutic range.[1,3] However, warfarin therapy is difficult due to a narrow therapeutic index and large inter-individual variability, which makes individual dosing necessary.[4,5] Unfortunately, the appropriate dose required is highly variable (up to 20-fold) such that routine dosing can lead to major and fatal bleeding. Current clinical warfarin guidelines are able to predict only 25% of variability in dosage.[6] This inter-individual variability is known to be affected not only by environmental factor, but also by genetic influence.[5,7]

Candidate-gene association studies have identified two genes responsible for the main proportion of the genetic effect: CYP2C9, which codes for the enzyme cytochrome P450 2C9 that metabolizes S-warfarin, and VKORC1, which codes for warfarin's target, vitamin K epoxide reductase.[8] Several single nucleotide polymorphisms (SNPs) in VKORC1 have been associated with a deficiency in vitamin-K dependent clotting factors, resulting in either increased sensitivity to

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arfarin or warfarin resistance.[9] Warfarin inhibits VKORC1 activity by reducing the regeneration of vitamin K and thus exerting its anticoagulation effect.[1,2,7] Reduced vitamin K is the essential cofactor for the activation of clotting factors II, VII, IX and X and proteins C, S and Z by γ-glutamyl carboxylase, encoded by GGCX.[4] If reduced vitamin K not regenerated, the biosynthesis of vitamin K-dependent coagulation/anticoagulation factors is suppressed.[8]

At least six SNPs of CYP2C9 have been found that may influence enzyme activity and subsequently account for slower metabolism. The most commonly reported variants, *2 and *3, are less effective than the wild-type *1 variant.[10] These alleles can cause alterations in initial warfarin dose sensitivities, delays in achieving a stable maintenance dose, and increased risk of serious or life-threatening bleeding complications.[9] Large body of previous studies on the inter-individual variability of warfarin have demonstrated that the genetic variations in the VKORC1 as well as CYP2C9, a warfarin metabolizing enzyme, influence warfarin responses.[3,4] Moreover, recently it has been revealed that a genetic variation of cytochrome P450 4F2 (CYP4F2) is also associated with a clinically relevant effect of warfarin.[2,11] Common genetic variation in CYP4F2 (V433M) results in lower steady-state enzyme levels, possibly owing to enzyme instability leading to reduced capacity to metabolic vitamin K.[12] However, there are few reports describing the effects of these SNPs in Korean patients treated with warfarin up to the present.

Numerous genetic and clinical factors have been associated with variability in maintenance warfarin dose requirements including age, race, weight, height, smoking status, medications and polymorphisms of the *CYP2C9* and *VKORC1* genes, which encode for enzymes important in warfarin pharmacology.[13] Genotype-guided dosage has been associated with fewer dosage changes, faster time to target INR, more time in target INR, and a decrease in out of range INRs in some studies. However, overall the results of these studies have been inconclusive and warfarin genotyping has not yet been incorporated into daily practice.[14]

To date, prospective studies using dosing algorithms have been conducted to test the validity of pharmacogenetics-based dosing; however, these algorithms could account ~40% of the variation and could not reduce adverse events.[7] Several clinical trials have been performed to investigate whether incorporation of genotype in warfarin dosing results in better patient management.

Although several similar algorithms of different ethnics have been reported, there has been no algorithm integrating genetic factor with environmental factors, which may influence warfarin-dose requirements in Korean patients. Ultimately, we tried to develop a novel algorithm incorporating genetic and clinical factors to predict effective warfarin dose. Additionally, we tried to analyze the influence of SNPs in VKORC1, CYP2C9, CYP4F2 and GGCX genes on the inter-individual variability of warfarin dose requirements in Korean patients.

MATERIALS AND METHODS

Subject recruitment

Eligible participants were recruited from the outpatient clinics of cardiology, neurology, and oncology department in four medical centers, Seoul St. Mary's hospital, Mary's hospital, Uijeongbu St Mary's Hospital and St. Vincent's hospital. Ethical permission for the study was obtained from the Institutional Review Boards of all participating hospitals. All patients provided written informed consent in accordance with the Declaration of Helsinki. The indications for anticoagulant therapy were mainly for prevention or treatment of thromboembolic diseases including atrial fibrillation, heart valve replacement, pulmonary thromboembolism, cerebral infarction and deep vein thrombosis.

A stable patient was defined as one whose warfarin-dose requirement had remained constant in the previous clinic visits over a minimum period of 3 months. Patients with abnormal blood tests in terms of hepatic or renal function and those receiving concurrent treatment interacting with warfarin were excluded.

Blood samples were taken for venous INR measurement and VKORC1, CYP2C9, GGCX and CYP4F2 genotyping. Demographics of age, gender, weight, height and body surface area (BSA), as well as indications for warfarin therapy, additional medical problems, and concurrent medications were also recorded during the clinic visit.

Genotyping

The polymorphisms for the 1173C>T, 3730G>A, 2255A>G and 1542G>C of VKORC1, CYP2C9*3, CYP2C9*2, 3115T>C (listed in http://www.nifds.go.kr/ for Koreans, rs9332092) and 4045G>A (http://www.nifds.go.kr/, rs9332098) in CYP2C9, CYP4F2 (7253233C>T; V433M) and GGCX (8016G>A) were analyzed. There is one SNP for the human *CYP4F2* gene listed in the National Center for Biotechnology Information SNP database Build 126 (http://www.ncbi.nlm.gov/SNP). CYP4F2 7253233C>T was located in an exon with a non-synonymous substitution amino-acid change. For each sample, the genomic DNA was isolated from whole blood using the QIAamp DNA blood mini kit (QIAGEN, Germantown, MD, USA) according to the supplier's instruction. PCR was performed using hot start Ace Taq DNA Polymerase Kit (Genenmed, Seoul, Korea). All the primers for PCR amplification and DNA sequencing were designed by using the Primer3 software (http://Frodo.wi.mit.edu/cgi-bin/primer3/primer3) and the sequences are available on request. PCR reaction was carried out in a final volume of 25 μl containing 10× buffer, 1.5 mmol/l $MgCl_2$, 20 μmol l^{-1} dNTP, 0.5 μmol l^{-1} each primer, 10 ng genomic DNA as template and 0.5 U polymerase. After confirming the purity and mobility of each PCR product by agarose gel electrophoresis, it was purified and subjected to DNA sequencing using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and the ABI Prism 3730 genetic analyzer. (Applied Biosystems). Each sample was sequenced for both strands to confirm the results.

Statistical analysis

The daily maintenance dose of warfarin in the different genotype groups was evaluated by *t*-test and ANOVA. Associations between warfarin dose and the age, BSA, height, weight and maintenance INR were analyzed using Pearson correlation test. We analyzed the distribution of warfarin dosage as a random variable using Shapiro–Wilk test. We estimated the differences of warfarin dosage according to genotypes by the nonparametric method. By Kruskal–Wallis test, we demonstrated the warfarin dosage as median and adjusted *P*-value. Multiple linear regression was performed to model the relationships of dose with other variables measured and used to develop a novel warfarin dosing algorithm. The results are presented as median ± interquartile range unless stated otherwise. A *P*-value of <0.05 was considered to be statistically significant. All analyses were performed using SAS Genetics version 9.13 (SAS Institute, Cary, NC, USA).

RESULTS

Patient characteristics

Demographics of the 564 patients are shown in Table 1. The recruited patients consisted of an elderly population with a mean age of 63.2 ± 11.7 years and males made up more than half of the patients recruited (n=306, 54.3%). The 56% of patients were on warfarin therapy due to atrial fibrillation (n=320) followed by cerebral infarction (n=117, 20.8%), heart valve replacement (n=114, 20.2%) and deep vein thrombosis/pulmonary embolism (n=13, 2.3%). Mean BSA estimated from height and weight was 1.7 ± 0.2 m^2. According to the underlying diseases, 140 (24.8%) had hypertension, 37 (6.6%) diabetes mellitus and 10 (1.8%) myocardial infarction or 377 unknown diseases. The average warfarin dose was 3.6 ± 1.4 mg per day and stable INR averaged 2.1 ± 0.8.

Genotype frequency of VKORC1, CYP2C9, CYP4F2 and GGCX

We analyzed nine candidate variants reported to be associated with warfarin dose. Table 2 showed their allelic frequencies and genotype distributions in warfarin treated patients. Four SNPs of *VKORC1* gene, 1173C>T, 3730G>A, 1542G>C and 2255A>G were correlated with pair-wise linkage disequilibrium in our study population (data not shown). For 3730G>A genotype, 500 patients (88.7%) were homozygous for the wild-type G allele, 56 patients (9.9%) were heterozygous and eight patients (1.4%) were homozygous for the variant A allele. In *CYP2C9* gene, the CYP2C9*3 genotype frequencies were 92.0% (AA), 8.0% (AC) and no CC genotypes were observed. However, the polymorphisms of CYP2C9*2 was absent in our study population (data not shown). In *GGCX* gene, the 8016G>A genotype frequencies were 47.0% (GG), 44.3% (GA) and 8.7% (AA). Moreover, CYP4F2 allele frequencies were 65.0% for C allele and 35.0% for T allele.

Table 1 Demographic characteristics of 564 study populations

Variable (unit)	*Value*
Number	564
Age (year)	63.2 ± 11.7
Male/Female, *n* (%)	306 (54.3)/258 (45.7)
Weight (kg)	65.1 ± 20.1
Height (cm)	163.0 ± 9.0
Body surface area (m^2)	1.7 ± 0.2
Therapeutic warfarin dose (mg per day)	3.6 ± 1.4
International normalized ratio, INR	2.1 ± 0.8
Primary reason for anticoagulation, n (%)	
Atrial fibrillation	320 (56.7)
Deep vein thrombosis/Pulmonary embolism	13 (2.3)
Heart valve replacement	114 (20.2)
Cerebral infarction, Others	117 (20.8)
History of concomitant therapy, n (%)	
Hypertension	140 (24.8)
Diabetes mellitus	37 (6.6)
Myocardial infarction, Others	10 (1.8)
Unknown	377 (66.8)

Abbreviation: INR, international normalized ratio.

Table 2 Allele frequencies and genotype distributions of the nine candidate SNPs

Gene	*SNP*	*Allele*	*Patient, N (%)*	*Genotype*	*Patient, N (%)*
VKORC1	1173T>C	T	1052 (93.3)	TT	493 (87.4)
		C	76 (6.7)	TC	66 (11.7)
				CC	5 (0.9)
	1542G>C	G	1047 (92.8)	GG	490 (86.9)
		C	81 (7.2)	GC	67 (11.9)
				CC	7 (1.2)
	2255A>G	A	1046 (92.7)	AA	489 (86.7)
		G	82 (7.3)	AG	68 (12.1)
				GG	7 (1.2)
	3730G>A	G	1056 (93.6)	GG	500 (88.7)
		A	72 (6.4)	GA	56 (9.9)
				AA	8 (1.4)
CYP2C9	CYP2C9*3	A	1081 (96.0)	AA	519 (92.0)
		C	45 (4.0)	AC	45 (8.0)
	3115T>C	T	1083 (96.0)	TT	519 (92.0)
	(rs9332092)	C	45 (4.0)	CT	45 (8.0)
	4045G>A	G	1083 (96.0)	GG	519 (92.0)
	(rs9332098)	A	45 (4.0)	GA	45 (8.0)
GGCX	8016G>A	G	780 (69.1)	GG	265 (47.0)
		A	348 (30.9)	GA	250 (44.3)
				AA	49 (8.7)
CYP4F2[a]	7253233C>T	C	524 (65.0)	CC	168 (41.7)
	(rs2108622)	T	282 (35.0)	CT	188 (46.6)
				TT	47 (11.7)

Abbreviation: SNP, single nucleotide polymorphism.
[a]CYP4F2 is genotyped in 403 patients.

Associations of genotypes of four genes with warfarin dose

We examined an association of the genotype with maintenance warfarin doses. There was a significant association of daily warfarin dose with nine SNPs including 1173T>C, 1542G>C, 2255A>G and 3730G>A in VKORC1, CYP2C9*3, 3115T>C and 4045G>A in CYP2C9, and 7253233C>T in CYP4F2 (Table 3).

The mean warfarin dose was higher in the patients with the VKORC1 1173CC genotype than in those with the 1173TT genotype (5.0 ± 0.0 mg per day vs 3.0 ± 1.5 mg per day, respectively; P=0.0003). The patients having VKORC1 3730AA genotype received significantly higher dose of warfarin (5.0 ± 0.8 mg/day) than those with the GG genotype (3.0 ± 1.5 mg per day; P=0.0001). Five hundred and nineteen patients (92.0%) having homozygous genotype for CYP2C9 (AA) required more warfarin dose than 45 patients (8.0%) having heterozygous genotype (AC), with the mean dose for 3.5 ± 2.0 mg per day

Table 3 Distributions of warfarin dose by each genotype

Gene	*SNP*	*Genotype*	N	*Warfarin dose (mg per day) (median ± IQR)*	P
VKORC1	1173T>C	TT	493	3.0 ± 1.5	0.0003
		TC	66	4.0 ± 2.0	
		CC	5	5.0 ± 0.0	
	1542G>C	GG	490	3.0 ± 1.5	<0.0001
		GC	67	4.0 ± 2.0	
		CC	7	5.0 ± 1.0	
	2255A>G	AA	489	3.0 ± 1.5	<0.0001
		AG	68	4.0 ± 2.0	
		GG	7	5.0 ± 1.0	
	3730G>A	GG	500	3.0 ± 1.5	0.0001
		GA	56	4.0 ± 2.0	
		AA	8	5.0 ± 0.8	
CYP2C9	CYP2C9*3	AA	518	3.5 ± 2.0	<0.0001
		AC	45	2.5 ± 1.0	
	3115T>C	TT	519	3.5 ± 2.0	<0.0001
		TC	45	2.5 ± 1.0	
	4045G>A	GG	519	3.5 ± 2.0	<0.0001
		AG	45	2.5 ± 1.0	
GGCX	8016G>A	GG	265	3.5 ± 2.0	0.0233
		GA	250	3.0 ± 1.8	
		AA	49	3.0 ± 2.0	
CYP4F2[a]	7253233C>T	CC	168	3.0 ± 1.5	0.0501
		CT	188	3.0 ± 1.5	
		TT	47	3.5 ± 2.5	

Abbreviations: IQR, interquartile range; SNP, single nucleotide polymorphism.
[a]CYP4F2 is genotyped in 403 patients.

and 2.5 ± 1.0 mg per day (CYP2C9 AA and AC), respectively. The mean warfarin dose in the CC genotypes of CYP4F2 (3.0 ± 1.5 mg/day) was significantly lower than that of TT homozygotes (3.5 ± 2.5 mg per day; P=0.0501). The mean warfarin dose was higher in the patients with the GGCX 8016GG genotype (3.5 ± 2.0 mg per day) compared with that of the GA (3.0 ± 1.8 mg/day) or AA (3.0 ± 2.0 mg/day) genotypes. A significant association was observed between warfarin dose and the 8016G>A of GGCX (P=0.0233).

Warfarin dosing algorithm

The non-parametric Pearson correlation analysis of the data revealed that warfarin dose was significantly negatively correlated with age (r=−0.41; P<0.0001) but significantly positively correlated with weight (r=0.14; P=0.0009), height (r=0.25; P<0.0001), INR (r=0.09; P=0.0410) and BSA (r=0.26; P<0.0001) (data not shown).

Multiple regression analysis was performed to estimate the relative contributions of age, gender, BSA, INR and four genetic polymorphisms to the inter-individual variations of warfarin dose. This result was presented in Table 4. When only age and BSA as clinical factors integrated with VKORC1 1173C>T, CYP2C9*3, GGCX 8016G>A, and CYP4F2 genotypes, 33% (Model 1, Table 4) of the variability could be explained, whereas the addition of gender and INR increased the R^2 to 35% (Model 2, Table 4). The multiple regression model included age, gender, BSA, INR and genotypes: dose=1.73−(0.03×age)+(0.20×gender)+(0.34×INR)+(1.77×BSA)+(0.76×VKORC1 1173T>C_TC)+(1.41×VKORC1 1173T>C_CC)−(1.18×CYP2C9*3)−(0.19×GGCX 8016G>A_GA)−(0.36×GGCX_AA_)+(0.17×CYP4F2_CT)+(0.58×CYP4F2_TT), where gender is coded as 1 if male, 2 if female; BSA is in m^2; the SNPs are coded 0 if absent, 1 if heterozygous and 2 if homozygous.

Of 564 patients, we also analyzed in 423 patients for the multiple regression tests. For the following reasons, the remaining 141 patients were excluded: (i) 27 patients had out of range INR value as >3.0. (ii) 114 patients having heart valve replacement were excluded due to higher warfarin maintenance dose than other diseases. In the multiple regression model, we can explain 40.0% of the variance in warfarin dose in Korean patients based on genetic polymorphisms and non-genetic factors (data not shown). The contribution of inter-individual variables in warfarin dose was 16.9% for age, 2.2% for gender, 1.1% for INR and 11.3% for BSA (data not shown). The clinical factors could explain 23.2% of the overall variability among patients in therapeutic warfarin dose (data not shown).

We also attempt to validate our pharmacogenetic dose algorithm comparing the predicted dose and actual dose of warfarin in same patients. According to our multiple regression model, the predicted dose is 4.9 mg per week (0.7 mg/per days) lower than the actual dose. Using Bland–Altman plot, we confirmed the smallest error between the predicted dose and the actual dose because the scatter diagram ranged around zero and distributed within a margin of error. Our data also showed a strong correlation between the predicted warfarin dose using the best regression model and actual dose (data not shown).

Table 4 Multiple linear regression models for predicting warfarin daily dose requirements (*N*=564)

		Model 1			*Model 2*		
Variables		*β*	*s.e.*	P	*β*	*s.e.*	P
Age		−0.035	0.005	<0.0001	−0.036	0.005	<0.0001
Gender					0.202	0.139	0.1465
INR					0.346	0.109	0.0017
BSA		1.506	0.317	<0.0001	1.773	0.374	<0.0001
1173C>T	TC	0.732	0.195	0.0002	0.767	0.193	<0.0001
	CC	1.349	0.633	0.0339	1.413	0.625	0.0244
CYP2C9*3	AC	−1.207	0.204	<0.0001	−1.182	0.201	<0.0001
8016G>A	GA	−0.183	0.118	0.1208	−0.199	0.117	0.0900
	AA	−0.317	0.208	0.1277	−0.361	0.205	0.0798
7253233C>T	CT	0.208	0.120	0.0846	0.177	0.119	0.1362
	TT	0.589	0.186	0.0017	0.583	0.184	0.0017
R^2		0.3286			0.3499		

Abbreviations: BSA, body surface area; INR, international normalized ratio; s.e., standard error.

DISCUSSION

Our results confirmed that VKORC1, CYP2C9, GGCX and CYP4F2 were significantly associated with warfarin dose requirement. However, the CYP4F2 SNP was not significantly associated with warfain dosage as borderline. Recent reports suggest that genetic polymorphisms in VKORC1 may explain a greater proportion of the variability in warfain dosing compared with polymorphisms in other candidate genes involved in the biochemical pathway of warfarin.[15] Lal S and colleagues also reported two polymorphisms involving base transitions in intron 1 (1173T>C) and the 3′-untranslated region (3730G>A). In the case of the intronic SNP, the average dose of warfarin in wild-type patients (CC) was 27% and 47% higher than in patients who were heterozygotes (CT) or variants (TT), respectively. Patients with 3730AA genotype required ∼25% higher dose of warfarin compared with patients who were heterozygotes or variants.[16] We observed that the mean warfarin dose was higher among patients with the VKORC1 1173CC genotype than in those with heterozygotes (TC) or the wild types (TT). Patients who are homozygous genotype AA for 3730G>A require more warfarin than patients with homozygous genotype GG or heterozygous genotype AG. Our data was consistent with previous studies, however, the minor allele frequencies of 1173T>C and 3730G>A in *VKORC1* gene were different in our study compared with Caucasians. Furthermore, we confirmed that the minor allele carriers of 1173T>C and 3730G>A require a higher daily dose of warfarin. Whether this is an ethnic specific association needs to be investigated further.

We also found that the CYP2C9*3 allele was a significant candidate influencing warfarin maintenance doses. There were significant differences in mean dose requirements of warfarin between the variant alleles and the wild type (P<0.0001). The allelic frequencies of CYP2C9*3 is 4.0% in our patients. In Caucasians, its allelic frequency is ∼6–10%. It has been known that this variant is less prevalent in Asian (1–4%) and African-American populations (1–2%) compared with Caucasians.[17] We could not find any CYP2C9*2 carrier in our patients (data not shown). In other studies, it was reported that CYP2C9*2 is not present in Asian populations and only 2–4% of African-American populations carry the CYP2C9*2 allele. The allelic frequencies of CYP2C9*2 and CYP2C9*3 are considerably different in ethnic populations.[2,17] Our data was consistent with previous studies on Asia populations.

The physiologic role of CYP4F2 in the vitamin K/warfarin pathway is unclear. It is known, however, that CYP4F2 hydroxylates the tocopherol phytyl side chain as the first step in the inactivation pathway of vitamin E. Given the similarity of the vitamin E and vitamin K side chains, CYP4F2 may hydroxylate the vitamin K phytyl side chain. As CYP4F2 is the major cytochrome responsible for synthesis of 20-hydroxyeicosatetraenoic acid in human kidney, the effect of CYP4F2 on warfarin dose could also be mediated through 20-HETE production. The rs2108622 polymorphism in CYP4F2

affects enzyme activity.[11] Recently, three independent white cohorts study demonstrated that a DNA variant in CYP4F2 was associated with warfarin dose and the difference of 1 mg per day in warfarin dose existed between CC and TT subjects.[2] We discovered that the daily warfarin doses of patients carrying TT genotype were significantly higher than those of patients carrying CC genotype. In whites and Asians, the minor allele frequency for rs2108622 is ~30% compared with 7% in blacks.[11] In our study, its frequency was 34.9%, which is similar to previous studies.

Several previous studies have examined the effect of a subset of GGCX polymorphism on warfarin dose. The commonly occurring non-synonymous polymorphism Q325R (rs699664, 8016G>A) was found not to be associated with warfarin dose, except in a recent Japanese populations.[19] In addition, a Japanese group reported an association of microsatellite marker in intron 6 with warfarin dose. In 183 warfarin-treated Swedes, a group of individuals carrying both alleles with 13 repeats or those with 14–16 repeats required significantly higher maintenance doses than patients with fewer repeats.[18,19] Our data suggested that a polymorphism in GGCX should have a low priority, certainly relative to CYP2C9 and VKORC1 and it was a promising candidate influencing warfarin maintenance doses significantly. Further studies with larger populations and additional ethnic groups are required to elucidate an association between variations in warfarin dosages and the GGCX 8016G>A genotypes.

Ethnicity is an important factor contributing to the warfarin maintenance dose. The warfarin maintenance dose in Asian patients was ~30–40% less than that of Caucasian patients and this considerable difference is, in part, attributable to genetic variations in CYP2C9 and VKORC1.[16] Most recently, several studies have insisted that VKORC1 and CYP2C9, age, gender and other environmental factors could explain almost one half (45–55%) of the variability in response to warfarin in Caucasian patients.[1,20,21] In Asian patients, the multiple regression model based on the genetic polymorphisms and the non-genetic factors of age and body weight can explain 30–40% of the variance in warfarin dose.[2,4] Cho HJ *et al.* suggested that it explained ~32% of the overall variability in warfarin dose requirements given all of the variables studied in Koreans.[22] In Japanese patients, the model using the multiple regression analysis including age, sex, weight and three genetic polymorphisms accounted for 33.3% of total variations in warfarin dose.[4] Also, a multiple regression model based on the genetic polymorphisms of VKORC1, CYP2C9, *EPHX1* and the non-genetic factors of age and body weight can explain 40.2% of the variance in warfarin dose in Han Chinese patients.[2]

In general, patients of Asian descent require a lower maintenance dose of warfarin for a similar degree of anticoagulation than patients of European descent.[23] Considering these racial differences in the anticoagulation therapy, we attempted to develop the pharmacogenetic dose algorithm for warfarin in Korean patients under low dose treatment. Our results can explain 35% of the variance in warfarin dose based on the genetic polymorphisms of VKORC1, CYP2C9, GGCX, and CYP4F2 and the non-genetic indicators of age, gender, INR and BSA. Our results indicated the inadequacy of the current algorithm of warfarin dose and the necessity to move toward a more individualized approach of warfarin therapy.

This study has demonstrated that the use of pharmacogenetics based dosing is suitable; however, there were several limitations. First, despite our current knowledge of pharmacogenomic and clinical factors, the source of more than 50% of the variability in warfain dose remains unclear. Additional genetic factors, including *EPHX1*, *MDR 1*, *apolipoprotein E*, and possible genes encoding additional components of the vitamin K epoxide reductase complex, as well as concomitant medications might be responsible for the observed inter-individual variability in warfarin dose requirement.[2,7,16] Second, a warfarin-dosing regimen using clinical data and pharmacogenomic information of VKORC1, CYP2C9, GGCX and CYP4F2 genotype could benefit patients treated with warfarin, but treatment algorithms incorporation pharmacogenomic data must be evaluated prospectively in a randomized controlled clinical trial before integrating into routine clinical practice.[16,20,24]

In this study, we can explain 35% of the variance in warfarin dose in Korean patients using a multiple regression model based on genetic polymorphisms and non-genetic factors. We expect that the current model will continue to evolve after the discovery of additional genes or new contributing factors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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