Influence of genetic, biological and pharmacological factors on warfarin dose in a Southern Brazilian population of European ancestry

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Warfarin is an oral anticoagulant widely used for thromboembolic disease prophylaxis. The pharmacokinetics and pharmacodynamics of this drug vary with environmental and genetic factors. Several algorithms including genetic and non-genetic factors have been developed for different populations. These investigations have shown that polymorphisms in *CYP2C9* and *VKORC1* genes are related to warfarin dose variation.

WHAT THIS STUDY ADDS

- This study confirms that *CYP2C9* and *VKORC1* genes are the main predictors of warfarin dose, but there are other genes with smaller effects that can improve the algorithm for better dose prediction.
- This study observed for the first time an effect of the *F2* gene on warfarin dose prediction.
- The inclusion for the first time of co-medication with amlodipine, carbamazepine, β -adrenoceptor blockers and diuretics refined the construction of an algorithm for dose prediction.

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AIMS

To investigate the influence of polymorphisms in *CYP2C9*, *VKORC1*, *CYP4F2* and *F2* genes on warfarin dose–response and develop a model including genetic and non-genetic factors for warfarin dose prediction needed for each patient.

METHODS

A total of 279 patients of European ancestry on warfarin medication were investigated. Genotypes for –1639G>A, 1173C>T, and 3730G>A SNPs in the *VKORC1* gene, *CYP2C9**2 and *CYP2C9**3, 1347C>T in the *CYP4F2* gene and 494C>T in the *F2* gene were determined by allelic discrimination with Taqman 5'-nuclease assays.

RESULTS

The *CYP2C9**2 and *CYP2C9**3 polymorphisms in the *CYP2C9* gene, –1639G>A and 1173C>T in the *VKORC1* gene and 494C>T in the *F2* gene are responsible for lower anticoagulant doses. In contrast, 1347C>T in the *CYP4F2* gene and 3730G>A in the *VKORC1* gene are responsible for higher doses of warfarin. An algorithm including genetic, biological and pharmacological factors that explains 63.3% of warfarin dose variation was developed.

CONCLUSION

The model suggested has one of the highest coefficients of determination among those described in the literature.

Introduction

Oral anticoagulants are extensively used in clinical practice to prevent and treat thromboembolic disorders [1]. The coumarins are the most used class of oral anticoagulants, to which warfarin belongs. The effect of this drug is influenced by several factors. Its pharmacokinetics and pharmacodynamics vary with bodyweight, diet, gender, drugs and genetic factors [2]. The dose to achieve the target INR ranges from an average of 4 mg to 80 mg weekly [3].

Single nucleotide polymorphisms (SNPs) in the cytochrome P450 complex (*CYP2C9*) and vitamin K epoxide reductase (*VKORC1*) genes affect the pharmacokinetics and pharmacodynamics of coumarins, respectively, and are strongly associated with warfarin dose. *CYP2C9**2 (rs1799853) in exon 3 (430C>T, Arg144Cys) and *CYP2C9**3 (rs1057910) in exon 7 (1075A>C, Iso359Leu) are associated with change in dose, since carriers of these alleles require a lower dose of warfarin [4–6]. The –1639G>A (rs9923231 – at promoter) and 1173C>T (rs9934438 – at intron 1) polymorphisms in the *VKORC1* gene are associated with a reduction of approximately 30% in warfarin dose [7]. In contrast, 3730G>A (rs7294 – 3'UTR) polymorphism, in the same gene, has been associated with an increased dose of the anticoagulant [7, 8]. Polymorphism 1347C>T (rs2108622) in *CYP4F2* [9, 10] and 494C>T (rs5896) in *F2* [11, 12] have also been associated with warfarin dose. Several studies proposed different pharmacogenetic algorithms [13–19] to determine the individual dose for each patient, but until now, most of them explained up to a maximum of 60% of warfarin dose variation.

The aim of this study was to propose a regression model with demographic, clinical and genetic factors to understand further warfarin dose variation. In addition, the individual influence of *CYP2C9**2 and *CYP2C9**3 polymorphisms in the *CYP2C9* gene, –1639G>A, 1173C>T and 3730G>A in the *VKORC1* gene, 1347C>T in the *CYP4F2* gene and 494C>T in the *F2* gene with warfarin dose in the weekly warfarin stable dose were evaluated.

Methods

Study population

A total of 302 patients of European ancestry recruited at the Cardiology and Internal Medicine Units from Hospital de Clínicas de Porto Alegre participated in the study. These patients were under regular use of warfarin, and attended the medical service once a month to adjust warfarin dose according to their INR target. The warfarin dose of each patient was defined as the dose at which the patient had the target INR for at least two consecutive medical visits. A questionnaire was completed for all patients by an interviewer, and their medical charts were reviewed to obtain details on ancestry, medicine intake and lifestyle variables such as smoking, physical activity, alcohol consumption

and warfarin stable dose. Patients who did not maintain the INR in target for at least two consecutive visits during the study period ($n = 23$) were not included in the study. Fourteen patients, based on clinical evidence were considered by the physicians as noncompliant to treatment and nine patients took the medication for a very short time, not reaching the target INR.

Only patients with all four grandparents born in Europe were included in the study in order to avoid population stratification. The Ethics Committee of the Hospital de Clínicas de Porto Alegre (Porto Alegre, Brazil) approved the study and all subjects provided written informed consent to participate.

Laboratory analysis

Genomic DNA was isolated from whole blood using PureLink™ Genomic DNA Purification Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Subjects were genotyped for –1639G>A (rs9923231), 1173C>T (rs9934438) and 3730G>A (rs7294) SNPs in the *VKORC1* gene, *CYP2C9**2 (rs1799853) and *CYP2C9**3 (rs1057910) SNPs in the *CYP2C9* gene, 1347C>T (rs2108622) in the *CYP4F2* gene and 494C>T (rs5896) in the *F2* gene in a 7500 Real-Time PCR System (Applied Biosystems, California, USA). Standardized TaqMan assays were used for detection of –1639G>A (C_30403261-20), 1173C>T (C_30204875-10) and 3730G>A (C_7473918-10) in *VKORC1* gene, *CYP2C9**2 (C_25625805-10) and *CYP2C9**3 (C_27104892-10) in *CYP2C9* gene, 1347C>T (C_16179493-40) in *CYP4F2* gene and 494C>T (C_11381382-20) in the *F2* gene. Genotyping were performed blind to clinical and demographic characteristics of patients. Positive and negative controls were run in all plates. Determination of the INR through prothrombin time was performed at the Haematology Laboratory of Hospital de Clínicas de Porto Alegre.

Statistical analysis

Chi-square test was used to assess Hardy-Weinberg equilibrium. The Multiple Locus Haplotype Analysis (version 3.0) [20] was used to estimate linkage disequilibrium between polymorphisms in the *VKORC1* gene and to derive haplotypes. The degree of linkage disequilibrium was assessed by D' and rho square (ρ^2). The correlation between weekly warfarin dose and quantitative variables (bodyweight, height, BMI and age) was assessed by Spearman correlation and differences between mean warfarin weekly dose and different genotypes, as well as between mean warfarin weekly dose and utilization of some medicines, were evaluated by ANOVA followed by Tukey's test. All ANOVA tests, as well as multiple linear regressions, were made using the logarithm to base ten of the warfarin weekly dose because of the assumption of the test, which requires that the dependent variable is consistent with normal distribution. To determine the variables to be included in the multiple linear regression models, univari-

ate analyses were performed to evaluate the independent influence of demographic, clinical and genetic factors in weekly warfarin dose. All variables with $P < 0.20$ and all polymorphisms studied were included in the model. The qualitative variables were entered as dummy variables, where 0 indicated absence and 1 indicated presence of the condition. The algorithm was elaborated with factors that presented at least $P < 0.05$ in the multiple linear regression models. Spearman correlation and mean absolute difference (AD) were used to compare between the observed stable warfarin dose of patients and the predicted dose algorithm. The AD for the model was calculated by (predicted dose–observed dose). A paired sample t -test was used to compare AD between the two proposed models. The Spearman correlation followed by r^2_s calculation was used to validate published algorithms in the investigated population. All statistical analyses were performed with the SPSS 16.0 statistical package.

Results

Characteristics of study population

The mean age of the 279 investigated subjects was 62.6 ± 14.1 years and ranged between 18 and 88 years. Most individuals were males (55.6%). The average warfarin dose in the studied sample was 33.52 ± 14.1 mg week⁻¹ and ranged from 7.5 mg to 85 mg. Table 1 shows the most commonly used medicines by the patients from this sample, along with other clinical features. About 5.4% of patients used warfarin only, 8.6% used warfarin and another drug, 6.4% used warfarin and two different drugs and the remaining used warfarin and three or more different medicines. The most common indications for oral anticoagulation were heart valve prosthesis and atrial fibrillation. The INR target of most patients was between 2 and 3, except in patients with heart valve mitral prosthesis, whose INR target was between 2.5 and 3.5. No differences in the mean INR targets achieved by patients were found for all polymorphisms tested ($P > 0.05$). Therefore, the algorithm presented in this paper is independent of target INR, because differences in average weekly dose and subsequent application of these data for dose prediction were not influenced by the different indications of anticoagulation/INR targets.

Association between non-genetic factors and warfarin dose

Positive correlations between weekly warfarin dose and bodyweight ($r_s = 0.220$, $P < 0.001$), height ($r_s = 0.164$, $P = 0.007$) and BMI ($r_s = 0.137$, $P = 0.026$) were detected. A negative correlation was detected between weekly warfarin dose and age ($r_s = -0.298$, $P < 0.001$). Bodyweight had a stronger association with warfarin dose compared with height and BMI. Because when two predictor variables that are highly correlated are included in the regression model, their estimated contributions and those of all other vari-

Table 1

Clinical characteristics of subjects

	n (%)
Indication for oral anticoagulation†	
Atrial fibrillation	147 (52.7)
Heart valve prosthesis	122 (43.7)
Thromboembolism	56 (20.1)
Cerebrovascular disease	49 (17.6)
Thrombophilias	17 (6.1)
Others	6 (2.2)
Other medicationst	
Aspirin	103 (36.9)
Amiodarone	22 (7.9)
Amlodipine	36 (12.9)
Captopril	101 (36.2)
Carbamazepine	7 (2.5)
Digoxin	62 (22.2)
Enalapril	62 (22.2)
Spironolactone	24 (8.6)
Furosemide	99 (35.5)
Hydralazine	25 (9.0)
Hydrochlorothiazide	86 (30.8)
Isosorbide	25 (9.0)
Levothyroxine	23 (8.2)
Losartan	21 (7.5)
Metformin	34 (12.2)
Metoprolol	108 (38.7)
Omeprazole	59 (21.1)
Propranolol	41 (14.7)
Simvastatin	105 (37.6)
Comorbiditiest	
Cardiopathy	77 (27.6)
Diabetes	59 (21.1)
Hypertension	168 (60.2)
Hypothyroidism	22 (7.9)
Renal failure	27 (9.7)

†The subjects can have more than one concomitant indication for anticoagulation, medication or comorbidity.

Table 2

Medications of prolonged use and their influence on warfarin dose

Medication	Dose without (n)	Dose with (n)	P
Amlodipine	34.53 mg (243)	26.74 mg (36)	0.001
Amiodarone	34.27 mg (257)	24.77 mg (22)	<0.001
β -adrenoceptor blockers	35.91 mg (113)	31.90 mg (166)	0.028
Carbamazepine	33.17 mg (272)	47.14 mg (7)	0.019
Diuretics	37.22 mg (99)	31.49 mg (180)	0.001
Statins	35.29 mg (162)	31.07 mg (117)	0.019

ables may be imprecise, we used only bodyweight in the regression model. The medicines of prolonged use that independently influenced warfarin dose are shown in Table 2. Amlodipine, amiodarone, β -adrenoceptor blockers, diuretics and statins decreased warfarin dose in 22.6%, 27.7%, 11.2%, 15.4% and 12.0%, respectively, whereas carbamazepine caused a 42.1% increase in the anticoagulant dose.

Linkage disequilibrium and haplotypic analysis

SNPs in the *VKORC1* gene showed linkage disequilibrium ($P < 0.001$). The values of D' between SNPs –1639G>A x 1173C>T, –1639G>A x 3730G>A and 1173C>A x 3730G>A were 0.961, 0.971 and 0.983; values for ρ^2 were 0.924, 0.362 and 0.371, respectively. The –1639A allele had a higher probability to segregate with the 1173T allele and the 3730G allele. The 3730G>A SNP showed weaker linkage disequilibrium than the others. We obtained seven different haplotypes, but the three most frequent haplotypes (–1639G/1173C/3730A (39.5%), –1639A/1173T/3730G (35.8%) and –1639G/1173C/3730G (23.1%)) accounted for more than 98% of the chromosomes investigated.

Association between genetic factors and warfarin dose

The genotype frequencies for all SNPs evaluated in this study are presented in Table 3 with their corresponding average weekly warfarin dose. The observed genotype distribution was in agreement with Hardy-Weinberg equilibrium (HWE) for all SNPs with the exception of 1347C>T in the *CYP4F2* gene ($P = 0.022$). Deviation from HWE can be due to several factors, but so far most researchers test for HWE primarily as a data quality check. Based on this assumption, it has been suggested that those loci with a significant level higher than 10^{-3} should be discarded from further investigations [21]. Since the P value for the *CYP4F2* gene was $P = 0.022$, we kept this polymorphism in the study.

The *CYP2C9* polymorphisms were strongly associated with weekly warfarin dose. As shown in Table 3, individuals

with *CYP2C9**1/*2, *CYP2C9**2/*2, *CYP2C9**1/*3 and *CYP2C9**2/*3 genotypes need 17.8%, 59.6%, 26.5% and 52.9% lower doses compared with homozygotes for the wild type allele. All the *VKORC1* polymorphisms also showed strong association with warfarin dose. Individuals with –1639GA and 1173CT genotypes and with –1639AA and 1173TT genotypes needed approximately 30% and 45% lower doses, respectively, when compared with homozygotes for the wild type. In contrast, individuals with 3730GA and 3730AA genotypes needed 18% and 53% higher doses when compared with individuals with the 3730GG genotype. Therefore, the 3730G>A polymorphism has an opposite effect on warfarin dose compared with the other two *VKORC1* SNPs. An association between *VKORC1* diplotypes and warfarin dose ($P < 0.001$) was observed. Diplotypes containing GCG or GCA haplotypes were responsible for higher doses when compared with diplotypes with an ATG haplotype. Neither 1347C>T in the *CYP4F2* gene ($P = 0.084$) nor 494C>T in the *F2* gene ($P = 0.807$) were associated independently with warfarin dose in our population. However, when these polymorphisms were included in the multiple linear regression, statistically significant results were obtained. We found that individuals with 1347CT and 1347TT genotypes for the *CYP4F2* gene need higher anticoagulant doses, whereas *F2* 494TT genotype carriers need lower warfarin doses. The Tukey analyses for *CYP2C9* and *VKORC1* genotypes are shown in Table 4.

About 20% of patients presented with minor bleeding, but this adverse effect was not associated with the genetic markers investigated herein. Major bleeding was not observed in this sample.

Table 3

Genotypic frequencies of SNPs and their respective average weekly dose ($n = 279$)

Polymorphism (MAF,%)	CYP2C9*2 (13.4) and CYP2C9*3 (5.4)						VKORC1 –1639G>A (36.7)		
Genotype	CYP2C9*1/*1	CYP2C9*1/*2	CYP2C9*1/*3	CYP2C9*2/*2	CYP2C9*2/*3	CYP2C9*3/*3	–1639GG	–1639GA	–1639AA
Frequencies (%)	182 (65.2)	64 (22.9)	25 (9.0)	3 (1.1)	5 (1.8)	0 (0)	112 (40.2)	129 (46.2)	38 (13.6)
Weekly dose (mg)	36.72	28.98	27.30	15.00	17.50	-	41.38	29.57	23.75
P (ANOVA)	<0.001						<0.001		
Polymorphism (MAF,%)	VKORC1 1173C>T (36.9)			VKORC1 3730G>A (39.8)			CYP4F2 1347C>T (32.4)		
Genotype	1173CC	1173CT	1173TT	3730GG	3730GA	3730AA	CC	CT	TT
Frequencies (%)	112 (40.1)	128 (45.9)	39 (14.0)	106 (38.0)	124 (44.4)	49 (17.6)	119 (42.7)	139 (49.8)	21 (7.5)
Weekly dose (mg)	41.36	29.86	23.01	29.10	33.63	42.81	31.53	34.93	35.48
P (ANOVA)	<0.001			<0.001			0.084		
Polymorphism (MAF,%)	Factor 2 494C>T (15.6)			VKORC1 diplotypes [–1639/1173/3730] †					
Genotype	494CC	494CT	494TT	GCG/GCG	GCG/GCA	GCG/ATG	GCA/GCA	GCA/ATG	ATG/ATG
Frequencies (%)	198 (71.0)	75 (26.9)	6 (2.1)	20 (7.2)	40 (14.3)	46 (16.5)	47 (16.8)	79 (28.3)	37 (13.3)
Weekly dose (mg)	33.51	33.80	30.42	41.13	40.75	28.75	42.50	30.19	23.44
P (ANOVA)	0.807			<0.001					

MAF, minor allele frequency. †Only the main diplotypes, which account for 96.4% of diplotypes.

Table 4

Mean difference in log-transformed weekly dose for CYP2C9 and VKORC1 genotypes

Genotypes		Mean difference	P	95% CI
VKORC1 1173C>T	CC x CT	−0.14	<0.001	−0.19, −0.09
	CC x TT	−0.26	<0.001	−0.33, −0.18
	CT x TT	−0.12	0.001	−0.19, −0.44
VKORC1 −1639G>A	GG x GA	−0.14	<0.001	−0.19, −0.09
	GG x AA	−0.24	<0.001	−0.31, −0.16
	GA x AA	−0.10	0.006	−0.17, −0.02
VKORC1 3730G>A	GG x GA	−0.07	0.006	−0.13, −0.02
	GG x AA	−0.18	<0.001	−0.26, −0.11
	GA x AA	−0.11	0.001	−0.18, −0.04
CYP2C9*2 and CYP2C9*3	*1/*1 x *1/*2	0.10	<0.001	0.04, 0.17
	*1/*1 x *1/*3	0.15	0.001	0.05, 0.25
	*1/*1 x *2/*2	0.40	0.001	0.12, 0.68
	*1/*1 x *2/*3	0.31	0.001	0.90, 0.52
	*1/*2 x *1/*3	0.05	0.776	−0.06, 0.16
	*1/*2 x *2/*2	0.30	0.035	0.01, 0.58
	*1/*2 x *2/*3	0.20	0.093	−0.02, 0.42
	*1/*3 x *2/*2	0.25	0.137	−0.04, 0.54
	*1/*3 x *2/*3	0.15	0.368	−0.08, 0.39
	*2/*2 x *2/*3	−0.09	0.948	−0.44, 0.25

Algorithm for warfarin dose prediction

The variables selected by univariate analyses ($P < 0.20$) were: bodyweight ($P < 0.001$), atrial fibrillation ($P < 0.001$), mitral valve prosthesis ($P = 0.040$), antiphospholipid syndrome ($P = 0.087$), venous thrombosis ($P = 0.191$), age ($P < 0.001$), alcohol consumption ($P = 0.172$), cardiopathy ($P = 0.119$), systemic arterial hypertension ($P = 0.002$), use of amlodipine ($P = 0.001$), amiodarone ($P = 0.001$), β -adrenoceptor blocker ($P = 0.006$), captopril ($P = 0.175$), carbamazepine ($P < 0.001$), digoxin ($P = 0.058$), diuretic ($P < 0.001$), statin ($P = 0.002$), CYP2C9 genotype ($P < 0.001$), VKORC1 haplotype/genotype ($P < 0.001$), CYP4F2 genotype ($P = 0.084$) and F2 genotype ($P = 0.807$). The factors that were statistically significant ($P < 0.05$) in the multiple linear regression are presented in Table 5. An algorithm that better explained the warfarin dose variation was derived and is shown as a footnote to Table 5. This algorithm can explain 63.3% of the warfarin dose variation. The clinical variable with the greatest effect was bodyweight and the genetic one was the VKORC1 gene. When CYP4F2 and F2 genes were excluded from the model, the adjusted r^2 became 0.604. CYP4F2 and F2 genes contribute together to 2.8% of variation in warfarin dose. Moreover, in addition to the exclusion of these two genes from the model, when we considered only the −1639G>A VKORC1 SNP instead of the haplotype with 1173C>T and 3730G>A, the model explained 57.7% of the warfarin dose variation (Table 5). However, the mean AD (absolute difference) value between the complete model and the one without the CYP4F2 and F2 genes and with only the −1639G>A VKORC1 SNP was not significant ($P = 0.245$).

As shown in Figure 1, the predicted dose of our model had a good correlation with the observed dose used by

patients ($r_s = 0.77$, $P < 0.001$). The mean AD value for this model was 6.9 mg week^{−1}. We compared the predicted dose of other models described in the literature with the observed dose in our patients (Table 6). The published algorithms [14–19, 22] poorly explained warfarin dose variation in this Southern Brazilian population of European ancestry. They explained between 36% and 48% of the warfarin dose variation when applied to our population. Even an algorithm elaborated from another Brazilian sample [17] explained only 41% of variation.

Discussion

In this study, the influence of common SNPs in CYP2C9, VKORC1, CYP4F2 and F2 genes and their influence on warfarin dose variation were investigated. All allele frequencies were similar to those described in previous studies with populations from the same ethnic background [8, 11, 17, 23, 24]. The independent association between lower warfarin dose and CYP2C9 SNPs [2, 17, 18, 25, 26] was also observed herein. Our findings showed that homozygous individuals for the wild type allele (CYP2C9*1/*1) needed higher doses when compared with individuals with CYP2C9*1/*2, CYP2C9*2/*2, CYP2C9*1/*3 and CYP2C9*2/*3 genotypes. As in previous studies [7, 8, 17, 18, 27, 28], in the present study the −1639G>A and 1173C>T VKORC1 polymorphisms were also associated with lower warfarin doses. On the other hand, carriers of the 3730A VKORC1 allele needed higher anticoagulant doses, as reported by D'Andrea *et al.* [8] and Kimura *et al.* [7]. In the present investigation the CYP4F2 1347C>T polymorphism was not associated with warfarin dose when analyzed independently,

Table 5

Multiple linear regression models with and without *CYP4F2* and *F2* genes and *VKORC1* haplotype

Variables	Model with <i>CYP4F2</i> gene, <i>F2</i> gene and <i>VKORC1</i> haplotype			Model without <i>CYP4F2</i> gene, <i>F2</i> gene and <i>VKORC1</i> haplotype		
	Regression coefficient (B)	P	95% CI for B	Regression coefficient (B)	P	95% CI for B
Constant	1.540	<0.001	1.429, 1.651	1.608	<0.001	1.498, 1.717
Age	-0.003	<0.001	-0.004, -0.002	-0.003	<0.001	-0.004, -0.002
Weight	0.004	<0.001	0.003, 0.005	0.004	<0.001	0.003, 0.005
Amiodarone	-0.077	0.004	-0.129, -0.025	-0.067	0.017	-0.121, -0.012
Carbamazepine	0.198	<0.001	0.102, 0.293	0.201	<0.001	0.099, 0.303
β-adrenoceptor blockers	-0.047	0.002	-0.077, -0.017	-0.037	0.025	-0.068, -0.005
Amlodipine	-0.065	0.004	-0.108, -0.021	-0.072	0.002	-0.118, -0.026
Diuretics	-0.037	0.024	-0.068, -0.005	-0.040	0.021	-0.074, -0.006
<i>CYP2C9</i> variant	-0.129	<0.001	-0.160, -0.098	-0.123	<0.001	-0.156, -0.091
2 <i>CYP2C9</i> variants	-0.280	<0.001	-0.370, -0.191	-0.290	<0.001	-0.385, -0.194
2 copies of GCG <i>VKORC1</i> haplotype	0.101	0.008	0.027, 0.174	-0.133†	<0.001†	-0.165, -0.101
1 copy of GCA <i>VKORC1</i> haplotype	0.049	0.018	0.008, 0.089	-0.254‡	<0.001‡	-0.301, -0.206
2 copies of GCA <i>VKORC1</i> haplotype	0.087	0.004	0.027, 0.147	–	–	–
1 copy of ATG <i>VKORC1</i> haplotype	-0.105	<0.001	-0.145, -0.064	–	–	–
2 copies of ATG <i>VKORC1</i> haplotype	-0.187	<0.001	-0.251, -0.123	–	–	–
<i>CYP4F2</i> CT	0.041	0.006	0.012, 0.070	–	–	–
<i>CYP4F2</i> TT	0.102	0.001	0.043, 0.160	–	–	–
<i>F2</i> TT	-0.120	0.013	-0.214, -0.025	–	–	–
<i>r</i> ²	0.656			0.595		
Adjusted <i>r</i> ²	0.633			0.577		
AD (mg week ⁻¹)	6.9			7.1		

The algorithm developed based on this regression model was: logarithm to base ten of warfarin weekly dose (mg) = 1.540–0.003 × (age in years) + 0.004 × (bodyweight in kg) – 0.077 × (1, if patient uses amiodarone, otherwise 0) + 0.198 × (1, if patient uses carbamazepine, otherwise 0) – 0.047 × (1, if patient uses β-adrenoceptor blocker, otherwise 0) – 0.065 × (1, if patient uses amlodipine, otherwise 0) – 0.037 × (1, if patient uses diuretic, otherwise 0) – 0.129 × (1, if patient has one *CYP2C9* variant, otherwise 0) – 0.280 × (1, if patient has two *CYP2C9* variants, otherwise 0) + 0.101 × (1, if patient has two copies of haplotype GCG, otherwise 0) + 0.049 × (1, if patient has one haplotype GCA, otherwise 0) + 0.087 × (1, if patient has two haplotype GCA, otherwise 0) – 0.105 × (1, if patient has one haplotype ATG, otherwise 0) – 0.187 × (1, if patient has two haplotype ATG, otherwise 0) + 0.041 × (1, if *CYP4F2* CT genotype, otherwise 0) + 0.102 × (1, if *CYP4F2* TT genotype, otherwise 0) – 0.120 × (1, if *F2* 494TT genotype, otherwise 0). †Values corresponding to GA genotype of –1639G>A polymorphism of *VKORC1* gene. ‡Values corresponding to AA genotype of –1639G>A polymorphism of *VKORC1* gene.

Figure 1

Relationship between the predicted dose by our algorithm and the observed dose in patients

in contrast with some results in the literature [10, 29]. However, in multivariate analyses, after controlling for covariates we observed that individuals with 1347CT and 1347TT genotypes needed higher doses, in agreement with Perini *et al.* [23] and Pautas *et al.* [30]. Results in the same direction were observed for 494C>T in the *F2* gene. D'Ambrosio *et al.* [11] and Shikata *et al.* [12] described an

Table 6

Comparison between predicted dose of published algorithms and the observed dose of our patients

Algorithm	<i>r</i> _s	<i>r</i> ² _s	Original <i>r</i> ²
Zhu <i>et al.</i> [19]	0.69	0.48	0.61
Herman <i>et al.</i> [14]	0.68	0.46	0.60
Klein <i>et al.</i> [15]	0.68	0.46	0.47
Wadelius <i>et al.</i> [22]	0.68	0.46	0.59
Gage <i>et al.</i> [13]	0.65	0.42	0.53
Perini <i>et al.</i> [17]	0.64	0.41	0.50
Sconce [18]	0.60	0.36	0.54
Miao <i>et al.</i> [16]	0.60	0.36	0.63

In the table are represented the Spearman correlation coefficient (*r*_s), the coefficient of determination based on *r*_s (*r*²_s) and the original *r*² obtained by the authors.

independent association between this SNP and warfarin dose. However, we corroborated their results only when the analyses were controlled for covariates; carriers of the 494TT genotype needed lower warfarin dose.

Our study proposed a regression model that explained 63.3% of the warfarin dose variation in a Southern Brazilian population of European ancestry. However, the model

needs to be validated in an independent sample. Several drug interactions with warfarin are known but usually few of them are taken into account in the development of algorithms. Our algorithm included medicines that are uncommon in other proposed algorithms, like amlodipine, β -adrenoceptor blockers (atenolol, metoprolol and propranolol) and diuretics (furosemide and hydrochlorothiazide). The inclusion of these medicines improved the algorithm by approximately 5%. These variables have no cost, and can be easily included for dose prediction. Statins were not included in the algorithm due to their high correlation with age in the studied sample. However, these drugs are widely known to influence warfarin dose [31, 32]. We have shown that co-medication is an important issue on warfarin dosing. Most studies do not take this factor into account. Nevertheless, drug interactions have a considerable value to explain the wide range of warfarin dose used and to improve algorithms to predict patients specific dose.

This study observed for the first time an effect of the *F2* gene on warfarin dose prediction. As previously reported [11, 12], carriers of the *F2* gene 494T allele needed a lower warfarin dose compared with 494C homozygotes. Based on our results, 494TT homozygous individuals needed 9.5% lower doses than heterozygote and homozygotes for the 494C allele. Notice, however that this effect is observed only when genotypes are controlled for covariates in the regression models. The possible explanation for the SNP effect is due to the substitution of a threonine, a polar amino acid, for a methionine, a non-polar one, in position 165 of the protein. This may induce conformational changes in the protein structure interfering with gamma-glutamyl carboxylase affinity [11]. However, this gene has never been included in a warfarin dosing algorithm before. Our results show that the *F2* gene does not have a predictive power as great as the *CYP2C9* and *VKORC1* genes SNPs, but it can be helpful to explain warfarin dose variation and improve algorithms. The inclusion of this polymorphism in the algorithm could explain a little more about the complexity of interacting factors involved in warfarin dose variation.

The inclusion of the *VKORC1* haplotype instead of -1639G>A SNP improved the model by 2.8% only. This effect occurred mainly due to the inclusion of the 3730G>A SNP, that presented an opposite influence in warfarin dose when compared with the -1639G>A and 1173C>T SNPs. However, the difference in mean AD values did not differ between models. Therefore, the results presented herein are in line with those previously published. The incorporation of additional *VKORC1* SNPs or haplotypes did not further improve dose prediction [33]. Thus, considering genotype cost, the model with better cost-benefit for clinical use, at present, in the studied population is the one without the *CYP4F2* gene, *F2* gene and *VKORC1* haplotype presented at Table 5. The inclusion of these three variables adds only 5.6% to the model.

The factors already described in the literature are those with the main influence on oral anticoagulant doses. It is unlikely that other variables with stronger influence will be found. Taking into account that warfarin dose variation is a multifactorial feature, there are many other factors that might have a very small effect. Thus, their detection becomes much more difficult, mainly when analyzed independently. In our study we did not investigate vitamin K intake due to methodological difficulties, but Franco *et al.* [34] showed that an increase in dietary vitamin K intake decreases the INR and a decrease in dietary vitamin K intake increases the INR. Thus, vitamin K intake is a factor that can improve the algorithms to predict the best warfarin dose for each patient. Additionally, other drugs may interact with warfarin, but their inclusion in the regression model often becomes difficult due to the small number of patients using these medicines. An increase in sample size would be needed for this detection. Genes like γ -glutamyl carboxylase [7, 35], protein C gene, apolipoprotein E [36], factor VII [11, 12], calumenin [37–39] and microsomal epoxide hydrolase [40] have already been associated with warfarin dose in some studies and can also potentially improve prediction of dose models.

The validation of other published algorithms confirms that the equations need to be population specific. All of them presented lower r^2 when applied to our population when compared with the value obtained in the original population, with exception of the IWPC's model, which was based in a sample ($n = 4043$) originating from nine different countries [15]. This model presented an initial $r^2 = 0.47$ and when it was validated in our sample the r^2 value was 0.46. Utilization of this significant sample from four different continents is more representative of different populations. Development of an algorithm with a great r^2 in a large sample may be an option for the elaboration of several specific models for each population. In a heterogeneous country like Brazil the relevant decrease in r^2 observed when data from another Brazilian sample [17] was used with the patients investigated herein is not unexpected. The population investigated in that study included individuals of mixed ancestry, whereas in the present study only Brazilians of European ancestry were investigated. The importance of ethnicity can be further observed by the difference observed between the original r^2 ($r^2 = 0.63$) and r^2 obtained by validation in our population ($r^2 = 0.36$) with a Chinese sample [16] that is easily explained by the different genetic and non-genetic features of the Chinese population as compared with a South American population.

Although a good prediction model was obtained in the present study, with a low AD and one of the highest coefficients of determination described in the literature, it still needs to be tested in an independent sample to be validated for possible clinical use. Prospective validation of algorithms is needed to evaluate their clinical efficacy and safety before their clinical use. In recent years, some prospective studies have been developed to validate the

algorithms in clinical practice and have shown positive results [22, 41, 42].

Competing Interests

There are no competing interests to declare.

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