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Effect of *NQO1* and *CYP4F2* genotypes on warfarin dose requirements in Hispanic–Americans and African–Americans

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Abstract

Aim—The objective of this study was to determine the additional contribution of *NQO1* and *CYP4F2* genotypes to warfarin dose requirements across two racial groups after accounting for known clinical and genetic predictors.

Patients & methods—The following were assessed in a cohort of 260 African–Americans and 53 Hispanic–Americans: clinical data; *NQO1* p.P187S (*1/*2); *CYP2C9**2, *3, *5, *6, *8 and *11; *CYP4F2* p.V433M; and *VKORC1* c.-1639G>A genotypes.

Results—Both the *CYP4F2* 433M (0.23 vs 0.06; $p < 0.05$) and *NQO1**2 (0.27 vs. 0.18; $p < 0.05$) allele frequencies were higher in Hispanic–Americans compared with African–Americans. Multiple regression analysis in the Hispanic–American cohort revealed that each *CYP4F2* 433M allele was associated with a 22% increase in warfarin maintenance dose ($p = 0.019$). Possession of the *NQO1**2 allele was associated with a 34% increase in warfarin maintenance dose ($p = 0.004$), while adjusting for associated genetic (*CYP2C9*, *CYP4F2* and *VKORC1*) and clinical factors. In this population, the inclusion of *CYP4F2* and *NQO1**2 genotypes improved the dose variability explained by the model from 0.58 to 0.68 ($p = 0.001$), a 17% relative improvement. By contrast, there was no association between *CYP4F2* or *NQO1**2 genotype and therapeutic warfarin dose in African–Americans after adjusting for known genetic and clinical predictors.

Conclusion—In our cohort of inner-city Hispanic–Americans, the *CYP4F2* and *NQO1**2 genotypes significantly contributed to warfarin dose requirements. If our findings are confirmed, they would suggest that inclusion of the *CYP4F2* and *NQO1**2 genotypes in warfarin dose prediction algorithms may improve the predictive ability of such algorithms in Hispanic–Americans.

Keywords

CYP2C9, *CYP4F2*, genotype; *NQO1*; *VKORC1*; warfarin

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Warfarin is the most commonly used oral anticoagulant and the leading cause of serious adverse drug events in the USA [1]. Warfarin exerts its anticoagulant effect by antagonizing VKORC1, thereby reducing the activation of vitamin K-dependent clotting factors II, VII, IX and X. Its usability is limited by a narrow therapeutic index and up to a 20-fold variation in therapeutic dose requirements [2]. Clinical factors, such as age, body size, comorbidities and interacting medications, contribute to this dose variability. Genetic variation of CYP2C9, the metabolizing enzyme of the more potent *S*-enantiomer of warfarin, and VKORC1, the target of warfarin, in combination with clinical factors, explain approximately 30–60% of the variation in therapeutic warfarin dose [3,4]. However, much of the variance remains unknown, particularly for African–Americans and Hispanic–Americans. In fact, Hispanic–Americans are largely excluded from warfarin pharmacogenetic studies; therefore, little is known about genetic determinants of warfarin dose requirements in this population.

NQO1 is an enzyme that catalyzes the reduction of quinones, including vitamin K. It is thought that NQO1 may participate in the reduction of vitamin K to vitamin K hydroquinone, which is the active cofactor for γ -carboxylation and activation of clotting factors [5]. Given its potential role in vitamin K recycling, *NQO1* is a candidate gene for further explanation of variance in therapeutic warfarin dose.

The *NQO1* gene is localized to chromosome 16. The *NQO1* p.P187S (rs1800566; *2) polymorphism in exon 6 destabilizes and inactivates the enzyme [6,7]. Individuals who are homozygous for the variant allele have no NQO1 activity, whereas heterozygotes have moderately reduced NQO1 function compared with wild-types [8]. The *NQO1**2 variant occurs in approximately 20% of African–Americans and Caucasians. *In vitro* data suggest differential NQO1 activity and *NQO1**2 functionality in Caucasians versus African–Americans, with African–Americans having higher baseline NQO1 activity [9]. *In vitro* data have also shown a direct correlation between NQO1 activity and both the enzyme's capacity to reduce vitamin K and the concentration of dicoumarol needed to inhibit NQO1 activity [10]. The *NQO1**2 allele has also been associated with reduced clotting factor activity and risk for ischemic stroke [11,12].

The CYP4F2 enzyme metabolizes vitamin K₁ to hydroxyvitamin K₁, thus reducing the amount of reduced vitamin K available for clotting factor activation. The p.V433M (rs2108622) SNP in *CYP4F2* has been associated with reduced enzyme activity *in vitro*, and warfarin dose in Caucasian and Japanese patients [13–17]. Specifically, the 433M allele has been associated with a higher warfarin maintenance dose, which would be expected based on the *in vitro* data. Inclusion of this variant in pharmacogenetic dosing equations has been shown to improve prediction of warfarin maintenance dose [18].

Our group previously reported no association between *NQO1* or *CYP4F2* genotype and warfarin dose requirements in African–Americans [19,20]. However, we did not examine the association of *NQO1* or *CYP4F2* genotype in the context of well-established genetic predictors of dose requirements. Neither *NQO1* nor *CYP4F2* genotype has been examined in warfarin-treated Hispanic–Americans. Given our mixed population of African– and Hispanic–Americans, we also examined the extent to which genetic ancestry contributed to warfarin dose requirements. Therefore, the objective of this study was to examine the additional effect of *NQO1* and *CYP4F2* genotypes on therapeutic warfarin dose in African–Americans and Hispanic–Americans while adjusting for well-known genetic (i.e., *VKORC1* and *CYP2C9*) and clinical predictors, as well as genetic ancestry.

Patients & methods

Study population & data collection

The patient population and procedures have been previously described [20,21]. Briefly, adult patients on a stable warfarin dose, defined as the same dose for at least three consecutive clinic visits with an average international Normalized Ratio (INR) within therapeutic range, were enrolled from the University of Illinois Hospital (IL, USA) and Health Sciences System Antithrombosis Clinic (IL, USA). Only patients who self-identified as African-American or Hispanic-American were included. Patients with a history of liver dysfunction or serum transaminase levels greater than three-times the upper limit of normal were excluded.

After obtaining written informed consent and authorization for medical record review, a buccal cell or venous blood sample was obtained for genetic analysis, as previously described [22]. Clinical data and social history were assessed via interview and review of the electronic medical record. The local institutional review board approved this study.

Genotyping & ancestry estimation

Genomic DNA was isolated from buccal cells or lymphocytes, and the *NQOI* rs1800566 (P187S; *2), *CYP2C9* rs1799853 (p.R144C; *2), rs1057910 (p.I359L; *3), rs28371686 (p.D360E; *5), rs9332131 (273frameshift; *6), rs7900194 (p.R150H; *8) and rs28371685 (p.R335W; *11), and *CYP4F2* rs2108622 (p.V433M) and *VKORC1* rs9923231 (c.-1639G>A) genotypes were determined, as previously described [20,23,24]. Individual genetic ancestry was determined for each person using 105 autosomal DNA ancestry informative markers for west African, Native American and European-American genetic ancestry using published methods [25–27]. Each participant was then scored from 0 to 100% for individual ancestry estimates of west African, Native American and European ancestry.

Data analysis

Average INR was calculated by averaging INR values from the three clinic visits when the warfarin dose was stable. Hardy-Weinberg equilibrium was tested by χ^2 analysis. Median daily warfarin dose was compared between genotype groups using the Mann-Whitney U test or the Kruskal-Wallis test.

The therapeutic warfarin dose was log transformed (natural log) prior to linear regression analysis to produce a more normal distribution of regression residuals. Pearson's correlations, unpaired t-test and χ^2 tests were used to identify clinical factors associated with warfarin maintenance dose. Variables potentially associated with warfarin dose requirements ($p < 0.10$) were included in the multivariate linear regression model.

Multivariate linear regression permitted tests for the additional effect of *NQOI**2 and *CYP4F2* genotypes on warfarin dose requirements while holding clinical and well-established genetic factors constant as well as adjusting for genetic ancestry. A dominant model was used for *CYP2C9* genotypes, with variant allele carriers coded as '1' and non carriers coded as '0', and an additive model was used for *CYP4F2* V433M and *VKORC1* -1639G>A, with the number of variant alleles coded 0, 1 or 2, as previously described [4,14,20]. The data for *NQOI* were analyzed using both a dominant and additive model, and the model providing the most robust fit for the data and requiring the least amount of assumptions was selected.

Clinical variables were retained in the model if they achieved statistical significance ($p < 0.05$) or were marginally significant ($0.05 < p < 0.10$) with strong biological plausibility.

Next, *CYP2C9* and *VKORC1* genotypes were added to the model, followed by *CYP4F2*, *NQO1* and genetic ancestry. The percentage of warfarin dose variability explained by a model including clinical variables plus *CYP2C9*, *VKORC1*, *CYP4F2* and *NQO1* genotypes was compared with the variability explained by a model containing clinical factors only, and a model containing clinical factors plus *CYP2C9* and *VKORC1* genotypes only. All statistical analyses were performed using SAS[®] software version 9.2 (SAS Institute, NC, USA).

Results

A total of 313 patients were enrolled, including 260 African–Americans and 53 Hispanic–Americans. The majority of Hispanic patients (89%) were of Mexican descent. Table 1 displays the characteristics of the study participants. African–Americans were of larger body size, more likely to have venous thromboembolism and less likely to have a history of diabetes, atrial fibrillation or aspirin use. Therapeutic warfarin dose and average INR were significantly higher in African–Americans compared with Hispanic–Americans ($p < 0.001$). Other characteristics were similar between racial groups.

Figure 1 displays the genetic ancestry results for 253 African–Americans and 50 Hispanic–Americans as determined by ancestral informative markers. African–Americans had an average of 82% west African, 13% European and 5% Native American ancestry, whereas Hispanic–Americans had 16% west African, 46% European and 38% Native American ancestry.

Genotype data were missing for one subject for *CYP2C9**8, one subject for *VKORC1* -1639G>A, eight subjects for *CYP2C9**6 and two subjects for *CYP4F2* p.V433M, owing to poor sample quality. All other genotype data were complete. No genotype deviated from Hardy–Weinberg equilibrium. There were significant differences in allele frequencies by self-reported race (Table 2), with higher frequencies of the *CYP2C9**2, *CYP2C9**3, *CYP4F2* 433M, *VKORC1* -1639A and *NQO1**2 alleles in Hispanic–Americans compared with African–Americans. The *NQO1**2 allele frequency observed in our Hispanic cohort was also higher than reported in Europeans [101].

Bivariate analysis between genotype & warfarin dosage

As previously reported, *CYP2C9* and *VKORC1* genotypes were significantly associated with warfarin dose requirements in both Hispanic–Americans and African–Americans (Table 3) [20,21]. The *CYP4F2* genotype was associated with warfarin dose in Hispanic–Americans but not in African–Americans. There was no association between the *NQO1**2 genotype and warfarin dose requirements in either race by bivariate analysis.

Multivariate linear regression analysis of association between *NQO1* & *CYP4F2* & warfarin dose

Clinical variables found on bivariate regression analysis to be potentially associated with log therapeutic warfarin dose in both self-reported racial groups were age, body surface area, history of venous thromboembolism and atrial fibrillation or flutter. History of stroke, hypertension, coronary artery disease, aspirin use and simvastatin use were associated with warfarin dose requirements in African–Americans only.

Tables 4 & 5 display the results of the multiple regression models in the African–American and Hispanic–American cohorts, respectively. For the *NQO1* genotype, both a dominant and additive model resulted in significant associations between genotype and therapeutic warfarin dose in the regression model. However, the dominant model provided the most robust fit and the fewest assumptions and was therefore used. Table 4 demonstrates that the

inclusion of *CYP2C9* and *VKORC1* genotypes into the clinical model increased the explained variance in warfarin dose for African–Americans. However, there was no further improvement in the model with the addition of *CYP4F2* or *NQOI*2* genotype (models 3 and 4). For Hispanic–Americans, Table 5 shows that the inclusion of *CYP4F2* improved the dose variability explained by the model from 0.58 to 0.63 ($p = 0.02$), and *NQOI*2* further improved the dose variability explained to 0.68 ($p = 0.004$). This equated to a 17% relative improvement in explanatory power (adjusted R^2) of therapeutic warfarin dose compared with model 2 ($p < 0.001$). Specifically, based on the regression coefficient, each *CYP4F2* 433M allele was associated with a 22% increase in warfarin maintenance dose, and possession of a *NQOI*2* allele was associated with a 34% ($p = 0.001$) increase in therapeutic warfarin dose requirements, while holding clinical and genetic predictors constant. Furthermore, model 4 in Hispanic–American explained 32% more variance in therapeutic warfarin dose than a similar model in African–Americans. After adjustment for Native American ancestry in model 5 in the Hispanic group, the explained variance increased to 0.70, although this was not significantly different from model 4. Adjusting for west African ancestry in self-reported African–Americans neither increased the variance explained nor did it significantly impact any of the β -coefficients in model 4.

It is reasonable to ask if the regression coefficients for the *NQOI*2* and *CYP4F2* variants are different between the African–American and Hispanic–American race groups. Therefore, we ran a test on the equality of the coefficients using an interaction product term in a multivariate regression model containing the total population with an indicator variable for race group. Both product terms for the interaction between the *NQOI*2* and *CYP4F2* variants with race and the race indicator variable were statistically significant ($p < 0.05$ for both interaction terms). This confirms there is an interaction between the effect of carrying *NQOI*2* and *CYP4F2* variants on warfarin dose by race.

Discussion

African–Americans and, to a greater extent, Hispanic–Americans, are under-represented in warfarin pharmacogenetic studies. In fact, this is one of the first studies to report on warfarin pharmacogenetics in Hispanic–Americans. In previous studies, we found that *CYP2C9* and *VKORC1* provide important contributions to understanding warfarin dose variability in both African–American and Hispanic–American populations [20,21]. We also found that the multi-ethnic pharmacogenetic warfarin dosing algorithm by the International Warfarin Pharmacogenetics Consortium (IWPC) performed as well in Hispanic–Americans as has been previously reported in populations of European descent [21], whereas there is evidence of poorer performance of the IWPC algorithm in those of African descent [28]. Importantly, a significant portion of the dose variability in both Hispanic–American and African–American populations remains unexplained by models including clinical factors as well as the *CYP2C9* and *VKORC1* genotypes [20,21]. The *CYP4F2* genotype has been associated with warfarin dose requirements in Caucasians and Asians, but not in African–Americans or Egyptians [13–16,29]. Its contribution to warfarin dose variability in Hispanic–Americans has not been previously reported. While the *NQOI* genotype has been examined for its contribution to acenocoumarol dose variability in Caucasians, in whom no association was found, it has not been adequately examined in other ethnic groups [30].

Our key finding from this study is that *CYP4F2* and *NQOI*2* genotypes were significantly associated with therapeutic warfarin dose in Hispanic–Americans when holding known associated clinical and genetic predictors constant. However, neither contributed to warfarin dose requirements in African–Americans. The multivariate regression model in Hispanic–Americans revealed that carrying a *NQOI*2* variant increased therapeutic warfarin dose by 34%, each *CYP4F2* variant increased dose requirements by 22% and consideration of

clinical and genetic factors, including *CYP4F2* and *NQO1*, explained 68% of the variance in therapeutic warfarin dose; a 17% improvement over a model without *CYP4F2* or *NQO1**2 genotype. Based on the median therapeutic dose of 4.5 mg/day observed in our Hispanic–American patients, the effect of carrying a *NQO1**2 variant equates to nearly a 2-mg/day higher warfarin dose requirement. Our data in Hispanic–Americans suggest each *CYP4F2* variant is associated with an approximately 1-mg/day higher therapeutic warfarin dose requirement, which is similar to data elucidated by Caldwell and coworkers for a mostly Caucasian cohort [14]. It is also important to note the large difference in R^2 for model 4 between the Hispanic–American and African–American groups. Essentially the same predictor variables explained nearly twice the variance in Hispanic–Americans compared with African–Americans. This further emphasizes the heterogeneity of treatment effect by ancestry and specifically highlights ancestry differences in the explanatory power of warfarin dose prediction models.

Hispanic–Americans are one of the largest and fastest growing minority groups in the USA and, to date, have been essentially absent in the largest warfarin pharmacogenetic data sets [4,21,102]. Given that nearly 4% have a history of atrial fibrillation, and 3% have a history of ischemic stroke, the number of Hispanic–Americans with indications for warfarin is substantial [31,32]. Therefore, identifying factors that are uniquely associated with warfarin dose in Hispanic–Americans is critical to improving care in this population. Two previous studies have examined warfarin pharmacogenetics in other Hispanic–American populations [33]. Compared with Hispanic–Americans of mostly Mexican descent in the current study, higher frequencies of the *CYP2C9**2 (19%) and *VKORC1*-1639A (41%) alleles were reported in a Puerto Rican population [33,34]. The percentage of Native American ancestry was higher in the current study than previously reported in Puerto Ricans, and in line with that reported in Mexican–Mestizos, potentially accounting for the observed differences in allele frequencies among Hispanic–American populations [35,36]. However, importantly, both the current study and previous studies in other Hispanic–American populations found significant reductions in the warfarin maintenance dose with *CYP2C9* and *VKORC1* variants [33,34]. Our study is unique in that it examined additional variants beyond those found in *CYP2C9* and *VKORC1*.

Our findings with *CYP4F2* are consistent with previous *in vitro* data demonstrating decreased enzyme activity with the 433M allele and previous association studies showing increased dose requirements in Caucasians with this allele [14,15]. However, our findings with *NQO1* are inconsistent with what might be expected. The *NQO1**2 genotype has been associated with decreased circulating clotting factor levels in patients in antithrombosis clinics in The Netherlands [11]. Furthermore, rats carrying the *NQO1**2 variant were shown to have decreased coagulation ability compared with wild-type rats [37]. Furthermore, in Taiwanese subjects, the *NQO1**2 genotype was associated with decreased risk of ischemic stroke [12]. Together, these data suggest that *NQO1**2 carriers have less circulating clotting factors and would therefore theoretically require less warfarin to maintain a therapeutic INR. This is in contrast to our finding of higher warfarin dose requirements in Hispanic–Americans carrying a *NQO1**2 (loss-of-function) variant. One possible explanation is that the *NQO1**2 variant is not the actual variant underlying the observed association with dose requirements. Rather, it may be in linkage disequilibrium with a functional locus associated with higher doses in Hispanic–Americans. This is supported by previous data suggesting a lack of association between *NQO1**2 genotype and warfarin dose requirements in African–Americans and Caucasians [30]. *NQO1**2 genotype was not associated with either warfarin dose or pharmacokinetic or pharmacodynamic response to warfarin in previously described, predominantly non-Hispanic, Caucasian populations, as well as in our African–American cohort [30]. Therefore, it is possible that *NQO1**2 is not in linkage with a causative SNP in other race/ethnic groups [30,38]. However, this is speculative and requires exploration.

Ancestral informative marker testing revealed an average Native American ancestry of 38% among our Hispanic–American population. By comparison, European–Americans reportedly have <10% Native American ancestry [39]. Genetic ancestry is a useful tool to control for genetic differences within heterogeneous populations such as Hispanic–American and African–Americans. This is especially the case for Hispanic–Americans who, depending on geographic origin, may possess a significant proportion of Native American (Mexican–American) or west African (Puerto Rican) genetic ancestry [40]. Given ancestral differences in allele frequencies and haplotype distribution, genetic studies limited to non-Hispanic whites may fail to detect variants predictive of dose in those of Hispanic ethnicity, who share a large portion of genetic architecture with Native Americans. In particular, genome-wide association studies assessing genetic determinants of warfarin dose requirements have been limited to European, Asian and, most recently, African populations [41–44]. Thus, whether variants beyond those identified in other populations are important for informing warfarin dose requirements in patients with Native American ancestry is unknown. Since the percentage of Native American ancestry varies among Hispanic–Americans depending on region of origin, this may lead to variability in genetic contributions to warfarin dose requirements across the Hispanic populations. For example, the *VKORC1*-1639G>A variant explains 30% of the variability in warfarin dose requirements among Mexican–Americans, but only 11% among Hispanic–Americans from Columbia, South America [21,34]. Therefore, it is important to identify variants of importance for different Hispanic populations.

Our sample size for Hispanic–Americans was relatively small and confined to those of Mexican descent and, thus, limits generalizability. As such, while we demonstrated a statistically and clinically significant association between *CYP4F2* and *NQO1**2 genotypes and therapeutic warfarin dose, our findings require confirmation in other Hispanic populations. Another limitation is that we did not assess dietary vita-min K, which can contribute to variation in warfarin dose. Although we only explored one *NQO1* variant, the *2 allele is reportedly more common than other *NQO1* variant alleles [45,46].

In summary, *CYP4F2* and *NQO1* genotypes did not impact warfarin dose requirements in our African–American cohort, even after controlling for clinical factors and genotypes associated with warfarin maintenance dose. However, in our cohort of inner-city Hispanic–Americans, both *CYP4F2* and *NQO1* were significantly associated with warfarin dose requirements, when holding clinical and genetic predictors constant. If our findings are confirmed, they would suggest that inclusion of *CYP4F2* and *NQO1**2 genotypes in warfarin dosing algorithms may improve the predictive ability of such algorithms in self-reported Hispanic–Americans of Mexican descent. Whether these genotypes are important for other Hispanic populations remains to be determined.

Future perspective

African–Americans, and especially Hispanic–Americans, remain under-represented in pharmacogenetic studies, including those involving warfarin. Our data support the need to investigate race-specific warfarin dose prediction models, which may provide more accurate dosing in under-represented patients. Moving forward, developing warfarin dose predication equations that take ancestry in concert with clinical and genetic characteristics into account may improve dose prescribing in under-represented populations.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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Figure 1. Percentage of patients of west African, Native American and European ancestry, as determined by ancestral informative markers, in subjects who self-identified as either African-American or Hispanic-American
(A, C & E) Patients self-identifying as African-American. **(B, D & F)** Patients self-identifying as Hispanic-American.

Table 1

Demographic and clinical characteristics of the study population by race.

Characteristics	African-American (n = 260)	Hispanic-American (n = 53)
Age (years), mean $\pm$ SD	55 $\pm$ 16	58 $\pm$ 15
Female sex, n (%)	192 (74)	33 (62)
Body surface area (m ²), mean $\pm$ SD	2.1 $\pm$ 0.3	2.0 $\pm$ 0.3 *
Past medical history		
Venous thromboembolism, n (%)	144 (55)	14 (26) *
Atrial fibrillation or flutter, n (%)	45 (17)	18 (34) *
Stroke or TIA, n (%)	79 (30)	22 (42)
Heart valve replacement, n (%)	18 (6.9)	4 (8)
Hypertension, n (%)	190 (73)	39 (74)
Diabetes mellitus, n (%)	72 (28)	25 (47) *
Heart failure, n (%)	50 (19)	11 (21)
Coronary artery disease, n (%)	39 (15)	11 (21)
Therapeutic warfarin dose (mg/day), median (interquartile range)	6.1 (4.7–7.9)	4.3 (2.9–7.1) *
Average INR (mean $\pm$ SD)	2.5 $\pm$ 0.3	2.0 $\pm$ 0.4 *
Concomitant medications		
Aspirin, n (%)	68 (26)	23 (43) *
Simvastatin, n (%)	80 (31)	16 (30)
Amiodarone, n (%)	7 (2.7)	1 (2)
Phenytoin or carbamazepine, n (%)	8 (3.1)	4 (8)
Current smoker, n (%)	46 (18)	7 (13)

INR: International Normalized Ratio; SD: Standard deviation; TIA: Transient ischemic attack.

*
p < 0.05

Table 2

Minor allele frequencies in African-Americans and Hispanic-Americans.

Allele	African-Americans (n = 260)	Hispanic-Americans (n = 53)
<i>NQO1</i> *2	0.18	0.27 [*]
<i>CYP2C9</i> *2	0.03	0.08 [*]
<i>CYP2C9</i> *3	0.01	0.07 [*]
<i>CYP2C9</i> *5	0.01	NA
<i>CYP2C9</i> *6	0.01	NA
<i>CYP2C9</i> *8	0.07	NA
<i>CYP2C9</i> *11	0.01	NA
<i>CYP4F2</i> V433M	0.06	0.23 [*]
<i>VKORC1</i> -1639G<A	0.09	0.33 [*]

NA: Not assessed.

^{*} p < 0.05 by χ^2 .

Table 3

Unadjusted analysis of therapeutic warfarin dose requirements by genotype in African-Americans and Hispanic-Americans.

Genotype	African-Americans (n = 260)			Hispanic-Americans (n = 53)		
	<i>n</i>	<i>Dose (mg/week), median (IQR)</i>	<i>p-value</i>	<i>n</i>	<i>Dose (mg/week), median (IQR)</i>	<i>p-value</i>
NQO1 <i>p.P187S</i>						
P/P	177	42.5 (32–56)	0.46	29	35(15–50)	0.78
P/S	74	42.5(34–52.5)		19	30(24–50)	
S/S	9	55(40–56.3)		5	30(27–30)	
CYP2C9						
*1/*1	198	45(35–58)	<0.001	37	35(24–52.5)	0.039
*2, *3, *5, *6, *8 or *11 allele	62	35 (27.5–48)		16	22 (15.5–35)	
CYP4F2 <i>p.V433M</i>						
V/V	229	42.5 (32.5–55)	0.84	33	27.5(18.5–35)	0.011
V/M	28	42.6 (35.5–60)		16	35 (18.75–51.3)	
M/M	1	37.5 (37.5–37.5)		4	59.5 (53.3–75)	
VKORC1 <i>c.–1639G>A</i>						
GG	213	45 (34–56.3)	<0.001	26	42.5 (27.5–52.5)	<0.001
AG	43	35 (25–42.5)		19	30 (15–35)	
AA	3	35 (13.8–36)		8	16.1 (13.1–24.8)	

IQR: Interquartile range.

Table 4

Multivariate regression model of log therapeutic warfarin dose in African-Americans.

	Model 1 [†]			Model 2 ^{‡*}			Model 3 [§]			Model 4 [¶]			Model 5 [#]		
	β (SE)	p-value		β (SE)	p-value		β (SE)	p-value		β (SE)	p-value		β (SE)	p-value	
Age (years)	-0.006 (0.002)	<0.001		-0.006 (0.001)	<0.001		-0.006 (0.001)	<0.001		-0.006 (0.001)	<0.001		-0.006 (0.001)	<0.001	
BSA (m ²)	0.32 (0.07)	<0.001		0.38 (0.06)	<0.001		0.38 (0.06)	<0.001		0.38 (0.06)	<0.001		0.39 (0.063)	<0.001	
Hypertension	-0.19 (0.05)	<0.001		-0.15 (0.049)	0.003		-0.15 (0.05)	0.003		-0.15 (0.049)	0.003		-0.15 (0.05)	0.003	
<i>CYP2C9</i>	-	-		-0.23 (0.05)	<0.001		-0.23 (0.05)	<0.001		-0.23 (0.050)	<0.001		-0.22 (0.05)	<0.001	
<i>VKORC1</i>	-	-		-0.24 (0.05)	<0.001		-0.24 (0.05)	<0.001		-0.25 (0.05)	<0.001		-0.24 (0.05)	<0.001	
<i>CYP4F2</i> V433M	-	-		-	-		-0.0002 (0.06)	0.98		-0.004 (0.06)	0.94		0.002 (0.06)	0.97	
<i>NQO1</i> *2	-	-		-	-		-	-		0.05 (0.04)	0.27		0.05 (0.04)	0.23	
West African ancestry	-	-		-	-		-	-		-	-		0.003 (0.16)	0.98	
Intercept	3.60	<0.001		3.52	<0.001		3.52	<0.001		3.50	<0.001		3.48	<0.001	

CYP2C9 variants includes the *2, *3, *5, *6, *8 and *11 alleles. *VKORC1* genotype was included as predictor with three levels (coded as 0 for GG, 1 for AG and 2 for AA). *NQO1* and *CYP2C9* genotypes were included as predictors with two levels (wild-type coded 0 vs variant carrier coded 1). Genetic ancestry is coded from 0 to 1 representing percentage west African ancestry. Clinical variables associated with therapeutic warfarin dose ($p < 0.10$) were tested in the model. Variables were retained in the model if they achieved statistical significance ($p \leq 0.05$) or were marginally significant ($0.05 < p \leq 0.10$) with strong biological plausibility.

For models 1, 2, 3 and 4, $n = 258$; for model 5, $n = 252$.

BSA: Body surface area; SE: Standard error.

* $p < 0.0001$ for incremental change in R^2 compared with model 1.

[†]Model 1 R^2 : 0.23; percentage variation in warfarin dose explained by clinical predictors only.

[‡]Model 2 R^2 : 0.37; percentage variation in warfarin dose explained by clinical predictors and *CYP2C9*, and *VKORC1* genotypes.

[§]Model 3 R^2 : 0.36; percentage variation in warfarin dose explained by clinical predictors and *CYP2C9*, *VKORC1* and *CYP4F2* genotypes.

[¶]Model 4 R^2 : 0.36; percentage variation in warfarin dose explained by clinical predictors and *CYP2C9*, *VKORC1*, *CYP4F2* and *NQO1* genotypes.

[#]Model 5 R^2 : 0.35; percentage variation in warfarin dose explained by clinical predictors and *CYP2C9*, *VKORC1* *CYP4F2* and *NQO1* genotypes adjusting for genetic ancestry.

Table 5

Multivariate regression model of log therapeutic warfarin dose in Hispanic-Americans.

	Model 1 [†]			Model 2 ^{‡*}			Model 3 [§]			Model 4 ^{¶**}			Model 5 [#]		
	β (SE)	p-value		β (SE)	p-value		β (SE)	p-value		β (SE)	p-value		β (SE)	p-value	
Age (years)	-0.009 (0.005)	0.055		-0.007 (0.004)	0.06		-0.008 (0.004)	0.03		-0.008 (0.003)	0.05		-0.008 (0.003)	0.0186	
BSA (m ²)	0.94 (0.24)	<0.001		0.55 (0.21)	0.01		0.48 (0.20)	0.02		0.53 (0.19)	0.006		0.553 (0.19)	0.0049	
Atrial fibrillation or flutter	-0.36 (0.14)	0.016		-0.39 (0.11)	0.002		-0.34 (0.11)	0.004		-0.37 (0.10)	<0.001		-0.398 (0.11)	0.0005	
<i>CYP2C9</i>	-	-		-0.39 (0.11)	0.001		-0.35 (0.11)	0.003		-0.29 (0.10)	0.006		-0.276 (0.10)	0.0096	
<i>VKORC1</i>	-	-		-0.34 (0.07)	<0.001		-0.33 (0.08)	<0.001		-0.36 (0.07)	<0.001		-0.373 (0.07)	<0.001	
<i>CYP4F2</i> V433M	-	-		-	-		0.20 (0.08)	0.02		0.17 (0.08)	0.03		0.181 (0.07)	0.02	
<i>NQO1</i> *2	-	-		-	-		-	-		0.29 (0.10)	0.004		0.354 (0.10)	0.0010	
Native American ancestry	-	-		-	-		-	-		-	-		-0.19 (0.23)	0.42	
Intercept	2.24	<0.001		3.24	<0.001		3.32	<0.001		3.12	<0.001		3.10	<0.001	

CYP2C9 variants includes the *2 and *3 alleles. *VKORC1* genotype was included as predictor with three levels (coded as 0 for GG, 1 for AG and 2 for AA). *CYP4F2* genotype was included as predictor with three levels (coded as 0 for CC, 1 for CT and 2 for TT). *NQO1* and *CYP2C9* genotypes were included as predictors with two levels (wild-type coded 0 vs variant carrier coded 1). Genetic ancestry is coded from 0 to 1 representing percentage Native American ancestry. Clinical variables associated with therapeutic warfarin dose ($p < 0.10$) were tested in the model. Variables were retained in the model if they achieved statistical significance ($p \leq 0.05$) or were marginally significant ($0.05 < p \leq 0.10$) with strong biological plausibility.

For models 1, 2, 3 and 4, $n = 53$; for model 5, $n = 50$.

BSA: Body surface area; SE: Standard error.

* $p < 0.0001$ for incremental change in R^2 compared with model 1;

** $p = 0.001$ for incremental change in R^2 compared with model 2.

[†]Model 1 R^2 : 0.36; percentage variation in warfarin dose explained by clinical predictors only.

[‡]Model 2 R^2 : 0.58; percentage variation in warfarin dose explained by clinical predictors and *CYP2C9*, and *VKORC1* genotypes.

[§]Model 3 R^2 : 0.63; percentage variation in warfarin dose explained by clinical predictors and *CYP2C9*, *VKORC1*, *CYP4F2* and *NQO1* genotypes.

[¶]Model 4 R^2 : 0.68; percentage variation in warfarin dose explained by clinical predictors and *CYP2C9*, *VKORC1*, *CYP4F2* and *NQO1* genotypes.

[#]Model 5 R^2 : 0.70; percentage variation in warfarin dose explained by clinical predictors and *CYP2C9*, *VKORC1*, *CYP4F2* and *NQO1* genotypes adjusting for genetic ancestry.