ARTICLE

Multi-site Investigation of Genetic Determinants of Warfarin Dose Variability in Latinos

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We conducted a multi-site investigation of genetic determinants of warfarin dose variability in Latinos from the U.S. and Brazil. Patients from four institutions in the United States ($n = 411$) and Brazil ($n = 663$) were genotyped for *VKORC1* c.-1639G> A, common *CYP2C9* variants, *CYP4F2*3*, and *NQO1*2*. Multiple regression analysis was used in the U.S. cohort to test the association between warfarin dose and genotype, adjusting for clinical factors, with further testing in an independent cohort of Brazilians. In the U.S. cohort, *VKORC1* and *CYP2C9* variants were associated with lower warfarin dose ($\beta = -0.29$, $P < 2.0 \times 10^{-16}$; $\beta = -0.21$, $P = 4.7 \times 10^{-7}$, respectively) whereas *CYP4F2* and *NQO1* variants were associated with higher dose ($\beta = 0.10$, $P = 2 \times 10^{-4}$; $\beta = 0.10$, $P = 0.01$, respectively). Associations with *VKORC1* ($\beta = -0.14$, $P = 2.0 \times 10^{-16}$), *CYP2C9* ($\beta = -0.07$, $P = 5.6 \times 10^{-10}$), and *CYP4F2* ($\beta = 0.03$, $P = 3 \times 10^{-3}$), but not *NQO1*2* ($\beta = 0.01$, $P = 0.30$), were replicated in the Brazilians, explaining 43–46% of warfarin dose variability among the cohorts from the U.S. and Brazil, respectively. We identified genetic associations with warfarin dose requirements in the largest cohort of ancestrally diverse, warfarin-treated Latinos from the United States and Brazil to date. We confirmed the association of variants in *VKORC1*, *CYP2C9*, and *CYP4F2* with warfarin dose in Latinos from the United States and Brazil.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Contribution of clinical and genetic factors within *VKORC1*, *CYP2C9* is documented in patients with European and African ancestry.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ This study investigated the contribution of clinical factors plus genotypes within *VKORC1-1639 G> A*, *CYP2C9* variants, *CYP4F2*2 and NQO1*2* among the largest cohort of Latinos from the U.S. and Brazil to date.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ This study confirmed the importance of warfarin associations within *VKORC1-1639 G> A*, *CYP2C9* variants,

*CYP4F2*3* and *NQO1*2* among Latinos from the U.S. and Brazil, which together explained ~ 42%–46% of warfarin dose variability.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ Clinical factors and genotypes within *CYP4F2 and NQO1,* in addition to *VKORC1-1639 G> A*, and *CYP2C9* variants may be considered to individualize warfarin dosing among Latinos, potentially providing further refinement of warfarin dose prediction.

Although the use of direct acting oral anticoagulants is increasing, warfarin remains commonly prescribed for prevention and treatment of thromboembolic events.[1,2] Complicating therapy with warfarin is the drug's narrow therapeutic index and high interpatient variability in dose requirements.[3] Latinos are at notably high risk for poor outcomes as a result of nontherapeutic anticoagulation with warfarin.[4–8] Particularly alarming is the increased risk for warfarin-related intracranial hemorrhage in Latinos compared with non-Latino whites.[7] Latinos also have a higher recurrence rate of thrombotic events and worse outcomes from these events compared with whites.[5,8,9]

Over a hundred studies have documented genetic associations with warfarin dose requirements.[10] However, the majority of data are in populations of European or Asian ancestry, in whom the most important genes affecting dose are *VKORC1* and *CYP2C9*.[11] The *VKORC1* −1639G>A genotype influences warfarin sensitivity, while *CYP2C9* genotype alters *S*-warfarin clearance.[12] *CYP2C9*2* and *3* are the primary *CYP2C9* variants affecting warfarin response in European ancestry populations, whereas *CYP2C9*2* is rare in Asians. Additional *CYP2C9* reduced function alleles impact warfarin dose requirements in persons of African ancestry and include *CYP2C9*5*, *6*, *8*, and *11*.[13] The *CYP4F2*3* polymorphism, which reduces metabolism of vitamin K, provides minor contribution to warfarin dose requirements in European and Asian populations, but not in African Americans.[14] Together, genotype plus clinical factors (e.g., age and body size) account for ~ 25–50% of the variability in warfarin dose requirements across populations studied to date.[15–17]

Latinos have either been excluded or marginally represented in most warfarin pharmacogenetic studies. Latinos are an admixed population with varying degrees of European, Native American, and West African ancestry. For example, the Amerindian population of Mexico is distinct from the more African populations from the Caribbean, Peru, and Colombia.[18] Genotype frequencies vary by ancestry, and thus, genetic associations in Europeans may not replicate in Latinos or may have less influence on the warfarin dose, as has been shown to be true for African Americans.[19,20] Similarly, Brazilians are highly admixed populations with varying contributions of European, Amerindian, and African ancestry.[21] Because of the significant admixture among Latinos and evidence that genetic associations with warfarin response vary by ancestry,[19] there may be uncertainty as to how best apply pharmacogenetic test results to warfarin prescribing decisions in Latinos. Therefore, we sought to investigate the contribution of common genetic variants in *VKORC1*, *CYP2C9*, and *CYP4F2*, and clinical factors to warfarin dose variability in a cohort of Latinos from four sites within the United States, with presumed diverse ancestry. Given emerging evidence that *NQO1*2* allele contributes to warfarin dose requirements in patients of Asian ancestry or Hispanic ethnicity,[19,22] this allele was also studied. We further tested associations in an independent cohort of Brazilians, also documented to have diverse ancestral background.[21,23] Unlike previous warfarin pharmacogenetic studies that were conducted in Latino patients from a single site or country,[24,25] we aimed to document the genetic contributions to warfarin dose requirements and quantify their effect sizes in a large cohort of warfarin-treated Latinos of heterogeneous ancestry (note that throughout the paper, we use the term "Latino," which includes individuals from Latin American countries, including Brazil, according to the National Advisory Committee on Racial, Ethnic, and Other Populations, 2020 Census, to refer collectively to our cohort of patients from U.S. sites and Brazil).

METHODS
Study population
Two cohorts, one from the U.S. and one from Brazil, were included. A total of 411 Latinos (by self-report) comprised the U.S. cohort, consisting of patients from the University of Arizona (UAZ, *n* = 76), University of Illinois at Chicago (UIC, *n* = 54), University of Puerto Rico (UPR, *n* = 260), and Icahn School of Medicine at Mount Sinai (*n* = 21). The Brazilian cohort (*n* = 663) was enrolled from the University of São Paulo and included 421 white Brazilians and 242 non-white Brazilians, who were defined as black or intermediate/brown by self-report according to the Instituto Brasileiro de Geografia e Estatística classification. Patients were ≥ 18 years of age and receiving a stable dose of warfarin. Definitions of stable dose at each site are in **Table S1**. Patients provided written informed consent for collection of their clinical data and either a venous blood or mouthwash sample for genetic analysis. All studies were approved by the institutional review boards at each participating organization and conducted in accordance with the Declaration of Helsinki.

Genotyping
DNA isolation and genotyping were performed locally at each site, which included *VKORC1* c.-1639G>A (rs9923231), *CYP2C9*2* (p.R144C, rs1799853), *CYP2C9*3* (p.I359L, rs1057910), *CYP4F2*3* (p.V433M, rs2108622), and *NQO1*2* (p. P187S; rs1800566). Additional *CYP2C9* alleles were genotyped in the UPR and Icahn School of Medicine at Mount Sinai cohorts (**Table S1**). Genotyping methodology is summarized in **Table S1**. Individual genetic ancestry was determined for three cohorts (UIC, UPR, and UAZ). For UIC, genetic ancestry was determined using 105 autosomal DNA ancestry informative markers for West African (WA), Native American (NA), and European-American (EA) genetic ancestry, with each patient having a value for WA, NA, and EA ancestry from 0–100%.[26,27] UPR and UAZ provided data on genetic ancestry using DNA ancestry informative markers from the DMET Plus Panel[28] and Illumina Infinium Multi-Ethnic Global BeadChip Array, respectively.

Data collection
Each site contributed genotype and clinical data using a common data collection tool derived from the International Warfarin Pharmacogenetic Consortium (IWPC) to ensure uniformity in data collection procedures across sites.[29] Clinical data included age, body size, anticoagulant indication, target international normalized ratio, use of CYP2C9 inducers (phenytoin and carbamazepine) and inhibitors (amiodarone), and stable warfarin dose.

Data analysis
The Hardy–Weinberg equilibrium assumption was tested by χ^2 analysis. A univariate analysis for the association of warfarin dose with genotype was conducted separately for each site. The median weekly warfarin dose requirements (mg) were compared between genotype groups at each site using nonparametric tests for two groups (Mann–Whitney *U*) or three groups (Kruskal–Wallis test) comparisons. An additive model was considered for *VKORC1*, *CYP2C9*, and *CYP4F2* genotype comparisons. For *CYP2C9*, we compared differences in warfarin doses between the *1/*1*, heterozygous variant (*2, *3, *5, *6,* or *11* carriers) and homozygous variant (e.g. *2/*3*) genotypes.

The relation between warfarin dose requirement and *NQO1*2* genotype satisfied a dominant model, and, therefore, heterozygous and homozygous variant genotypes were combined into one group and compared to the *1/*1* genotype. To leverage the power of increased sample size, we analyzed the combined data from the four U.S. sites. A univariate analysis of the weekly warfarin dose was tested against the genotypes in the combined cohort using either Kruskal–Wallis or Mann–Whitney *U* tests for additive (*VKORC1*, *CYP2C9*, and *CYP4F2*) and dominant (*NQO1*) models, respectively.

Multiple regression analysis in Latinos from the United States

A stepwise multiple linear regression analysis was performed in the combined U.S. cohort to identify the contribution of clinical and genotypes to variability in warfarin dose requirements. One patient from UIC had missing genotypes at *VKORC1 c.-1639G>A*, *NQO1*2*, and *CYP4F2 *3*. Due to insufficient amount of DNA from collected samples, 97 patients from UPR could not be tested for *CYP4F2* and *NQO1* single-nucleotide polymorphisms (SNPs). Another three patients in this cohort did not have genotypes for *VKORC1 c.-1639G>A* because of poor DNA quality. Analyses for warfarin dose associations included U.S. Hispanic patients who had available genotypes at all four genes (*n* = 310).

Adjusted R-squared (R^2) and Akaike Information Criterion (AIC) were used to determine improvement in warfarin dose prediction when *NQO1*2* and *CYP4F2*3* genotypes were added to a model including clinical predictors alone (model 1) or clinical predictors plus *VKORC1* and *CYP2C9* genotypes (model 2). To account for heterogeneity across the U.S. sites, the analysis was repeated adjusting for site assignment (site-adjusted model). The model with the highest R^2 and lowest AIC values was deemed as the model with the best fit and was tested in the Brazilian cohort. The analysis was conducted using R (version 1.3.959). An example dataset and R codes for model development and assessment are provided in the **Supplementary Material**.

In order to account for admixture and ancestral diversity among Latino patients in the United States, we repeated the warfarin association analysis, adjusting for estimates of African and Native American ancestry in a subgroup of patients who had ancestry marker information available (UIC, UPR, and UAZ). Measurements of individual ancestral proportions and the related admixture analysis was conducted in STRUCTURE using 105 ancestry informative markers and 71 ancestry informative markers from the DMET Plus at UIC and UPR, respectively. For UAZ, the same analysis was conducted in STRUCTURE using genomewide data from the Illumina Multi-Ethnic Global Array.

Multiple regression analysis in Latinos from Brazil

The varying minor allele frequencies of some SNPs between white and non-white Brazilian cohorts suggested different ancestral composition between white and non-white individuals. Therefore, separate association analyses were performed in each subgroup.

Finally, a meta-analysis of summary statistics of warfarin genotype associations between the U.S. (model 3) and Brazilian cohorts was performed separately in white and non-white Brazilians, using fixed effect, inverse-variance, weighted method, implemented in Comprehensive Meta-analysis Software version 3.

RESULTS

The mean age of patients in the U.S. and Brazilian cohorts was 66 ± 13 and 65 ± 14 years, respectively (**Table 1**). Most patients were taking warfarin for atrial fibrillation or flutter. The median and interquartile range of weekly warfarin dose

Table 1 Patient characteristics across the U.S. and Brazilian cohorts (white and non-white cohorts)

Characteristic	U.S. Latino cohort (*n* = 411)	White Brazilian cohort (*n* = 421)	Non-white Brazilian cohort (*n* = 242)
Age, years	66 ± (13)	66± (13)	63 ±(14)
Female sex	82 (20)	196 (47)	123 (51)
BSA, m²	2.0 ± 0.2	1.8 ± 0.2	1.9 ± 0.3
Medical history			
Venous thromboembolism	82 (20)	8 (2)	4 (2)
Atrial fibrillation or flutter	249 (61)	294 (70)	179 (74)
Stroke or TIA	56 (14)	23 (5)	9 (4)
Heart valve replacement	30 (7)	24 (6)	11 (5)
Diabetes mellitus	141 (34)	94 (22)	58 (24)
Stable warfarin dose, mg/week	32 [22.5–41.5]	27.5 [20.0–35.0]	30 [20–40.0]
Concomitant medications			
Amiodarone	10 (2)	50 (12)	42 (17)
Phenytoin	3 (1)	0 (0)	0 (0.0)
Carbamazepine	3 (1)	1 (0.2)	1 (0.4)
Current smoker	31 (8)	19 (5)	16 (7)

Categorical variables were expressed as *N* (%). Numerical variables were expressed as mean (SD) as in age and BSA, or median (interquartile range) as in stable weekly warfarin doses.
BSA, body surface area; TIA, transient ischemic attack.

Table 2 Minor allele frequencies in the U.S. and Brazilian Latinos

| Allele | U.S. cohort[a] (*n* = 411) | Brazilians | | $P^{\#}$ comparing U.S. vs. white Brazilian cohort | $P^{\#\#}$ comparing U.S. vs. non-white Brazilian cohort |
		Whites[b] (*n* = 421)	Non-whites[c] (*n* = 242)		
VKORC1–1639A	0.37	0.35	0.25	0.32	<0.001
CYP2C9*2	0.09	0.11	0.09	0.18	0.86
CYP2C9*3	0.05	0.05	0.05	0.14	0.88
NQO1*2	0.27	0.23	0.24	0.19	0.46
CYP4F2*3	0.25	0.30	0.23	0.07	0.60

The U.S. cohort included cohorts from University of Illinois at Chicago, University of Puerto Rico, University of Arizona, and Icahn School of Medicine at Mount Sinai.
$P^{\#}$ and $P^{\#\#}$ are P values for comparing genotype frequencies between U.S. and white Brazilians and U.S. and non-white Brazilians, respectively using χ^2 test.
[a]For the U.S. cohort, four patients had a missing genotype for VKORC1 c.-1639G>A, 98 patients had missing genotypes for NQO1*2 or CYP4F2*3.
[b]For white Brazilians, five patients had a missing genotype for VKORC1 c.-1639G>A; five and three patients had a missing genotype for CYP2C9*2 or *3, and NQO1*2, respectively.
[c]For non-white Brazilians, two patients had a missing genotype for VKORC1 c.-1639G>A and three had a missing genotype for CYP2C9*2 or *3.

in the U.S. and Brazilian cohorts were 32.0 mg (22.5–41.5) and 27.5 mg (20.0–35.0), and 30 (20–40.0), respectively (**Table 1**). Allele frequencies in the U.S. and Brazilian cohorts are shown in **Table 2** and **Table S2**. Frequencies of *CYP2C9*2*, *CYP2C9*3*, *NQO1*2*, and *CYP4F2*3* were similar between U.S. Latinos and Brazilians, whereas the frequency of *VKORC1 c.-1639G>A* was lower among non-white Brazilians (0.25) compared with Latinos (0.37) in the U.S. (*P* < 0.001). All genotypes were in Hardy–Weinberg equilibrium.
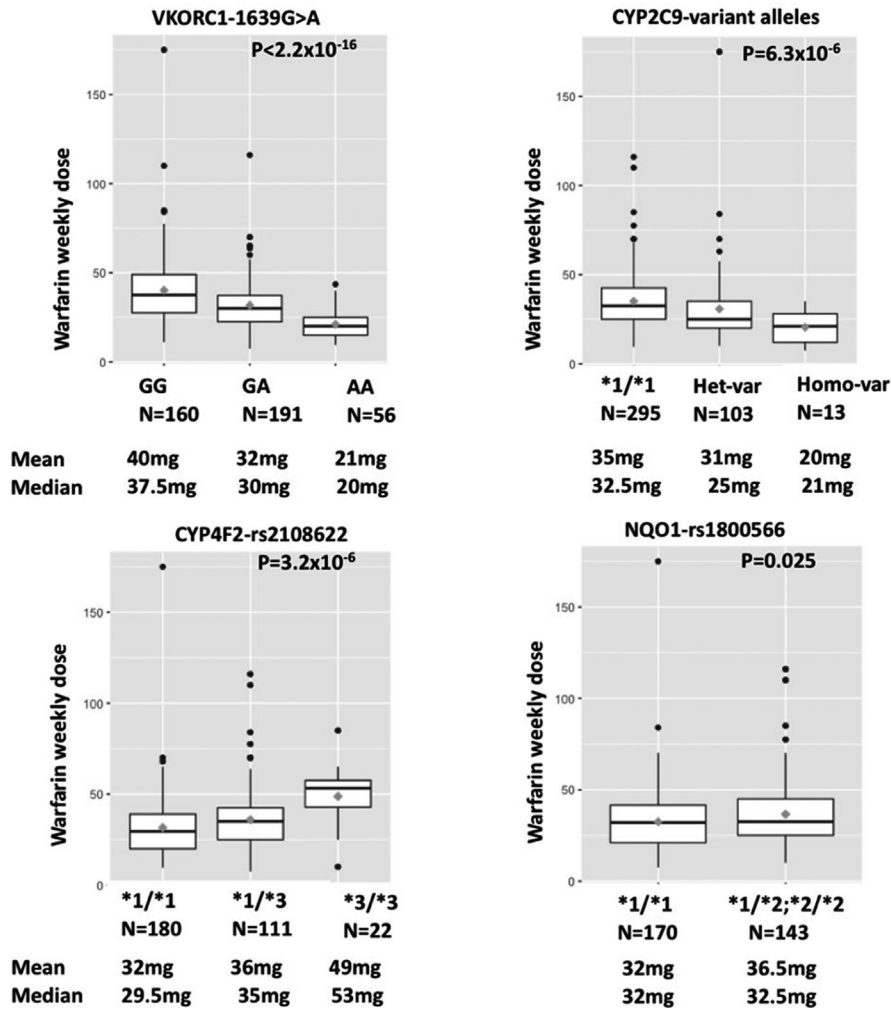
Genotype associations with warfarin dose requirements

The univariate associations between the evaluated genotypes and weekly warfarin dose requirements in mg for the four individual U.S. sites are shown in **Supplementary Figure S1**. The *VKORC1*, *CYP2C9*, *CYP4F2*, and *NQO1* genotypes were significantly associated with warfarin dose requirements in the combined U.S. cohort on both univariate analysis (**Figure 1**) and multiple linear regression (**Table 3**). Together, the evaluated genotypes and clinical factors (model 3; **Table 3**) explained 46% of the interpatient variability in dose requirements among U.S. Hispanics. This was significantly higher than that explained by clinical factors alone (model 1; **Table 3**, R^2 = 20%) or clinical factors plus *VKORC1* and *CYP2C9* genotypes (model 2, **Table 3**, R^2 = 42%). The full model (model 3) was characterized by a better fit as suggested by a lower AIC value (AIC = 246) compared with a model with clinical predictors only (model 1, AIC = 363, $P = 2.2 \times 10^{-16}$), or clinical predictors plus *VKORC1* c.-1639G>A and *CYP2C9* (model 2, AIC = 265, $P = 1.6 \times 10^{-5}$). Similar effect size for warfarin association (β and P values) were obtained when the analysis was repeated with site-adjustment (site-adjusted model, **Table 3**).

In a model adjusted for clinical factors and genotypes, each additional *CYP4F2*3* allele was associated with a 11% increase in weekly warfarin dose, and *NQO1*2* carriers had an 11% increase in their weekly dose. On the other hand, each *VKORC1–1639A* and *CYP2C9* variant allele was associated with 25% and 18% reduction in warfarin weekly dose, respectively.

The average ancestry estimates for patients enrolled at UIC, UPR, and UAZ are shown in **Table S3**. Contribution of EA, WA, and NA ancestry significantly differed across the three sites, suggesting a diverse ancestral background. The Puerto Rican cohort had the greatest WA ancestry and least NA ancestry, whereas the cohort at UAZ had the greatest European and least WA ancestry. We entered ancestry estimates in the model for the combined UIC-UPR-UAZ data. Neither the NA ancestry (β = –0.04, *P* = 0.79) nor WA ancestry (β = 0.15, *P* = 0.38) ancestry were associated with warfarin dose variability in the regression model that included genotypes, clinical predictors, and ancestry proportions (**Table S4**).

Univariate associations of genotypes and weekly warfarin dose requirements in mg are shown for the Brazilian cohort in **Figure S2**. On univariate analysis, *VKORC1*, *CYP2C9*, and *CYP4F2* genotypes were associated with warfarin dose requirement in the overall Brazilian cohort (**Figure S2, panel A**). A model with clinical predictors (age, body surface area, carbamazepine, and amiodarone) plus *VKORC1*, *CYP2C9*, and *CYP4F2* genotypes explained 42.4% of warfarin dose variability (**Table 4**). This model resulted in a slight improvement in the model fit (AIC = –591) compared with a model with clinical predictors plus *VKORC1* and *CYP2C9* genotypes only (AIC = –585, adjusted R^2 = 41.7%, *P* = 0.004). *NQO1*2* was not associated with warfarin dose requirement in the overall Brazilian cohort (β = 0.01, *P* = 0.30).

Given the admixture of the Brazilian cohort, the white and non-white cohorts were examined separately. In the white Brazilians, there was a significant association between warfarin dose requirements and *CYP4F2* genotype (β = 0.03, *P* = 0.002), but not *NQO1* genotype (β = –0.001, *P* = 0.94, **Table 4**). A model with clinical predictors plus *VKORC1*, *CYP2C9*, and *CYP4F2* genotypes explained 43% of warfarin dose requirements (AIC = –396). This model contributed an additional 1% to the percent of variability in warfarin dose explained by a model with clinical predictors plus *VKORC1* and *CYP2C9* genotypes (AIC = –388, adjusted R^2 = 42%, *P* = 0.002). In non-white Brazilians, there was no association between dose and *CYP4F2* genotype (β = 0.02, *P* = 0.19), but

Figure 1 Univariate analysis of genotype associations with warfarin dose in the combined U.S. cohort. Genotype groups were coded as 0, 1, 2, for *VKORC1*, *CYP2C9*, and *CYP4F2*, genotype groups respectively, and as 0 and 1 for *NQO1* genotype groups. From left top panel, weekly warfarin doses were compared across *VKORC1* c.-1639G>A, *CYP2C9*, *CYP4F2*3*, and *NQO1*2* genotypes in a univariate analysis using Kruskal–Wallis or Mann–Whitney *U* tests. P: Unadjusted *P* values for comparisons. Grey dots and horizontal lines within boxplots represent mean and median doses. *CYP2C9 *2*, **3*, **5*, **6*, **8*, **11*, and **12* variants were included in the analysis.

a trend toward association with *NQO1* genotype (β = 0.03, P = 0.11). A model with clinical predictors plus *VKORC1* and *CYP2C9* genotypes explained 40% of the dose variability. Inclusion of the *NQO1* genotype in the model for non-whites did not significantly improve the percent of variability explained (adjusted R^2 = 41.0%, P = 0.09).

In the U.S. non-white Brazilian meta-analysis, *VKORC1*, *CYP2C9*, *NQO1*, and *CYP4F2*3* genotypes were associated with warfarin dose (**Table 5**). In the meta-analysis for the U.S. and white Brazilian cohort, *VKORC1*, *CYP2C9*, and *CYP4F2* genotypes, but not *NQO1* genotype, were associated with warfarin dose (**Table 5**).

DISCUSSION

Latinos are the largest minority group in the United States, comprising 18% of the nation's population. Yet, Latinos have been the focus of few warfarin pharmacogenetic studies to date.[30] Previous studies have been conducted in relatively small and specific Latino cohorts, such as cohorts from Puerto Rico[24] or Brazil.[25] We included a more heterogenous cohort of Latinos from four urban sites in the United States and Brazil to examine whether genetic associations identified in smaller cohorts remained when tested in a larger cohort with variable admixture patterns. Studies in a population with a broad range of ancestry, such as ours, can facilitate extrapolating predictions to the Latino population at large. Ultimately, identifying pharmacogenetic associations in underserved populations, including Latinos, is critical to ensure that research and subsequent clinical implementation in the field extend across populations rather than widening disparities.

We confirmed that, similar to other populations, *VKORC1* c.-1639G>A and *CYP2C9* genotypes plus clinical factors (age, body size, and use of CYP2C9 inhibitors or inducers) are important determinants of warfarin dose requirements in Latinos across the United States and Brazil.

Table 3 Multiple linear regression analysis of determinants of warfarin dose requirements in the U.S. cohort

	Model 1		Model 2		Model 3		Site-adjusted	
	β (SE)	*P* value	β (SE)	*P* value	β (SE)	*P* value	β (SE)	*P* value
Intercept	3.3 (0.2)	2×10^{-16}	3.6 (0.2)	$<2 \times 10^{-16}$	3.5 (0.2)	$<2 \times 10^{-16}$	3.6 (0.2)	$<2 \times 10^{-16}$
Age, years	−0.01 (0.002)	1.8×10^{-7}	−0.01 (0.002)	3.1×10^{-8}	−0.01 (0.002)	1.2×10^{-8}	−0.01 (0.002)	2.5×10^{-9}
BSA, m^2	0.43 (0.1)	6.9×10^{-6}	0.35 (0.08)	2.6×10^{-5}	0.34 (0.08)	2.3×10^{-5}	0.3 (0.08)	6.1×10^{-5}
Carbamazepine	0.48 (0.2)	0.056	0.57 (0.2)	0.008	0.57 (0.2)	0.006	0.7 (0.2)	0.001
Amiodarone	−0.61 (0.2)	0.0002	−0.32 (0.1)	0.03	−0.27 (0.1)	0.06	−0.28 (0.1)	0.05
VKORC1–1639G>A	–	–	−0.30 (0.03)	$<2 \times 10^{-16}$	−0.29 (0.03)	$<2.0 \times 10^{-16}$	−0.3 (0.03)	$<2.0 \times 10^{-16}$
CYP2C9 variant genotype	–	–	−0.22 (0.03)	4.2×10^{-8}	−0.20 (0.04)	4.7×10^{-7}	−0.2 (0.04)	3.0×10^{-7}
*CYP4F2*3*	–	–	–	–	0.1 (0.03)	2×10^{-4}	0.1 (0.03)	5×10^{-4}
*NQO1*2*	–	–	–	–	0.1 (0.04)	0.01	0.1 (0.04)	0.01
Adjusted R^2; AIC	20%; 363		42%; 265		46%; 246 ($P^{\#} = 2.2 \times 10^{-16}$; $P^{\#\#} = 1.6 \times 10^{-5}$)		47%; 245 ($P^{*} = 0.07$)	

The multiple linear regression was performed in the combined cohorts of U.S. Latinos (University of Illinois at Chicago, University of Puerto Rico, University of Arizona, and Icahn School of Medicine at Mount Sinai). Analysis included 310 patients who had clinical data and genotypes at all four genes.
Model 1 adjusted for age, BSA, and medications (carbamazepine and amiodarone).
Model 2 adjusted for the same covariates as in model 1 in addition to *VKORC1 c.-1639G>A* and *CYP2C9* genotypes.
Model 3 adjusted for same covariates as in model 2 in addition to *CYP4F2* and *NQO1* variants.
$P^{\#}$: *P* value comparing model 3 vs. model 1; $P^{\#\#}$: *P* value comparing model 3 vs. model 2; P^{*}: *P* value comparing model 3 vs. site-adjusted model.
AIC, Akaike information criterion; BSA, body surface area.

Table 4 Associations with warfarin dose in Brazilians

	White		Non-white		Overall Brazilians	
Model	β (SE)	*P* value	β (SE)	*P* value	β (SE)	*P* value
Age	−0.002 (0.001)	2.6×10^{-5}	−0.004 (0.001)	6.9×10^{-8}	−0.003 (0.00)	2.3×10^{-12}
BSA	0.19 (0.03)	4.0×10^{-9}	0.17 (0.04)	5.1×10^{-5}	0.17 (0.02)	4.6×10^{-12}
Carbamazepine use	0.43 (0.15)	0.004	0.09 (0.09)	0.35	0.18 (0.08)	0.02
Amiodarone use	−0.13 (0.02)	3.8×10^{-8}	−0.10 (0.03)	0.0005	−0.11 (0.02)	4.5×10^{-11}
VKORC1–1639G>A	−0.15 (0.01)	$<2.0 \times 10^{-16}$	−0.13 (0.016)	1.3×10^{-14}	−0.14 (0.01)	$<2.0 \times 10^{-16}$
CYP2C9 variant genotype	−0.08 (0.01)	2.1×10^{-8}	−0.06 (0.02)	0.004	−0.07 (0.01)	5.6×10^{-10}
*NQO1*2*	−0.001 (0.01)	0.94	0.03 (0.02)	0.11	0.01 (0.01)	0.3
*CYP4F2*3*	0.03 (0.011)	0.002	0.02 (0.02)	0.19	0.03 (0.01)	0.003
Adjusted R^2	43%		41%		42%	

Multiple linear regression was performed in white, non-white and overall Brazilians (white and non-white). Analysis was adjusted for age, BSA, medications (carbamazepine and amiodarone) and genotypes at *VKORC1* c.-1639G> A, CYP2C9 genotypes, *NQO1*2*, and *CYP4F2*3* genotypes.
BSA, body surface area.

Table 5 Meta-analysis of warfarin genotype associations for U.S. Hispanics and Brazilians

	Meta-analysis U.S.-white Brazilians		Meta-analysis U.S. non-white Brazilians	
	β (SE)	*P* value$^{\#}$	β (SE)	*P* value$^{\#\#}$
VKORC1–1639G>A	−0.16 (0.01)	0.000	−0.17 (0.01)	0.000
CYP2C9 variant	−0.09 (0.01)	2.2×10^{-12}	−0.09 (0.02)	8.7×10^{-7}
*NQO1*2*	0.01 (0.01)	0.42	0.05 (0.02)	0.01
*CYP4F2*3*	0.05 (0.02)	0.0002	0.05 (0.02)	0.002

Summary statistics of warfarin genotype associations of U.S. and Brazilian cohorts were meta-analyzed using fixed effect, inverse variance, weighted meta-analysis implemented in Comprehensive Meta-analysis version 3, following software steps.
$P^{\#}$ and $P^{\#\#}$ are meta-analysis *P* values for U.S. white Brazilians and U.S. non-white Brazilians, respectively.

The consideration of the *CYP4F2*3* and *NQO1*2* genotypes provided additional contribution to warfarin dose requirements in U.S. Latinos, whereas *CYP4F2*, but not *NQO1*, contributed to a minor extent in Brazilians. The contributions of *VKORC1* and *CYP2C9* genotypes plus clinical factors to warfarin dose requirements is consistent with that reported

in European populations and greater than that reported in African Americans or Asians.[31] The *CYP4F2*3* variant has been associated with higher warfarin dose requirements in European and Asian populations, but not in African Americans,[14] likely due to its lower variant allele frequency in persons of African ancestry. Consistent with data in Europeans and Asians, we observed higher warfarin doses in U.S. Latinos with the *CYP4F2*3* allele.

Brazilians have variable European, African, and Native Amerindian ancestry, depending on the geographic location.[32] Similar to the data in African Americans,[14] the *CYP4F2* genotype was not associated with warfarin dose in non-white Brazilians, who were defined as black or intermediate brown. However, *CYP4F2* was linked to higher warfarin dose requirements in white Brazilians, which is consistent with previous data in Brazilians with predominantly European ancestry from Porto Alegre.[25] In that study, *CYP4F2*3* together with a variant in the *F2* gene contributed to nearly 3% of the variability in warfarin dose. In our combined Brazilian cohort consisting of mostly white Brazilians, the inclusion of *CYP4F2*3* in the regression model slightly increased the contribution to warfarin dose variability (~ 1%). In contrast, Perini *et al*.[33] found no association between *CYP4F2*3* and warfarin response in an admixed population from Rio de Janeiro, in which 50% of patients were described as intermediate/brown or black.

Genetic ancestry has been correlated with self-declared race, with a higher percentage of African ancestry in individuals self-declaring race as non-white and a higher percentage of European ancestry in those self-declaring as white.[34] Taken together, the data suggest that *CYP4F2* genotype is associated with warfarin dose among Brazilian populations of mostly European ancestry, but not in those with significant African ancestry; although, these data are only speculative as we did not have genetic ancestry data in our Brazilian cohort.

The data with *NQO1* genotype are intriguing. NAD(P) H:quinone oxidoreductase (NQO1) catalyzes the two-electron reduction of quinones, including vitamin K, to hydroquinone. NQO1 was once thought to be involved in the reduction of vitamin K during gamma carboxylation of vitamin K-dependent clotting factors, although this has been refuted by other investigators.[35,36] The gene encoding NQO1 is located on chromosome 16q22.1, and the *NQO1*2* allele is a missense variant in exon 6 that leads to a proline to serine substitution. It has previously been associated with loss of NQO1 activity, reduced coagulation factor activity, and reduced risk for ischemic stroke.[35,37–39] Given this evidence and the once putative role of NQO1 in vitamin K recycling, we and others have examined warfarin dose requirements by the *NQO1*2* genotype with mixed results. Although no association has been identified in European or African ancestry populations,[19,40] accumulating data suggest an association in some Latino and Asian populations; although data are not entirely consistent.[19,22,41,42]

Latinos who have varying European, African, and Native American ancestral composition share certain haplotypes with Asians,[43] in whom *NQO1* associations with warfarin were documented. Our U.S. cohort included Latinos from the UAZ, who are presumably of mostly Mexican descent, as suggested by the genetic ancestry analysis, and UIC, who we previously reported were of predominantly Mexican descent,[19] with mostly European and Native American ancestry. The population from Puerto Rico, in contrast, had more African ancestry. Despite the variable ancestral contribution, the association with *NQO1*2* genotype was evident in our combined U.S. Latino population, with individuals requiring ~ 4 mg/week higher doses with each *NQO1*2* allele. Although not significant in the overall Brazilian cohort or in white Brazilians, there was a trend toward higher warfarin requirements with the *NQO1*2* allele in non-white Brazilians. A meta-analysis of the U.S. and non-white Brazilian cohorts showed a significant association between *NQO1*2* genotype and warfarin dose requirements. Without a formal analysis of ancestral estimates in all U.S. and Brazilian Latinos presented in our study, it is difficult to draw conclusions as to the ancestral origin of the potential association between *NQO1*2* and warfarin dose variability.

The mechanism underlying higher warfarin dose requirements with the *NQO1*2* variant is unclear. Although *NQO1*2* reduces NQO1 activity,[37] it likely does not directly affect warfarin response given its variable association with dose requirements across populations. It is possible that the *NQO1*2* allele is in linkage disequilibrium with the functional SNP(s) underlying the association in some populations but not others. The *NQO1*2* SNP is also an expression quantitative trait locus for *CLEC18C* and *PDXDC2P* according to Haploreg version 4[44] and Genotype-Tissue Expression.[45,46] The function of these genes is not obviously related to warfarin metabolism or vitamin K cycling. Rather, the ontology of these genes points to their role in carbohydrate metabolism (*CLEC18C*), pyridoxal phosphate binding, and carboxylase activity (*PDXDC2P*). Future studies are therefore warranted to elucidate the exact underpinnings of the *NQO1* association.

Although the pharmacogenetic model in our U.S. Latino and Brazilian cohorts explains less variability in warfarin dose (43–46%) than has been previously observed in Puerto Ricans[24] and Brazilians,[25] previous models were derived from smaller, less heterogenous populations, which likely influenced the genetic effect size and test statistics including R^2. Our model specifically included patients with Mexican ancestry (UIC and UAZ).

Currently, dosing algorithms originally developed from patients of predominately European ancestry, such as the IWPC and Gage dosing algorithms,[29,47] are recommended to estimate warfarin dose requirements.[10] However, neither of these algorithms includes *CYP4F2*3* or *NQO*2*; although the *CYP4F2*3* genotype is included in the dosing tool available through warfarindosing.org. In a smaller cohort, we previously showed that a model including *NQO1*2* and *CYP4F2*3* outperformed the IWPC algorithm in predicting the warfarin dose among Puerto Ricans.[48] In this larger cohort, the *CYP4F2*3* and *NQO*2* variants together explained an additional 4% of the variability in warfarin dose beyond that explained by other genotypes and clinical factors in U.S. Latinos. Our findings suggest that consideration of *CYP2F4*3* and *NQO1*2* variants in U.S. Latino patients may provide further refinement of warfarin dose

prediction, and specifically allow for identifying those who may require higher warfarin doses beyond that expected based on *VKORC1−1639A* and *CYP2C9* genotypes alone.

A strength of this study is that it included the largest sample of warfarin-treated Latinos of diverse ancestry to date. We also acknowledge some limitations. First, we focused on a limited number of candidate genes and SNPs, and, therefore, may have missed other important variants influencing warfarin response. Nevertheless, we were able to explain up to 46% of warfarin dose variability focusing on these SNPs in four genes. As mentioned above, we did not have data on ancestry markers for all patients from the United States and Brazil. We also did not evaluate the effects of *CYP2C9*5*, *6*, *8*, and *11* in all cohorts, which may have underestimated the effect of *CYP2C9* genotype in Latinos with higher African ancestral proportions and introduced heterogeneity in our data. Additionally, we recognize some nonuniformity in our data across sites inherent with the data being collected through individual efforts. Nevertheless, our approach to combining the data was similar to that taken with other multi-site investigations of genetic associations with warfarin, including the IWPC.[16,29] Further, our results did not change following adjustment for site of enrollment to account for heterogeneity in data across sites.

In summary, *VKORC1*, *CYP2C9*, and *CYP4F2* genotypes were associated with warfarin dose requirements in Latino populations across the United States and Brazil. Additionally, *NQO1*2* was associated with warfarin dose variability among Latinos from the United States, and in the combined U.S. and non-white Brazilian cohort. Taken together, these data support the importance of genetic information for individualizing warfarin dosing in Latinos and underscore the increased need for pharmacogenetic algorithms in under-represented Latino populations.

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