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

Nihal El Rouby1,2, Leiliane Rodrigues Marcatto3, Karla Claudio4, Leticia Camargo Tavares3, Heidi Steiner5, Marianna R. Botton6, Steve A. Lubitz7, Echo N. Fallon8, Kevin Yee8, Justin Kaye9, Stuart A. Scott6,†, Jason Karnes3,†, Paulo Caleb Junior de Lima Santos10,†, Jorge Duconge4,† and Larisa H. Cavallari1,*,†

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 (β = −0.29, P < 2.0 × 10−16; β = −0.21, P = 4.7 × 10−7, respectively) whereas CYP4F2 and NQ01 variants were associated with higher dose (β = 0.10, P = 2 × 10−4; β = 0.10, P = 0.01, respectively). Associations with VKORC1 (β = −0.14, P = 2.0 × 10−16), CYP2C9 (β = −0.07, P = 5.6 × 10−10), and CYP4F2 (β = 0.03, P = 3 × 10−3), but not NQ01*2 (β = 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 NQ01*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 NQ01*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 NQ01, 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.9 Latinos also have a higher recurrence rate of thrombotic events and worse outcomes from these events compared with whites.5,8,9

1These authors jointly supervised the work.
2Department of Pharmacotherapy and Translational Research and Center for Pharmacogenomics and Precision Medicine, University of Florida, Gainesville, Florida, USA; 3Department of Pharmacy Practice and Administrative Sciences, University of Cincinnati James L. Winkle College of Pharmacy, Cincinnati, Ohio, USA; 4Laboratory of Genetics and Molecular Cardiology, Faculdade de Medicina FMUSP, Heart Institute (InCor), Universidade de São Paulo, São Paulo, Brazil; 5University of Puerto Rico, San Juan, Puerto Rico, USA; 6Department of Pharmacy Practice and Science, University of Arizona College of Pharmacy, Tucson, Arizona, USA; 7Icahn School of Medicine at Mount Sinai, New York, New York, USA; 8Cardiac Arrhythmia Service and Cardiovascular Research Center, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA; 9Banner University Medical Center-Tucson, Tucson, Arizona, USA; 10Department of Pharmacology, Escola Paulista de Medicina, Universidade Federal de São Paulo, EPM-Unifesp, São Paulo, Brazil. *Correspondence: Larisa H. Cavallari (lcavallari@cop.ufl.edu)

Received: March 4, 2020; accepted: June 16, 2020. doi:10.1111/cts.12854
Over a hundred studies have documented genetic associations with warfarin dose requirements. 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. The VKORC1 -1639G>A genotype influences warfarin sensitivity, while CYP2C9 genotype alters S-warfarin clearance. 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. 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. 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.

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. 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. Similarly, Brazilians are highly admixed populations with varying contributions of European, Amerindian, and African ancestry. Because of the significant admixture among Latinos and evidence that genetic associations with warfarin response vary by ancestry, 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*12 allele contributes to warfarin dose requirements in patients of Asian ancestry or Hispanic ethnicity, this allele was also studied. We further tested associations in an independent cohort of Brazilians, also documented to have diverse ancestral background. Unlike previous warfarin pharmacogenetic studies that were conducted in Latino patients from a single site or country, 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.I535L, 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%. UPR and UAZ provided data on genetic ancestry using DNA ancestry informative markers from the DMET Plus Panel 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. 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 χ² 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 \( R^2 \) 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 data set 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

| 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.
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.

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 R² = 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 R² = 42%, P = 0.002). In non-white Brazilians, there was no association between dose and CYP4F2 genotype (β = 0.02, P = 0.19), but

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

| Allele    | U.S. cohorta (n = 411) | Whitesb (n = 421) | Non-whitesc (n = 242) | P* comparing U.S. vs. white Brazilian cohort | P# comparing U.S. vs. non-white Brazilian cohort |
|-----------|------------------------|-------------------|-----------------------|---------------------------------------------|-----------------------------------------------|
| VKORC1−1639A | 0.37 | 0.35 | 0.25 | 0.32 | <0.001 |
| CYP2C9∗2 | 0.09 | 0.11 | 0.09 | 0.18 | 0.06 |
| 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 χ² 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.
a trend toward association with NQO1 genotype ($\beta = 0.03$, $P = 0.11$). 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. Previous studies have been conducted in relatively small and specific Latino cohorts, such as cohorts from Puerto Rico or Brazil. 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.
The consideration of the \textit{CYP4F2*3} and \textit{NQO1*2} genotypes contributed to a minor extent in Brazilians. The contributions of \textit{VKORC1} and \textit{CYP2C9} genotypes plus clinical factors to warfarin dose requirements is consistent with that reported in the U.S. Latino cohort.
in European populations and greater than that reported in African Americans or Asians.\textsuperscript{31} The CYP4F2*3 variant has been associated with higher warfarin dose requirements in European and Asian populations, but not in African Americans,\textsuperscript{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.\textsuperscript{32} Similar to the data in African Americans,\textsuperscript{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.\textsuperscript{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 \textit{et al}.\textsuperscript{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 as race non-white and a higher percentage of European ancestry in those self-declaring as white.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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,\textsuperscript{19,40} accumulating data suggest an association in some Latino and Asian populations; although data are not entirely consistent.\textsuperscript{19,22,41,42}

Latinos who have varying European, African, and Native American ancestral composition share certain haplotypes with Asians,\textsuperscript{33} 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,\textsuperscript{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,\textsuperscript{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\textsuperscript{44} and Genotype-Tissue Expression.\textsuperscript{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\textsuperscript{24} and Brazilians,\textsuperscript{25} previous models were derived from smaller, less heterogenous populations, which likely influenced the genetic effect size and test statistics including R\textsuperscript{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,\textsuperscript{29,47} are recommended to estimate warfarin dose requirements.\textsuperscript{10} However, neither of these algorithms includes CYP4F2*3 or NQO1*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.\textsuperscript{38} In this larger cohort, the CYP4F2*3 and NQO1*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.18,19 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.

Supporting Information. Supplementary information accompanies this paper on the Clinical and Translational Science website (www.cts-journal.com).

Acknowledgments. The authors would like to thank Andrea Peralta, BS, Juanita Gonzalez, RN, and Amy Kennedy, PharmD, for their assistance with this project. We also want to acknowledge the patients of the San Juan Veterans Affairs Caribbean Healthcare Center for voluntarily participation in this study. This material is the result of work supported in part with resources and the use of facilities at the Veterans Affairs Caribbean Health Care Center in San Juan, Puerto Rico.

Funding. This work was supported by an American Heart Association Midwest Affiliate Grant-In-Aid (10GRNT3750024, L.H.C.), institutional career development award from the University of Health Care Science Center (J.H.K), Flinn Foundation under a Seed Grant to Promote Translational Research in Precision Medicine (J.H.K), FAPESP (Saao Paulo Research Foundation: 2013/09295-3, 2016/22507-8, and 2016/23454-5), National Institutes of Health (NIH) grant 1R01HL139731 and American Heart Association 18SRN34250007 (S.A.L.). This work was also supported in part by grant HL123911 (J.D.) from the National Heart, Lung, and Blood Institute (NHLBI) and by the RCMI award U54 MD007600 (J.D.) from the National Institute on Minority Health and Health Disparities (NIMHD).

Conflict of Interest. S.A.S. is a paid employee of Sema4. L.B.R. declared no competing interests for this work. S.A.L. received sponsored research support from Bristol Myers Squibb/ Pfizer, Bayer AG, and Boehringer Ingelheim, and has consulted for Bristol Myers Squibb/ Pfizer and Bayer AG. All other authors declared no competing interests for this work.

Author Contributions. N.E. and L.H.C. wrote the manuscript. S.A.S., J.H.K., P.C.S., J.D., L.H.C., designed the research. L.R.M., L.C.T., K.C., H.S., M.R.B, S.A.L., E.N.F, K.Y., and J.K. performed the research. N.E. and L.C.T. analyzed the data.

Disclaimer. Karla Claudia-Campos and Jorge Duconge held a without compensation (WOC) employment status with the Pharmacy Service, VA Caribbean Healthcare Systems (VACHS), in San Juan, Puerto Rico, at the time of conducting the study. The contents of this paper do not represent the views of the Veterans Affairs Caribbean Healthcare System, the Department of Veterans Affairs, the National Institutes of Health, or the United States Government.

1. Barnes, G.D., Lucas, E., Alexander, G.C. & Goldberger, Z.D. National trends in ambulatory oral anticoagulant use. Am. J. Med. 128, 1300–1306 (2015).
2. Desai, N.R. et al. Patterns of initiation of oral anticoagulants in patients with atrial fibrillation - quality and cost implications. Am. J. Med. 127, 1075–1082 (2014).
3. Budnitz, D.S., Lovegrove, M.C., Shehab, N. & Richards, C.L. Emergency hospitalizations for adverse drug events in older Americans. N. Engl. J. Med. 356, 2002–2012 (2011).
4. Go, A.S. et al. Heart disease and stroke statistics–2014 update: a report from the American Heart Association. Circulation 129, e28–e292 (2014).
5. White, R.H., Dager, W.E., Zhou, H. & Munir, S. Racial and gender differences in the incidence of recurrent venous thromboembolism. Thromb. Haemost. 96, 287–293 (2006).
6. Shen, A.Y. et al. Racial/ethnic differences in the prevalence of atrial fibrillation among older adults—a cross-sectional study. J. Natl. Med. Assoc. 102, 906–913 (2010).
7. Shen, A.Y., Yas, J.F., Sarr, S.S., Jorgensen, M.B. & Chen, W. Racial/ethnic differences in the risk of intracranial hemorrhage among patients with atrial fibrillation. J. Am. Coll. Cardiol. 50, 309–315 (2007).
8. Simpson, J.R. et al. Mexican Americans with atrial fibrillation have more recurrent strokes than do non-Hispanic whites. Stroke 41, 2132–2136 (2010).
9. Tang, Y. et al. Ethnic differences in out-of-hospital fatal pulmonary embolism. Circulation 123, 2219–2225 (2011).
10. Johnson, J.A. et al. Clinical pharmacogenetics implementation consortium (CPIC) guideline for pharmacogenetics-guided warfarin dosing: 2017 update. Clin. Pharmacol. Ther. 102, 397–404 (2017).
11. Kaye, J.B. et al. Warfarin pharmacogenomics in diverse populations. Pharmacotherapy 37, 1150–1163 (2017).
12. Johnson, J.A. et al. Clinical pharmacogenetics implementation consortium guidelines for CYP2C9 and VKORC1 genotypes and warfarin dosing. Clin. Pharmacol. Ther. 90, 625–629 (2011).
13. Cavallari, L.H. et al. Genetic and clinical predictors of warfarin dose requirements in African Americans. Clin. Pharmacol. Ther. 87, 459–464 (2010).
14. Danese, E. et al. Effect of CYP4F2, VKORC1, and CYP2C9 in influencing coumarin dose: a single-patient data meta-analysis in more than 15,000 individuals. Clin. Pharmacol. Ther. 105, 1477–1491 (2019).
15. Cha, P.C. et al. Genome-wide association study identifies genetic determinants of warfarin responsiveness for Japanese. Hum. Mol. Genet. 19, 4735–4744 (2010).
16. Perera, M.A. et al. Genetic variants associated with warfarin dose in African-American individuals: a genome-wide association study. Lancet 382, 790–796 (2013).
17. Cooper, G.M. et al. A genome-wide scan for common genetic variants with a large influence on warfarin maintenance dose. Blood 112, 1022–1027 (2008).
18. Rodriguez, C.J. et al. Status of cardiovascular disease and stroke in Hispanics/ Latinos in the United States: a science advisory from the American Heart Association. Circulation 130, 593–625 (2014).
19. Bress, A. et al. Effect of NG01 and CYP4F2 genotypes on warfarin dose requirements in Hispanics-American and African-Americans. Pharmacogenomics 13, 1925–1935 (2012).
20. Momary, K.M. et al. Factors influencing warfarin dose requirements in African-Americans. Pharmacogenomics 8, 1535–1544 (2007).
21. Manta, F.S. et al. Analysis of genetic ancestry in the admixed Brazilian population from Rio de Janeiro using 46 autosomal ancestry-informative indel markers. Ann. Hum. Biol. 40, 84–98 (2013).
22. Luo, Z. et al. Identification of novel variants associated with warfarin stable dosage by use of a two-stage extreme phenotype strategy. J. Thromb. Haemost. 15, 28–37 (2017).
23. Salum de Neves Manta, F. et al. Revisiting the genetic ancestry of Brazilians using autosomal AIM-Indels. PLoS One 8, e75145 (2013).
24. Ramos, A.S. Development of a pharmacogenetic-guided warfarin dosing algorithm for Puerto Rican patients. Pharmacogenomics 13, 1937–1950 (2012).
25. Botton, M.R., Bandinelli, E., Rohde, L.E., Amon, L.C. & Hutz, M.H. Influence of genetic, biological and pharmacological factors on warfarin dose in a Southern Brazilian population of European ancestry. Br. J. Clin. Pharmacol. 72, 442–450 (2011).
26. Giri, V.N. et al. Race, genetic West African ancestry, and prostate cancer prediction by prostate-specific antigen in prospectively screened high-risk men. Cancer Prev. Res. (Phila). 2, 244–250 (2009).
27. Tian, C. et al. A genomewide single-nucleotide-polymorphism panel with high ancestry information for African American admixture mapping. Am. J. Hum. Genet. 79, 640–649 (2006).
28. Claudio-Campos, K. et al. Warfarin anticoagulation therapy in Caribbean Hispanics of Puerto Rico: a candidate gene association study. Front. Pharmacol. 8, 347 (2017).
29. Klein, T.E. et al. Estimation of the warfarin dose with clinical and pharmacogenetic data. N. Engl. J. Med. 360, 753–764 (2009).
30. Vespa, J., Armstrong, D.M. & Medina, L. Demographic turning points for the United States: population projections for 2020 to 2060. <https://www.census.gov/centerd/Census/library/publications/2018/demo/P25_1144.pdf>. March 20, 2020.
31. Linds, N.A. et al. Warfarin pharmacogenetics: a single VKORC1 polymorphism is predictive of dose across 3 racial groups. Blood 115, 3827–3834 (2010).
32. Suarez-Kurtz, G., Paula, D.P. & Struchiner, C.J. Pharmacogenomic implications of population admixture: Brazil as a model case. Pharmacogenomics 15, 209–219 (2014).
33. Perini, J.A., Struchiner, C.J., Silva-Assuncao, E. & Suarez-Kurtz, G. Impact of CYP4F2 rs2108622 on the stable warfarin dose in an admixed patient cohort. Clin. Pharmacol. Ther. 87, 417–420 (2010).
34. Bernardes-Pereira, S. et al. Genomic ancestry as a predictor of haemodynamic profile in heart failure. Open Heart 3, e000434 (2016).
35. de Visser, M.C. et al. Haplotypes of VKORC1, NQ01 and GGCX, their effect on activity levels of vitamin K-dependent coagulation factors, and the risk of venous thrombosis. Thromb. Haemost. 106, 583–585 (2011).
36. Ingrain, B.O., Turbyfill, J.L., Bledsoe, P.J., Jaiswal, A.K. & Stafford, D.W. Assessment of the contribution of NAD(P)H-dependent quinone oxidoreductase 1 (NQ01) to the reduction of vitamin K in wild-type and NQ01-deficient mice. Biochem. J. 456, 47–54 (2013).
37. Traver, R.D. et al. Characterization of a polymorphism in NAD(P)H: quinone oxidoreductase (DT-diaphorase). Br. J. Cancer. 75, 69–75 (1997).
38. Siegel, D., McGuinness, S.M., Winski, S.L. & Ross, D. Genotype-phenotype relationships in studies of a polymorphism in NAD(P)H: quinone oxidoreductase 1. Pharmacogenetics 9, 113–121 (1999).
39. Shyu, H.Y. et al. Genotype polymorphisms of GGCX, NQ01, and VKORC1 genes associated with risk susceptibility in patients with large-artery atherosclerotic stroke. Clin. Chim. Acta. 411, 840–845 (2010).
40. Wadellius, M. et al. Association of warfarin dose with genes involved in its action and metabolism. Hum. Genet. 121, 23–34 (2007).
41. Chung, J.E., Chang, B.C., Lee, K.E., Kim, J.H. & Gwak, H.S. Effects of NAD(P)H quinone oxidoreductase 1 polymorphisms on stable warfarin doses in Korean patients with mechanical cardiac valves. Eur. J. Clin. Pharmacol. 71, 1229–1236 (2015).
42. Li, J. et al. Impact of VKORC1, CYP4F2 and NQ01 gene variants on warfarin dose requirement in Han Chinese patients with catheter ablation for atrial fibrillation. BMC Cardiovasc. Disord. 18, 96 (2018).
43. Starkovskaya, Y.B., Sukernik, R.I., Schurr, T.G., Kogelnik, A.M. & Wallace, D.C. mtDNA diversity in Chukchi and Siberian Eskimos: implications for the genetic history of Ancient Beringia and the peopling of the New World. Am. J. Hum. Genet. 63, 1473–1491 (1998).
44. Ward, L.D. & Kellis, M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res. 40, D930–D934 (2012).
45. Consortium, G.T. The genotype-tissue expression (GTEx) project. Nat. Genet. 45, 580–585 (2013).
46. GTEx Consortium. Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multisissue gene regulation in humans. Science. 348, 648–660 (2015).
47. Gage, B.F. et al. Use of pharmacogenetic and clinical factors to predict the therapeutic dose of warfarin. Clin. Pharmacol. Ther. 84, 326–331 (2008).
48. Duconge, J. et al. A novel admixture-based pharmacogenetic approach to refine warfarin dosing in Caribbean Hispanics. PLoS One 11, e0145480 (2016).

© 2020 The Authors. Clinical and Translational Science published by Wiley Periodicals LLC on behalf of the American Society of Clinical Pharmacology and Therapeutics. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.