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Prediction of Warfarin Dose Reductions in Puerto Rican Patients, Based on Combinatorial *CYP2C9* and *VKORC1* Genotypes

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Abstract

BACKGROUND—The influence of *CYP2C9* and *VKORC1* polymorphisms on warfarin dose has been investigated in white, Asian, and African American populations but not in Puerto Rican Hispanic patients.

OBJECTIVE—To test the associations between genotypes, international normalized ratio (INR) measurements, and warfarin dosing and gauge the impact of these polymorphisms on warfarin dose, using a published algorithm.

METHODS—A retrospective warfarin pharmacogenetic association study in 106 Puerto Rican patients was performed. DNA samples from patients were assayed for 12 variants in both *CYP2C9* and *VKORC1* loci by HILOmet PhyzioType assay. Demographic and clinical nongenetic data were retrospectively collected from medical records. Allele and genotype frequencies were determined and Hardy-Weinberg equilibrium (HWE) was tested.

RESULTS—Sixty-nine percent of patients were carriers of at least one polymorphism in either the *CYP2C9* or the *VKORC1* gene. Double, triple, and quadruple carriers accounted for 22%, 5%, and 1%, respectively. No significant departure from HWE was found. Among patients with a given *CYP2C9* genotype, warfarin dose requirements declined from GG to AA haplotypes; whereas, within each *VKORC1* haplotype, the dose decreased as the number of *CYP2C9* variants increased. The presence of these loss-of-function alleles was associated with more out-of-range INR measurements (OR = 1.38) but not with significant INR >4 during the initiation phase. Analyses based on a published pharmacogenetic algorithm predicted dose reductions of up to 4.9 mg/day in carriers and provided better dose prediction in an extreme subgroup of highly sensitive patients, but also suggested the need to improve predictability by developing a customized model for use in Puerto Rican patients.

CONCLUSIONS—This study laid important groundwork for supporting a prospective pharmacogenetic trial in Puerto Ricans to detect the benefits of incorporating relevant genomic information into a customized DNA-guided warfarin dosing algorithm.

Keywords

CYP2C9; genotyping; pharmacogenomics; *VKORC1*; warfarin

Warfarin is considered to be the standard-of-care therapy for many thromboembolic disorders.^{1,2} In 2010, there were more than 32 million warfarin prescriptions in the US.³ Warfarin is frequently associated with unpredictable pharmacologic responses, ranging from occult bleeding to hemorrhage, due in part to its narrow therapeutic index.⁴ Therefore, its action is closely monitored by means of frequent blood testing for the international normalized ratio (INR) determination, and dosage adjustments are often necessary.^{2,5,6}

The difference in successful outcomes during warfarin therapy is a multifactorial issue.^{7–9} Apart from the clinical and environmental variables, the individual's unique genetic make-up plays a fundamental role in the warfarin response.^{10,11} In January 2010, the Food and Drug Administration revised the warfarin label to include dosing recommendations based on genetic polymorphisms in genome-wide association studies and identified *CYP2C9* and vitamin K epoxide reductase subunit C1 (*VKORC1*) as candidate genes.^b *CYP2C9* encodes the cytochrome P450, subfamily IIC, polypeptide 9, an enzyme responsible for metabolizing

the more active *S*-enantiomer of warfarin.^{12–15} Approximately 25–35% of the general population has a *CYP2C9* variant that leads to deficient enzyme activity,^{16–20} resulting in warfarin accumulation, hemorrhagic complications,²¹ alterations in initial dose sensitivities, and delays in achieving stable maintenance doses.^{11,17,19,22–30} The *CYP2C9* status alone accounts for approximately 15–20% of the variance in warfarin dose.^{11,20,25,30,31}

Warfarin exerts its anticoagulant effect through inhibition of the *VKORC1* gene product.^{11,30,32–34} Carriers of a common polymorphism in the *VKORC1* promoter sequence (–1639 G>A) may require a lower warfarin maintenance dosage.^{35,36} The –1639 G>A genotype and related haplotype can independently determine 20–25% of warfarin dose variance.^{29,36} Together, the *CYP2C9* and *VKO-RC1* combinatorial genotypes account for up to 45% of warfarin dose variability.^{11,22,24,30,31,36}

Nongenetic warfarin dosing algorithms may incorporate clinical factors such as body size and age but they rely on trial-and-error to achieve therapeutic dose, after an initial warfarin dose in the range of 2–10 mg.⁴ Clinicians can now estimate the therapeutic dose a priori by genotyping their patients for single nucleotide polymorphisms (SNPs) that affect warfarin metabolism or sensitivity, theoretically reducing the time needed to achieve target dose and reducing the risk of adverse events.^{11,30}

The primary objective of this retrospective study was to predict daily warfarin dose reduction in a Puerto Rican cohort of patients treated with warfarin by using their combinatorial *CYP2C9* and *VKORC1* genotypes. Secondary objectives included an assessment of the impact of *CYP2C9* and *VKO-RC1* genotypes on time to dose stabilization and number of out-of-range INRs. Finally, a comparison of dose predictability between a published pharmacogenetic-guided model and both the clinical algorithm and fixed-dose approach was performed, using collected data from our study cohort.

Methods

STUDY COHORT

A total of 106 warfarin-treated, stable patients from the Veterans Affairs Caribbean Healthcare System (VACHS)–affiliated anticoagulation clinic at San Juan, Puerto Rico, were recruited, and they provided written consent as required by the institutional review board. Stable warfarin dose is the steady-state dose that leads to stable anticoagulation levels, defined as 3 consecutive clinic visits for which INR measurements are within therapeutic range for the same mean daily dose.³⁷ Finally, the therapeutic range was set at 2–3 or 2.5–3.5, according to indication for warfarin use. Patients were unrelated Hispanic Puerto Ricans whose parents were also born in Puerto Rico. The patients were receiving warfarin for diverse thromboembolic disorders.

Demographic data such as age, sex, height, weight, and clinical nongenetic data (eg, stable warfarin daily dose, INR measurements from the beginning of warfarin therapy until patient stabilization, target INR range, most recent actual INR value, primary indication for warfarin therapy, smoking status, concurrent medications, refill histories, concomitant diseases) were obtained retrospectively from the computerized patient record system (CPRS).

^bA glossary of genetic terminology is maintained by the National Human Genome Research Institute at www.genome.gov/glossary.cfm.

LABORATORY ANALYSIS

Five milliliters of EDTA-treated blood samples were obtained from each patient at the time of routine INR testing. Genomic DNA sample was extracted and purified from whole fresh blood using QIAamp DNA Blood Midi Kit (QIAGEN Inc., Valencia, CA) following the manufacturer's protocol. Extracted DNA was stored at -80°C in TRIS-EDTA (TE) buffer. Quantification of DNA was performed by fluorescent staining of double-stranded DNA (PicoGreen dsDNA Quantitation Kit, Molecular Probes, Eugene, OR). Fluorescent intensity was measured using a fluorescent micro-titer plate reader (BMG Labtech, Fluo-Star Optima, Germany).

Genotyping of the *CYP2C9* and *VKORC1* genes at 12 variable sites, 5 SNPs in *CYP2C9* and 7 SNPs in *VKORC1* (Table 1), was performed at Genomas (Clinical Laboratory Improvement Amendments–certified Laboratory of Personalized Health, Hartford, CT). The Tag-It Mutation Detection assays (Luminex Molecular Diagnostics, Austin, TX) were used for genotyping, following the HILOmet PhyzioType system. A full explanation of this assay can be found elsewhere.^{38,39}

STATISTICAL ANALYSIS

A cross-sectional analysis, retrospective association study was conducted. Allelic distribution frequencies were determined in this Puerto Rican population of warfarin-treated patients for the loci of interest. Departure from Hardy-Weinberg equilibrium (HWE) was estimated under the null hypothesis of the predictable segregation ratio of specific matching genotypes ($p > 0.05$) by use of χ^2 goodness-of-fit test with 1 degree of freedom. Statistical calculation indicated 80% power to detect SNPs with allele frequencies of 0.1–0.4 in the study sample, accounting for 10% of phenotypic variation at less than 5% of significance level.

To explore the study population–wide impact of polymorphisms on warfarin dose, we classified patients by their combinatorial *VKORC1* and *CYP2C9* genotypes. The expected reduction in dose for each combination was calculated by using a DNA-guided equation published by the International Warfarin Pharmacogenetics Consortium (IWPC).³⁷ Effective daily warfarin dose was estimated for each combinatorial genotype, using the IWPC-derived equation,³⁷ by substituting for average demographic characteristics of recruited patients. Mean (SD) predicted warfarin daily dose was computed for each combinatorial genotype observed in the study cohort as well as wild types (reference genotypes). The reduction in warfarin daily dose for each combinatorial genotype was then calculated as the difference between the mean dose predicted for each particular combinatorial genotype and the mean dose predicted for the wild-type genotypes.

Using data from the study cohort, we also compared dose predictions from the IWPC-derived pharmacogenetic model with those from 2 other models (ie, a clinical model that did not include genetic factors and the fixed dose of 5 mg/day of warfarin). The mean absolute error, defined as the mean of the absolute values for the difference between the predicted and actual effective daily warfarin doses, and the coefficient of determination (R^2) were used to evaluate each model's predictive accuracy. We evaluated the potential clinical value of each algorithm, following the same approach described by others,³⁷ calculating the percentage of patients whose predicted effective warfarin dose was within 20% of the actual effective dose. In addition, we calculated the percentage of patients for whom the predicted dose according to each algorithm was at least 20% higher than the actual dose (overestimation) or at least 20% lower than the actual dose (underestimation). These values represent a difference of 1 mg/day relative to the traditional starting dose of 5 mg daily, a difference that clinicians usually accept as clinically relevant. We also assessed the

performance of the algorithms in 3 dose groups: participants requiring a low dose (< 3 mg/day), those requiring a high dose (> 7 mg/day), and those requiring intermediate doses (> 3 and < 7 mg/day) for effective anticoagulation. Patients requiring doses of less than 3 mg/day would be at risk for excessive anticoagulation if they were started on the standard dose of 5 mg/day. Conversely, patients requiring doses of more than 7 mg/day would be at risk for inadequate anticoagulation if treatment were started with 5 mg/day. The McNemar test of paired proportions was used for comparison of the pharmacogenetic algorithm with the clinical algorithm and the fixed-dose approach.

Nonparametric 2-tailed, unpaired Mann-Whitney *U*-Wilcoxon rank-sum test for warfarin dose, time-to-target INR, age, height, and weight and Fisher exact test for comedications, smoking status, and primary indications were used to compare genotype groups ($p < 0.05$). Odds ratios (OR, 95% CI) adjusted by warfarin dose (milligrams per day) were estimated to show association between the *CYP2C9* and *VKORC1* genotypes and the estimates of the out-of-range INR ratio above 0.5 in Puerto Rican patients treated with warfarin at VACHS–San Juan, Puerto Rico. The out-of-range INR ratio is equal to the sum of the number of INRs above range plus the number of INRs below range divided by the total INR determinations during the initiation of therapy (ie, from initiation of warfarin therapy until achievement of therapeutic dose). An out-of-range INR ratio of 0.5 means that 50% of INR determinations are out-of-range during the initiation of therapy. Range is the INR therapeutic range as defined above.

Our ability to detect a 1-mg/day difference in dose due to genotypes, assuming a standard deviation of 2.2 mg/day, required a minimum of 30 subjects per group (ie, carriers vs noncarriers) at 5% of significance and 80% power.

Results

Mean age of the participants was 65 years (range, 44–86), and 99% were men. Three patients were excluded from further analyses because of poor genotyping call rate, which left the sample size at 103. In addition, 6 individuals were excluded from some assessments because of the lack of complete clinical data from CPRS ($N = 97$).

GENOTYPE DISTRIBUTION IN PUERTO RICANS

Table 2 summarizes patient characteristics by presence of 1 or more *CYP2C9* and *VKORC1* variant alleles (carriers) and wild-type (noncarrier) status. Notably, no significant differences between carriers and noncarriers with regard to clinical nongenetic and demographic variables were found, except for the daily warfarin dose ($p < 0.001$). Consequently, statistical analysis showed that these factors do not confound the genetic influence of the *CYP2C9* and *VKORC1* polymorphisms. Table 3 presents the alleles, genotypes, and haplotype frequencies for the *CYP2C9* and *VKORC1* markers. The frequency of the *CYP2C9**2, *3, and *5 alleles was 19.1%; 5.4%, and 1%, respectively. The *VKORC1*-1639A promoter allele was detected with a 41% frequency.

The carrier prevalence calculated in this study sample, combining polymorphisms in both genes, was 68.9%, whereas noncarriers accounted for 31.1% of the study population. The percentage of subjects with polymorphisms in only a single gene was 41.7%. Thirty-two patients were identified with a single polymorphism in *CYP2C9* (*1/*2, *1/*3, or *1/*5), and 54 presented the *VKORC1*-1639 GA haplotype. Double carrier patients with either a single polymorphism in both *CYP2C9* and *VKORC1* genes or with 2 allelic variants in 1 of them accounted for 21.4% of the study population. Among them were 9 patients who carried a single *CYP2C9* and single *VKORC1* polymorphism (eight *1/*2/GA, one *1/*3/GA). Triple-carrier patients with a single polymorphism in *CYP2C9* and double in *VKORC1* (one

*1*2/AA), or double polymorphism in *CYP2C9* and single in *VKORC1* (one *2*2/GA, three *2*3/GA), accounted for 4.9%. Only 1 quadruple carrier with double polymorphisms in both genes was detected (ie, *2*2/AA, ~1%, n = 103). No significant departure from HWE was found in this study ($\chi^2 < 3.84$, Table 3). Notably, the observed frequencies of least common *VKORC1*-1639 AA and *CYP2C9* allelic variants in our study population were higher than expected (15 vs 10 and 10 vs 4.27, respectively) for perfect HWE, under a model of ascertainment of 1 group.

WARFARIN DOSING PER GENOTYPE

Median actual effective doses of warfarin were compared by genotypes in a subset of 97 patients with complete clinical and genotyping data. Patients were grouped according to *CYP2C9* and *VKORC1* carrier status as wild-types or noncarriers, single, double, triple, and quadruple carriers. Significant differences were observed between carriers and noncarriers ($p < 0.001$, Table 2) and across carrier groups (Figure 1), with a clear decline in the median warfarin dose by increasing number of nonfunctional alleles (wild-type group 5.71 mg/day, single carriers 4.64 mg/daily, double carriers 3.43 mg/day, and 2.36 mg/day and 1.86 mg/day for triple and quadruple carriers, respectively).

Figure 1 depicts warfarin daily dose in 97 patients according to *CYP2C9* and *VKORC1*-1639 G>A polymorphisms. The *CYP2C9* genotypes were grouped as wild-type, 1 and 2 variant alleles according to the number of variant alleles (*2, *3, and *5) present. Average effective warfarin dose was stratified based on *CYP2C9* and *VKORC1* carrier status. Two distinct trends emerged. First, among Puerto Rican patients with a given *CYP2C9* genotype, the daily warfarin dose requirements declined from the GG to the GA and AA genotypes. Second, within each *VKORC1*-1639 G>A genotype, the warfarin dose decreased as the number of variant *CYP2C9* alleles increased. There was substantial overlap in the warfarin dose requirement across the combined genotype groups. Statistical analysis predicted that the effective warfarin daily dose in our population is dependent on genotypes.

No significant difference for the time to target INR between both groups was found (Table 2, $p = 0.34$). The presence of a variant allele in *CYP2C9* and/or *VKORC1* was associated with slightly higher odds for more than 50% out-of-range INR measurements during the initial phase of warfarin therapy. However, its detrimental impact was not significant among recruited patients after adjustment by warfarin daily dose (adjusted OR 1.38; 95% CI 0.43 to 4.39, $p = 0.24$). Figure 2 depicts the projected effect of *CYP2C9* and *VKORC1* combinatorial genotypes on warfarin dose reduction, using an early published DNA-driven equation by the IWPC.³⁷ The estimation procedure was based on combinatorial *CYP2C9* and *VKORC1* genotypes observed in the study population, average age (66.5 years), body size (85.9 kg), height (171 cm), mixed race, and comedications used, according to the clinical data collected from the CPRS. Patients who were carriers of a single polymorphism would require a dose decrease of 1.57 mg/day relative to a predicted wild-type's mean dose of 6 mg/day. Double carriers need a dose reduction in the range of 1.86–3.0 mg/day. Triple carriers may require a decrease in dose between 3.0 and 3.86 mg/day, whereas quadruple carriers require a dose reduction of 4.0–4.86 mg/day.

The performance of the pharmacogenetic, clinical, and fixed-dose models in the study cohort is shown in Tables 4 and 5. In the low-dose subgroup (ie, highly sensitive patients at risk of serious bleeding episodes due to excessive anticoagulation), the pharmacogenetic algorithm provided dose estimates that were more accurate and thus significantly closer to the actual doses required than the estimates derived from the clinical algorithm or the fixed-dose approach, as evidenced by a mean absolute error that was lower than that for both the clinical algorithm and the fixed-dose approach (0.57 mg/day; 95% CI 0.37 to 0.77 vs 1.79 mg/day; 95% CI 1.45 to 2.13 and 3.51 mg/day; 95% CI 3.27 to 3.75, respectively; $p < 0.001$

for both comparisons). These data show how the addition of genetic information alters the dose prediction from the clinical algorithm and suggest that a significant percentage of difference in dose requirements is explained by genotype within this subgroup of individuals (Table 4). Indeed, the addition of genetic information to clinical information decreased the absolute error in the dose estimate of this subgroup and increased the fraction of variability explained (R^2 ; from 3.62% to 45.3%). The differences in the performance of the 3 approaches in the intermediate dose (>3 and <7 mg/day) and high dose (≥ 7 mg/day) groups are also shown. No significant differences were observed in the intermediate range, although a marginal difference was found between the pharmacogenetic and the clinical algorithms in the high-dose subgroup ($p = 0.042$).

For patients whose warfarin dose requirement was 3 mg/day or less (~20% of the total cohort), the pharmacogenetic algorithm provided a significantly better prediction of dose than did the clinical algorithm or the fixed-dose approach; 53% of the dose predictions fell within 20% of the actual (ie, ideal) dose with the pharmacogenetic algorithm compared with 6% with the clinical algorithm ($p < 0.001$) and 0% with the fixed-dose approach ($p < 0.001$). In addition, the pharmacogenetic algorithm provided significantly fewer overestimations of dose in the low-dose group (12% vs 94% with the clinical algorithm; $p < 0.001$, and 100% with the fixed-dose approach; $p < 0.001$) (Table 5). However, for patients requiring 7 mg or more per day (~12% of the total cohort), both the pharmacogenetic and the clinical algorithms underestimated the actual effective dose of warfarin in our study cohort (both 100%). In the intermediate-dose group, the accuracy of the dose prediction was similar between the fixed-dose approach and the pharmacogenetic algorithm but poorer for the latter with respect to the clinical approach. The analysis highlights the fact that this IPWC-derived pharmacogenetic algorithm provided consistently better dose prediction in an extreme subgroup of highly sensitive patients, but also suggests the need to improve predictability by developing a customized model for the Puerto Rican population.

Overall, our study provides evidence on significant associations between genotypes and effective warfarin dosing in Puerto Rican patients, but not for the time to target INR or the number of out-of-range INRs during the initial phase of warfarin therapy. The published pharmacogenetic algorithm yields stepwise dose reductions dependent on the number of polymorphisms carried by the patient.

Discussion

Findings in this study confirm the extensive interindividual variability of effective warfarin dose required to maintain the INR within the target range for prevention of different thromboembolic disorders. We evaluated the *CYP2C9* and *VKORC1* gene SNPs in view of previous evidence that polymorphisms on these 2 genes are independent predictors of warfarin dose in multistep linear regression models.^{8,11,22,24,25,30,37} The study demonstrated a pharmacogenetic association between effective warfarin dose and combinatorial *VKORC1* and *CYP2C9* genotypes. To our knowledge, this is the first time that such an association has been described in Puerto Rican patients.

There has been uncertainty about frequencies of minor alleles expected in the Puerto Rican population because of a diverse mixture of Amerindian, European, and African ancestries. A few reports have included a limited number of Puerto Ricans as part of single Hispanic clusters,^{37,39–41} but ignore stratification and difference in SNP distributions within. We previously found that 60% of subjects in a pilot study with 92 nontreated Puerto Rican neonates were carriers of polymorphisms in either *CYP2C9* or *VKORC1*, predicting deficient warfarin metabolism or responsiveness.⁴² Notably, one sample carried the rare allele *CYP2C9**6 (818delA, decreased activity) that is preferentially found in African

descendants. This study extends those findings to a different population in a clinical setting. We found that 69% (~7 of 10) of patients were carriers of at least 1 functional polymorphism in either of the 2 warfarin-relevant genes.

The observed allele frequencies in this study (Table 3) were similar to those reported in SNP databases such as the International HapMap Project and studies analyzing populations of mostly white ancestry. The observed frequencies of common, functionally deficient *CYP2C9**2, *3, and *VKORC1*-1639 G>A polymorphisms were 18.9% (resembling whites, 10–20%^{43–47}), 5.3% (resembling whites, 5–10%^{43–47}), and 41% (resembling whites, 37.5–45%^{22,24,25,35,44,45}), respectively. Current literature suggests significant differences in prevalence of *CYP2C9* and *VKORC1* polymorphisms between and within ethnic groups.^{25,35,43–49} Regarding *VKORC1* haplotypes, these ethnic differences contribute to lower warfarin doses in Asians and Mexicans,^{50,51} whereas African Americans have a high incidence of rare haplotypes.^{25–52}

The uniqueness of the frequency distribution of *VKORC1* haplotypes in the Amerindians compared with other populations has been previously discussed by Perini et al.⁵³ Whites have the highest frequency of the *2 and *3 alleles, ranging from 10–20% and 5–10%, respectively. Africans have a very low *2 and *3 allele frequency, about 1% each.^{17,21,22,25,31,54} Accordingly, our study has contributed to filling a gap in existing knowledge of frequencies for *CYP2C9* and *VKORC1* polymorphisms in the admixed Puerto Rican population.

Based on existing algorithms, Puerto Rican patients with 1 or 2 of these SNPs are expected to have reduced warfarin metabolism, lower initial dose requirements, a longer time to dose stabilization, and a 2- to 3-fold elevated risk of an adverse event when beginning warfarin.^{11,21,23,30,52,55–57} According to our findings, *VKORC1*-1639 G>A genotype proved to be the strongest predictor of effective warfarin daily dose, and thus needs to be considered in the further development of a Puerto Rican customized, pharmacogenetic-driven, multivariate warfarin dosing model. This conclusion may be extended to the *CYP2C9* genotype-specific differences, because the effective warfarin daily dose requirement in our patient cohort decreased significantly with the increase in the number of variant *CYP2C9* nonfunctional alleles (Figure 1).

Combinatorial genotypes yield valuable information for potential DNA-guided adjustments in warfarin dose in Puerto Rican patients. Individuals with the greatest number of deficient polymorphisms will benefit most from this information (Figure 2). The predicted warfarin dose reduction ranged from 1.6 mg/day to 4.9 mg/day for patients having the observed combinatorial genotypes. This finding confirms that a pharmacogenetic approach can be recommended for reducing warfarin-induced adverse events in Puerto Ricans. Currently, we are using a clinical protocol to develop a genomic-based warfarin dosing algorithm for this population.

Data analysis results shown in Tables 4 and 5 suggest that the IWPC pharmacogenetic-guided algorithm provides better predictions of the optimal dose of warfarin than do either the clinical or a fixed-dose approach among patients in the study cohort whose stable therapeutic warfarin doses were 3 mg or less per day, but not among those whose stable doses were 7 mg or more per day. The former are patients for whom an overdose could have adverse clinical consequences such as life-threatening bleeds and intracranial hemorrhage. In this VACHS cohort, patients who require intermediate doses are likely to obtain little benefit from use of the IWPC-derived pharmacogenetic algorithm. However, these results do not address the issue of whether a precise initial dose of warfarin translates into improved clinical endpoints, such as reduced time to achieve a stable therapeutic INR, fewer out-of-

range INRs, and reduced incidence of bleeding or thromboembolic events. The IWPC pharmacogenetic algorithm performed less predictably among patients who required high doses of warfarin (>7 mg/day), because the common genetic markers included in the IWPC pharmacogenetic model primarily explain increased sensitivity to warfarin, not increased resistance. Some polymorphisms of *VKORC1* have been associated with resistance to warfarin, but these variants are rare in the majority of populations and, therefore, not usually considered as independent predictors of dose variability.

In general, published pharmacogenetic-guided warfarin dosing algorithms, such as the one developed by the IWPC, have performed poorly in predicting the actual effective warfarin dose among Puerto Rican patients, which explains the less than 36.3% of total variation in warfarin dose (absolute error of 7.9 mg/wk; 25% mean bias). This may be attributed to greater genetic diversity in the admixed Puerto Rican population and the possible influence of additional genes or other functional variants within the *VKORC1* and *CYP2C9* loci. A genomic-based algorithm can eventually improve model predictability in patients, particularly those who require high doses of warfarin.

To date, most of these algorithms are based on studies carried out in people of white ancestry. However, the rich genetic repertoire found in the Puerto Rican population is likely to contribute substantially to variations in warfarin dose requirements, a component likely to be missed by traditional studies in more homogeneous populations. This addressable oversight is of great concern, since it will tend to exacerbate the health care disparity already experienced by Hispanic populations. Therefore, it is important to provide information on samples from other geographic regions, preferably from highly heterogeneous groups such as Puerto Ricans, to better understand the potential impact of such polymorphisms at the population level.

Accordingly, our data have important implications in terms of the application of universally useful warfarin dosing algorithms, because differences in allele frequency distribution between groups of individuals of diverse ancestry can have an impact on the amount of variance in required warfarin dosing explained by the algorithms.

In addition to providing the first report to our knowledge on the frequency distribution of *CYP2C9* and *VKORC1* polymorphisms in the heterogeneous warfarin-treated Puerto Rican patient population in Puerto Rico, our study has other significant strengths. First, we had access to complete information on all drugs being prescribed and dispensed, since the participants were provided with their medications at the study site. Such information could be relevant to identifying potential drug-warfarin interactions or for future clinical and epidemiologic studies in this population. Second, this study reflects real-life prescribing and dispensing of warfarin in the context of a health care facility in a Latino/Hispanic territory. Third, the recruitment of warfarin-treated patients from a highly mixed population⁵⁸ provided evidence to support the notion that, in such populations, self-reported race/skin color categorization is not a reliable predictor of drug dosing (or drug choice) in the context of pharmacogenetic-informed clinical practice.

We recognize that this study has some limitations, most of which have been described in previous studies with a similar design. First, given that our study was retrospective, the associations described must be prospectively tested in an appropriately large cohort. Second, although the clinical nongenetic covariates (eg, time to target INR, effective warfarin daily dose, comedications) were readily available in the patient's medical record, the genetic marker covariates, namely the *CYP2C9* and *VKORC1*-1639 G>A genotypes, cannot be determined in a time frame compatible with the urgency of starting warfarin administration to many patients. Third, almost all patients enrolled in this study were elderly males from 1

medical facility; therefore, caution should be observed when trying to extrapolate our findings to other groups in this population. Finally, we did not address the impact of the covariates on the clinical response to warfarin, but instead used the INR as a surrogate for it. Still, limited sample size may preclude the estimation of statistically significant odds ratios, given the high interindividual variability in warfarin response at clinical settings.

Our work provides new evidence of significant prevalence of polymorphisms in warfarin-related genes within the Puerto Rican population and puts emphasis on the need to genotype high-risk warfarin-treated Puerto Rican patients before the induction phase of anticoagulation therapy. In so doing, we might be better able to understand dose variability in Puerto Rican patients so that anticoagulation therapy can be validated in the context of their unique genetic make-up.

This study lays important groundwork for supporting a prospective pharmacogenetic trial in the Puerto Rican population to detect the benefits of incorporating relevant genomic information into a customized DNA-guided warfarin dosing algorithm, particularly for patients who require low daily doses—the subgroup in this study for whom dose estimates based on the published pharmacogenetic algorithm differed significantly from those based on merely clinical, nongenetic variables.

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Figure 1.

Box and whisker plots representing association of *CYP2C9* and *VKORC1*-1639 G>A polymorphisms (abscissa) with actual warfarin dose (ordinate) in 97 Puerto Rican patients at San Juan Veterans Affairs Caribbean Healthcare System. *CYP2C9* genotype is labeled as wild-type ($*1/*1$), 1 variant allele ($*1/*2$, $*1/*3$, or $*1/*5$), or 2 variant alleles ($*2/*2$, $*2/*3$, $*2/*5$). Within each *CYP2C9* genotype, the *VKORC1* genotype at -1639 G>A is shown as GG, GA, or AA. Individual values for each group are depicted as open circles. Median warfarin daily doses in each of the 9 subgroups by genotypes are represented by horizontal bars. Two-tailed p values correspond to nonparametric Mann-Whitney *U* (rank-sum) test results after comparing 2 different pairs of observed genotype groups.

Figure 2.

Predicted mean decreases in warfarin dose in the average patient (N = 97; aged 66.5 years, 171 cm, 85.9 kg), based on observed genotypes and clinical/demographic data. The filled areas indicate ranges of predicted dose reductions based on the published genetic algorithm.³⁷

Table 1

CYP2C9 and VKORC1 Variants^a

CYP2C9			VKORC1		
Allelic Variant	Change to Protein	Activity	Allelic Variant	Change to Protein	Activity
*1 (WT) ^b	Reference	Normal	WT ^b	Reference	Normal
*2 (430C>T)	Arg144 Cys	Decreased	−1639 G>A	Promoter	Deficient
*3 (1075A>C)	Ile359Leu	Null	+85 G>T	Val29Leu	Null
*4 (1076T>C)	Ile359Tyr	Decreased	+121 G>T	Ala41Ser	Null
*5 (1080C>G)	Asp360Glu	Decreased	+134 T>C	Val45Ala	Null
*6 (818delA)	Frameshift	Null	+172 A>G	Arg58Gly	Null
			+1331 G>A	Val66Met	Null
			+3487 T>G	Leu128Arg	Null

^aDetected with the HILO^{met} warfarin system on Luminex 100 xMap technology; effects on enzymatic activity are also depicted.

^bWild-types are assigned as a result of the absence of other single nucleotide polymorphisms.

Table 2Demographic and Clinical Characteristics of the Study Population^a

Characteristic	Noncarriers (n = 32)	Carriers (n = 71)	p Value ^b
Warfarin dose (mg/day)			
mean (SD)	6.01 (1.78)	4.14 (1.46)	<0.001
median (second quartile)	5.71	4.00	
interquartile range width	2.23	1.93	
Smoker, n (%)			
yes	6 (19)	10 (14)	0.99 (NS)
no	26 (81)	61 (86)	
Comedications, ^c n (%)			
yes	12 (38)	31 (44)	0.76 (NS)
no	20 (62)	40 (56)	
Primary indication, n (%)			
			0.60 (NS)
atrial fibrillation	24 (75)	51 (73)	
deep vein thrombosis	6 (19)	14 (20)	
pulmonary embolism	2 (6)	4 (6)	
cerebrovascular accident/stroke	5 (16)	15 (21)	
atrial valve replacement	4 (13)	5 (7)	
transient ischemic attack	1 (3)	6 (9)	
congestive heart failure	8 (25)	14 (20)	
bioprosthetic mitral valve replacement	1 (3)	3 (4)	
aortobifemoral bypass	0 (0)	1 (1)	
myocardial infarction	0 (0)	1 (1)	
aortic valve disease	1 (3)	0 (0)	
internal carotid artery stenosis	0 (0)	1 (1)	
Time-to-target INR (days)			
mean (SD)	244 (268)	203 (244)	0.34 (NS)
median (second quartile)	124	121	
interquartile range width	368	199	
Age (y)			
mean (SD)	67.6 (6.5)	66.9 (8.6)	0.83 (NS)
median (second quartile)	67.5	68.0	
interquartile range width	9.0	12.5	
Height (cm)			
mean (SD)	170.3 (6.2)	170.9 (6.5)	0.41 (NS)
median (second quartile)	170.2	170.2	
interquartile range width	6.3	7.6	
Weight (kg)			

Characteristic	Noncarriers (n = 32)	Carriers (n = 71)	p Value ^b
mean (SD)	84.69 (14)	84.15 (18.54)	0.97 (NS)
median (second quartile)	84.15	82.80	
interquartile range width	12.96	21.15	

INR = international normalized ratio; NS = not significant ($p = 0.05$).

^a N = 103; for warfarin daily dose and time to target INR, data were available for only 97 individuals.

^b p Values for the difference between carriers and noncarriers were calculated using the 2-tailed Mann-Whitney *U*-Wilcoxon rank-sum tests for warfarin dose, time-to-target INR, age, height, and weight and Fisher exact test for comedications, smoking status, and primary indications.

^c Comedications: amiodarone, statins (simvastatin, rosuvastatin, fluvastatin, and pravastatin), and azoles (fluconazole, itraconazole, ketoconazole, and trimethoprim/sulfamethoxazole).

Table 3Allele and Genotype Frequency Distributions of *CYP2C9* and *VKORC1*-1639 G>A Polymorphisms^a

Genotype/Allele	n (%); 95% CI	χ^2 Test ^b
<i>VKORC1</i> -1639 G>A		0.6 (p < 0.05)
GG	34 (33); 25 to 43	
GA	54 (52); 42 to 61	
AA	15 (14.7); 9 to 23	
G	122 (59); 52 to 66	
A	84 (41); 34 to 47	
<i>CYP2C9</i>		1.22 (p < 0.05)
*1/*1	61 (59); 49 to 68	
*1/*2	26 (25); 18 to 34	
*1/*3	5 (5); 1.8 to 11	
*1/*5	1 (1); 0.1 to 5	
*2/*2	3 (3); 0.6 to 8	
*2/*3	6 (6); 2.5 to 12.5	
*2/*5	1 (1); 0.1 to 5	
*1	154 (74.8); 68 to 80	
*2	39 (18.9); 14.3 to 25	
*3	11 (5.3); 2.9 to 9.5	
*5	2 (1); 0.04 to 3.7	

^aN = 103.

^bThe χ^2 value indicates the difference between expected and observed values for genotype counts; the likelihood of observing differences by chance is determined from a χ^2 table (1 degree of freedom) at 5% significance level; since the calculated χ^2 values are <3.84, the null hypothesis that the population is in Hardy-Weinberg equilibrium is not rejected.

Table 4Predicted Warfarin Daily Dose^a

Parameter	IWPC Algorithm	Clinical Algorithm	Fixed-Dose Approach ^b
Low dose (3 mg/day)			
MAE, mg/day (95% CI) ^c	0.57 (0.37 to 0.77)	1.79 (1.45 to 2.13)	3.51 (3.27 to 3.75)
R ² (%)	45.3	3.62	
p Value ^d		<0.001	<0.001
Intermediate dose (>3 and <7 mg/day)			
MAE, mg/day (95% CI) ^c	1.4 (1.2 to 1.7)	1.3 (1.1 to 1.6)	1.8 (1.6 to 2.1)
R ² (%)	15.8	1.18	
p Value ^d		NS	NS
High dose (7 mg/day)			
MAE, mg/day (95% CI) ^c	3.9 (3.2 to 4.6)	3.5 (2.7 to 4.2)	2.2 (1.5 to 2.8)
R ² (%)	12.6	~0	
p Value ^d		0.042	<0.001

IWPC = International Warfarin Pharmacogenetic Consortium; MAE = mean absolute error; NS = not significant; R² = coefficient of determination.

^aN = 97. To predict daily doses, the IWPC pharmacogenetic-guided algorithm, a clinical-based algorithm, and a fixed-dose approach were compared with actual doses of warfarin for therapeutic effect in patients requiring low, intermediate, or high exposure.

^bThe fixed dose was 5 mg/day.

^cThe 95% CIs on the estimates of MAE were calculated.

^dp Values for the pharmacogenetic algorithm, compared with the clinical and fixed-dose algorithms, were derived with the McNemar test of paired proportions.

Table 5Percentage of Patients^a with Ideal, Underestimated, or Overestimated Warfarin Dose

Prediction Model	Pts., n	Dose ^b		
		Ideal (%)	Underestimated (%)	Overestimated (%)
Low dose (3 mg/day)				
IWPC-derived algorithm	17	53	35	12
Clinical algorithm		6	0	94
Fixed-dose approach		0	0	100
Intermediate dose (>3 and <7 mg/day)				
IWPC-derived algorithm	69	34.83	63.72	1.45
Clinical algorithm		66.7	0	33.3
Fixed-dose approach		42	0	58
High dose (7 mg/day)				
IWPC-derived algorithm	11	0	100	0
Clinical algorithm		0	100	0
Fixed-dose approach		45.5	54.5	0

IWPC = International Warfarin Pharmacogenetic Consortium.

^aN = 97.

^bIdeal dose was defined as a predicted dose within 20% of the actual stable therapeutic dose, underestimated dose was defined as a predicted dose at least 20% lower than the actual dose, and overestimated dose was defined as a predicted dose at least 20% higher than the actual dose.