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Development of a pharmacogenetic-guided warfarin dosing algorithm for Puerto Rican patients

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Ethical conduct of research

The authors state that appropriate institutional review board (IRB) approvals from the Veteran Affairs Caribbean Healthcare System (VACHS), Hartford Hospital and UPR-MSC were granted and the principles outlined in the Declaration of Helsinki for human experimental procedures were followed at any time. In addition, written informed consents have been obtained from all the participants involved in this survey.

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Abstract

Aim—This study was aimed at developing a pharmacogenetic-driven warfarin-dosing algorithm in 163 admixed Puerto Rican patients on stable warfarin therapy.

Patients & methods—A multiple linear-regression analysis was performed using log-transformed effective warfarin dose as the dependent variable, and combining *CYP2C9* and *VKORC1* genotyping with other relevant nongenetic clinical and demographic factors as independent predictors.

Results—The model explained more than two-thirds of the observed variance in the warfarin dose among Puerto Ricans, and also produced significantly better ‘ideal dose’ estimates than two pharmacogenetic models and clinical algorithms published previously, with the greatest benefit seen in patients ultimately requiring <7 mg/day. We also assessed the clinical validity of the model using an independent validation cohort of 55 Puerto Rican patients from Hartford, CT, USA ($R^2 = 51\%$).

Conclusion—Our findings provide the basis for planning prospective pharmacogenetic studies to demonstrate the clinical utility of genotyping warfarin-treated Puerto Rican patients.

Keywords

algorithm; CYP2C9; genotyping; personalized medicine; pharmacogenetics; VKORC1; warfarin

Despite the advent of new oral anticoagulants (e.g., dabigatran, rivaroxaban and apixaban), warfarin therapy remains the mainstay for prevention of thromboembolic complications, which are life-threatening manifestations of cardiovascular disease. Cardiovascular diseases show a disproportionately high prevalence in US Hispanics, particularly in Puerto Ricans. Inherited factors may account for substantial variability in response to warfarin. Pharmacogenetic studies with warfarin have ascertained functional *CYP2C9* and *VKORC1* polymorphisms and numerous population-specific DNA-guided warfarin dosing algorithms

exist [1–6]; however, given their poor performance in Puerto Ricans [7], they need to be improved before being implemented in this population. No DNA-guided personalized medicine paradigm exists for Hispanic Puerto Ricans, a medically underserved population in need of better strategies to address healthcare disparities in cardiovascular and thromboembolic disorders.

In a recent publication, we found a significant association between stable warfarin daily dose and combinatorial *CYP2C9* and *VKORC1* genotypes in a cohort of Puerto Rican patients receiving warfarin therapy. We also predicted dose reductions of up to 4.9 mg/day in carriers and suggested the need to improve predictability by developing a customized model for use in Puerto Rican patients [7].

In the present study, we developed a pharmacogenetic-driven warfarin dosing algorithm in Puerto Ricans by combining *CYP2C9* and *VKORC1* genotypes with other relevant nongenetic clinical and demographic factors. Next, we prospectively validated this dosing algorithm in an independent cohort of Puerto Rican patients receiving warfarin therapy by comparison with two previously published pharmacogenetic algorithms developed in larger populations [2,8]. We also determined whether the dosage recommendations based on the pharmacogenetic algorithm developed in Puerto Ricans were significantly better than those predicted by two pharmacogenetic-guided algorithms published earlier [2,8], as well as a clinical nongenetic algorithm and the fixed-dose scheme.

Patients & methods

Study cohort

A total of 175 warfarin-treated stable patients from the Veterans Affairs Caribbean Healthcare System (VACHS)-affiliated anticoagulation clinic at San Juan, Puerto Rico, were recruited between January 2009 and July 2010, and provided written informed consent as approved by the VACHS institutional review board. Subjects were selected from the study population based on the warfarin initiation date (after 1997) at the VACHS anticoagulation clinic. Although varying from case to case, the majority of patients were initiated on warfarin therapy following induction regimens that rely on age-adjusted fixed-dose schemes. A stable patient on warfarin was defined as having at least three consecutive International Normalized Ratio (INR) measurements within the expected therapeutic range (2–3 or 2.5–3.5, according to indication for warfarin use) for the same average weekly dose [2,6].

Demographic data such as age, gender, height, weight and clinical nongenetic data (i.e., complete warfarin dosing information, INR measurements, target INR range, most recent actual INR value, smoking status, bleeding complications, primary indication for warfarin therapy, concurrent medications and comorbidities) were retrospectively obtained from the computerized patient record system (CPRS). Participants also completed a questionnaire about their vitamin K-rich beverage and food intake, town of origin and self-reported race (i.e., White, Black or Mestizo, according to the last US Census [in 2010], which relies on self-perception of skin color).

Laboratory analysis

A 5-ml ethylenediaminetetraacetic acid (EDTA)-treated blood sample was obtained from each patient at the time of routine INR testing. A genomic DNA sample was extracted and purified from whole fresh blood using QIAamp[®] DNA Blood Midi Kit (QIAGEN Inc., CA, USA) following the manufacturer's protocol. Extracted DNA was stored at –80°C in TRIS-EDTA (TE) buffer (Promega Co., WI, USA). Quantification of DNA was performed by fluorescent staining of dsDNA (PicoGreen[®] dsDNA Quantitation Kit, Molecular Probes,

OR, USA). Fluorescence intensity was measured using a fluorescent microtiter plate reader (FluoStar® Optima, BMG Labtech, Germany).

Genotyping the *CYP2C9* and *VKORC1* genes at 12 variable sites – five SNPs in *CYP2C9* and seven SNPs in *VKORC1* – was performed at Genomas, Inc. (Clinical Laboratory Improvement Amendments [CLIA]-certified Laboratory of Personalized Health, Genomas, CT, USA). The Tag-It™ Mutation Detection assays (Luminex Molecular Diagnostics, TX, USA) were utilized for genotyping, following the HILOmet PhyzioType™ System (Genomas Inc., CT, USA). A full explanation of this assay and a complete list of SNPs ascertained can be found elsewhere [9,10]. Departure from Hardy–Weinberg equilibrium 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 one 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 <5% significance level.

Multiple linear-regression model

We selected all patients with complete genetic and clinical data from the VACHS ($n = 163$) as the ‘derivation cohort’ for developing dose-prediction models. A sample of 55 patients from the Brownstone outpatient clinic in Hartford Hospital (Hartford, CT, USA) constituted the external ‘validation cohort’, which was used for testing validity of the pharmacogenetic model developed in Puerto Ricans (**Equation 1**; see page 1941), in comparison with two other early published DNA-guided warfarin dosing algorithms published earlier: a pharmacogenetic refinement algorithm developed by Lenzini and coworkers on the basis of INR values, clinical factors and genotypes [8], and the algorithm developed by the International Warfarin Pharmacogenomics Consortium (IWPC) in a larger multiethnic population [2]. Patients in the validation cohort were selected based on the same inclusion criteria as those in the derivation cohort. The investigators who performed the modeling and analysis did not have access to this validation set until after the final model was selected.

To select candidate variables for the multiple linear-regression model of warfarin dosing prediction in Puerto Ricans, we initially examined the independent effects of demographic (i.e., age, gender, race, height and weight), clinical (i.e., treatment indication, comorbidities, comedications, INR/dose at the third day and smoking status) and genetic (i.e., *VKORC1* -1639G>A genotype and number of variant *CYP2C9* alleles) variables individually. All discrete covariates entered the analysis transformed as dummy variables to quantify how the individual categories affected daily warfarin dose.

Log transformation was used to help stabilize the variance (i.e., limit heteroscedasticity) and improve model fit by removing residual patterns identified in the diagnostic plots. Variables were included in the final regression model if they were significantly ($p < 0.05$) associated with the log-transformed daily warfarin dose or were marginally significantly associated ($0.05 < p < 0.15$) with strong biological plausibility. The mean absolute error (MAE; mg/day), defined as the mean of the absolute values for the difference between the predicted and actual doses, was used to evaluate the model’s predictive accuracy. The MAE was computed in the original units rather than in the log-transformed units to allow a fair comparison of all models. We selected the final model as the one that had the lowest predictive MAE. The bias of the dosing algorithm estimates (precision) was assessed by calculating the mean percentage of difference from the observed dose, where mean percentage of difference is equal to the MAE between predicted and actual dose divided by the actual dose ($[\text{predicted dose} - \text{observed dose}] / \text{observed dose} \times 100\%$). Finally, the effect size of each independent predictor covariate on the log-transformed daily dose of warfarin was also computed.

Using the available data set, we compared dose predictions from our pharmacogenetic model with those from a fixed-dose approach and three other separate models: a clinical model that did not include genetic factors, the Lenzini and coworkers model [8] and the IWPC algorithm [2]. The clinical model was built with the use of the same methods as the developed pharmacogenetic model in Puerto Ricans, but without the incorporation of genetic variables. We evaluated the potential clinical value of each algorithm by calculating the percentage of patients in the validation cohort whose predicted dose of warfarin was within 20% of the actual stable therapeutic 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/day, a difference clinicians would define as clinically relevant. We also assessed the performance of the algorithms in three 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 <3 mg/day would be at risk of 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 of inadequate anticoagulation if they were started on a dose of 5 mg/day. McNemar's test of paired proportions was used for comparison of our pharmacogenetic algorithm with either the two clinically-based nomograms or the two published algorithms. Significance level of all statistical analyses was set at $p < 0.05$. Statistical analyses were performed using the SPSS® software version 11.0 (IBM® Co., NY, USA). Because this study required a blood sample at enrollment followed by a retrospective CPRS medical record review, no patients were withdrawn or lost to follow-up.

Results

Derivation cohort

A total of 177 eligible patients (i.e., those meeting the inclusion criteria) were approached regarding participation in this study. Two patients declined to participate, resulting in 175 enrolled patients. A total of 12 patients were excluded from further analysis: three samples were excluded because of poor genotyping call rate; two patients were subsequently excluded owing to mildly impaired decision-making capability; and another seven individuals were removed owing to lack of complete clinical data from the CPRS. Therefore, there were 163 patients available for the regression analysis. The majority of patients in this derivation cohort were part of a previous study [7].

Model development

In the derivation cohort ($n = 163$), the daily therapeutic warfarin dose ranged from 1.4 to 11.0 mg/day, a difference representing approximately two standard deviations. Notably, more than one-quarter (i.e., 27.6%) of individuals in the study cohort were stabilized at a dose level that was either above or below the standard 'intermediate' range (i.e., 3–7 mg/day). The mean age was 68 years (range: 31–90 years), 99% were male and 89.6% were self-identified as white. The (arithmetic) mean daily warfarin dose was 4.5 mg (Table 1). The most common indications for warfarin therapy were atrial fibrillation ($n = 120$) and pulmonary thromboembolism ($n = 9$; one of whom also had atrial fibrillation). Patients in the validation cohort ($n = 55$) were younger (mean age: 60.7 years; range: 23–90 years) and less often male (~36%), and 60% had atrial fibrillation as their primary indication for warfarin therapy (Table 1).

The *CYP2C9* and *VKORC1* alleles were highly prevalent in the study population (Table 2). There are 49 (30.1%) participants with the wild-type genotype and 114 (69.9%) with a

variant genotype, who are subdivided in to 42% single, 23% double, 4% triple and 1% quadruple carriers. Among all patients, 26.4% had at least one polymorphism in *CYP2C9*, whereas, 60.1% were carriers of the *VKORC1* -1639G>A variant. In both cohorts, all alleles were in Hardy–Weinberg equilibrium.

A multiple, least-squares, linear regression modeling approach to develop a DNA-guided algorithm that predicts the log-transformed effective warfarin dose and incorporates genetic, demographic and clinical variables proved to be the best option for the available data, according to the lowest MAE criterion. The performance of this pharmacogenetic model is shown in Figure 1. Variables that entered into the regression model that were significantly associated ($p < 0.05$) with the log-transformed stable warfarin daily dose are listed in Table 3 with their respective regression coefficients, p-values, percentage effect on dose and the contribution to explain the observed variability that is accounted for by the model, as measured by the partial R^2 statistics. The *VKORC1* -1639G>A haplotype was the first genetic variable to enter the stepwise regression model; combined *VKORC1* -1639AA and GA genotypes explained approximately 24% (95% CI: 19.4–31.6) of the dose variability in this population. Moreover, this covariate was associated with a 21% reduction in the therapeutic warfarin dose per number of A alleles. The number of loss-of-function *CYP2C9* polymorphisms explained another 9.5% (95% CI: 7.4–14.2) of the observed intersubject variability in dose requirements, and it was associated with a 17% decrement in the warfarin dose per mutated allele.

INR/dose on day 3 was one of the nongenetic clinical variables to enter the model, and each 0.25-unit increase in this interaction variable was associated with a 14% decrease in the therapeutic warfarin dose. This variable explained approximately 19% (95% CI: 10.3–30.6) of the observed dose variability.

Other factors that entered the regression model were age, amiodarone usage and prior venous thromboembolism as the indication for warfarin therapy. Overall, the pharmacogenetic model contained seven significant ‘explanatory’ variables; these independent predictors explained more than two-thirds of the observed variance in the therapeutic warfarin dose ($R^2 = 67.8\%$; $p = 0.033$; mean square error: 0.054; standard error of estimate: 0.23 mg/day). Interaction terms between the genotypes and key medications (e.g., amiodarone, statins, azoles and sulfamethoxazole) were not statistically significant. The best-fitted pharmacogenetic model for predicting optimal warfarin dose (mg/day) in the study population was **Equation 1**:

$$\exp \left[\begin{array}{l} 2.602 - \left(0.569 \times \frac{\text{INR}}{\text{dose}} \right) - (0.463 \times \text{VKORC1 AA}) - \\ (0.153 \times \text{VKORC1 GA}) - (0.174 \times \text{CYP2C9}) \\ - (0.272 \times \text{PE}) - (0.276 \times \text{amiodarone}) - (0.0086 \times \text{age}) \end{array} \right]$$

Where exp is the exponential function; INR/dose is the ratio of these two variables on day 3 of initiation of warfarin therapy; *VKORC1* AA status is a carrier code, where 1 = AA and 0 = otherwise; *VKORC1* GA status is a code, where 1 = GA and 0 = otherwise; *CYP2C9* status is a code, where 0 = wild-type, 1 = one mutated allele and 2 = two mutated alleles; pulmonary embolism (PE) is 1 if the indication for warfarin is PE and 0 = otherwise; amiodarone = 1 if the patient is taking that drug and 0 = otherwise; and age is in years. Because the resulting algorithm computes the log-transformed dose, the output must be exponentially transformed to compute the predicted warfarin daily dose.

The differences in the performance of the pharmacogenetic and clinical models, as well as the fixed-dose scheme, in the low-dose (< 3 mg/day), intermediate-dose (>3 and <7 mg/day)

and high-dose (≥ 7 mg/day) groups of the derivation cohort, are shown in Table 4. The pharmacogenetic-driven algorithm in Puerto Ricans provided more accurate dose estimates (i.e., significantly closer to the actual dose requirements) than those derived from the clinical algorithm or the fixed-dose approach, as demonstrated by an overall MAE, which was lower than that for both the clinical algorithm and the fixed-dose approach (mean ± standard deviation: 1.22 ± 0.34 mg/day vs 2.84 ± 0.06 mg/day and 2.22 ± 0.06 mg/day, respectively; $p < 0.001$ for both comparisons). In general, the addition of relevant pharmacogenetic information to clinical and demographic data decreased the absolute error in the estimation of the effective dose and increased the fraction of variability explained (R^2) by 20% on average. Moreover, when comparing with two previous pharmacogenetic algorithms [2,8], our customized algorithm performed better for our study population than these two published models, with higher R^2 (i.e., 0.67 [Puerto Rican model] vs 0.36 [IWPC model]) or 0.33 ([Lenzini and coworkers model [8]], respectively), less scatter and lower MAE and mean bias percentage (i.e., MAE = 0.79 mg/day and 17.7% mean bias [Puerto Rican model] vs MAE = 1.13 mg/day and 25% mean bias [IWPC model] or MAE = 1.27 mg/day and 28% mean bias [Lenzini and coworkers model [8]], respectively).

For patients who required 3 mg or less of warfarin per day (18.4% of the total cohort), our pharmacogenetic algorithm provided a significantly better prediction of dose than the clinical algorithm or the fixed-dose approach; 43.3% of the dose predictions resulted within 20% of the actual dose ('ideal dose') with the pharmacogenetic algorithm as compared with 10% with the clinical algorithm ($p < 0.001$) and 0% with the fixed-dose approach ($p < 0.001$). In addition, our pharmacogenetic algorithm provided significantly fewer overestimations of dose in the low-dose group (50 vs 90% with the clinical algorithm [$p < 0.001$]; and 100% with the fixed-dose approach [$p < 0.001$]). Similarly, for patients requiring between 3 and 7 mg/day (intermediate-dose group: 72.4% of the total cohort), the pharmacogenetic algorithm predicted doses in the ideal range for significantly more patients than the clinical algorithm or the fixed-dose approach (77 vs 55 and 55%, respectively; $p < 0.001$ for both comparisons), with significantly fewer dose overestimations (12.7 vs 39 and 38%, respectively; $p < 0.001$ for both comparisons). In the high-dose group (9.2% of the total cohort), the accuracy of the dose prediction was similar with the fixed-dose approach, although lower than the clinical approach. In general, the pharmacogenetic algorithm in Puerto Ricans provided consistently better dose prediction, particularly for patients who required either low doses (i.e., highly sensitive to warfarin) or intermediate doses, who, combined, accounted for 90% of the entire cohort. Strikingly, in more than half of patients, the MAE value was <1 mg/day (i.e., falling within 20% of the actual dose, which is a well-accepted criterion for accuracy in dose estimation).

A correlation plot of the predicted warfarin daily dose based on the pharmacogenetic algorithm developed for Puerto Ricans as compared with the actual daily dose in the validation cohort is shown in Figure 2A. As can be observed, our pharmacogenetic model predicted 51% of the variance in warfarin dose when externally validated in 55 genotyped individuals from the independent Hartford's patient cohort. On the contrary, the two pharmacogenetic algorithms showed a poorer predictability of effective dose in this independent cohort, with $R^2 = 19\%$ (IWPC model) and 27% (Lenzini and coworkers model), as depicted in Figure 2B and Figure 2C, respectively.

Discussion

Our previous analysis of significant pharmacogenetic and physiogenomic data from Puerto Ricans revealed that each individual within the Puerto Rican population is a 'genetic mosaic', with contributions from each of the three historical parental groups (i.e., Iberian Caucasians, west Africans and Amerindians), but in widely different proportions, resulting

in a considerably larger pharmacogenetic divergence among individuals and a corresponding richer repertoire of combinatorial genotypes for key pharmacological pathways [11]. Since this component is likely to be missed by traditional studies in more homogeneous populations (e.g., non-Hispanic Caucasians), we would expect that earlier-developed DNA-guided warfarin dosing algorithms will perform badly in our population. In addition, as recently postulated by Suarez-Kurtz and coworkers [12], pharmacogenetics is local but also highly sensitive to within-population diversity, particularly in admixed populations. Therefore, the predictive power of a pharmacogenetic algorithm for effective warfarin dose estimation is likely to be population dependent.

This retrospective study of 163 Puerto Rican patients receiving warfarin for different thromboembolic disorders at the VACHS-affiliated anticoagulation clinic in San Juan confirms the extensive interpatient dose variability required to maintain the INR levels within the target range for prevention of bleeding or strokes in Puerto Ricans.

In this study we developed a new Puerto Rican-oriented pharmacogenetic algorithm using the data derived from our VACHS derivation cohort, with 163 patients in total, and compared its performance with a fixed-dose scheme, a clinically-driven algorithm and two published pharmacogenetic models in larger multiethnic populations [2,8]. To our knowledge, this is the first external validation of the publicly available IWPC-derived pharmacogenetic algorithm, as well as the pharmacogenetic refinement model by Lenzini and coworkers [8], in an entirely Hispanic population of Puerto Rican origin. Notably, Hispanics (and Puerto Ricans in particular) have either been excluded or under-represented (<1%) in most of the previous pharmacogenetic studies of warfarin, even though they are considered the largest and most rapidly growing minority group in the USA [6].

The pharmacogenetic algorithm we developed in the present study provided significantly better predictions of the effective warfarin daily dose in Puerto Ricans than either the clinically-driven (i.e., clinical and fixed-dose approaches) or the available pharmacogenetic algorithms by Lenzini and coworkers [8] and the IWPC consortium [2]. The greatest differences among the dose-prediction approaches were noted among patients whose stable warfarin doses were 3 mg or less per day (highly sensitive) and among those whose stable doses were between 3 and 7 mg/day (intermediate doses), representing 90% of the study cohort (Table 4). The former are the patients for whom overdosing could have adverse clinical consequences such as major bleeding episodes or intracranial hemorrhage. Patients who require high doses (i.e., 7 mg/day) are likely to obtain little benefit from the use of this customized pharmacogenetic algorithm because the genotyping information added to the final model relies on SNPs that increase sensitivity to warfarin but lack SNPs that cause resistance (e.g., *VKORC1* -106G>T [p.D36Y] missense variant).

Polymorphisms in the *VKORC1* gene locus have been previously associated with resistance to warfarin, but these variants are rare except in certain populations from the Middle East [13–15]. In addition, discovery of novel variants by resequencing candidate genes or performing genome-wide association studies in admixed populations may identify additional markers that can eventually improve model predictability, particularly in high-dose patients. Currently, we are evaluating some polymorphisms on other candidate genes such as *CYP4F2*, *CALU*, *EPHX1* and *GGCX* to determine whether they can further improve predictability in the Puerto Rican population. We also seek to include other potentially important independent predictors, such as vitamin K intake score and an admixture vector component, which could help the model perform more predictably among all Puerto Rican patients.

Such admixture vectors are expected to capture a significant proportion of the missing ‘genetic’ effect on dose variability. Due to its large genetic diversity [11], the admixed population of Puerto Rico may harbor missed variants that can eventually improve model predictability. In this regard, we have demonstrated that interindividual variations in ancestral contributions (mainly Amerindian) have significant implications for the way each Puerto Rican responds to warfarin therapy because of the large interethnic differences in *VKORC1* and *CYP2C9* allele frequencies [16]. To account for this ancestry-based population stratification as a potential confounder, admixture can be incorporated into our pharmacogenetic model by adding a continuous function of the relative proportion of individual Amerindian, European and African ancestries.

The percentage of dose variability among patients that is explained by our model ($R^2 = 67\%$) is similar to, or even higher than, that in other early published models (31–71%) [1–6], which confirmed the clinical validity of the developed algorithm in Puerto Ricans. Furthermore, we did not restrict our algorithm to patients with a target INR range of 2–3, so it can also provide guidance on dosage to achieve INRs range of 2.5–3.5. The *VKORC1* -1639G>A haplotype proved to be the most important genetic predictor in our model, with a partial R^2 value of 24.5%. The -1639G>A SNP, located in the promoter region of *VKORC1*, is a well-known genetic marker for a patient’s sensitivity to warfarin, affecting gene expression at transcription level. Haplotypes comprising the A allele are associated with reduced *VKORC1* expression in the human liver and, therefore, with low-dose requirements [15]. Owing to high linkage disequilibrium, we would not expect the haplotype to explain more of the variance in warfarin dose than the individual *VKORC1* -1639G>A polymorphism. The *CYP2C9* genotype, the other pharmacogenetic factor ascertained in this study, had a partial R^2 value of 9.5% in our multivariate model. Previously reported contributions of either *VKORC1* or *CYP2C9* polymorphisms to the total variance of the warfarin daily dose have shown large inter- and intra-ethnic differences, which preclude further comparison [17–19]. Nonetheless, in a previous algorithm in Brazilians by Perini and coworkers [4], the *VKORC1* -1639G>A and *CYP2C9* genotypes explained the 23.8% and 6.9% of dose variability, respectively. In the model developed by Wu and coworkers from a multiethnic population that includes 22 Hispanics, *CYP2C9* and *VKORC1* genotypes explained 5 and 34% of dose variance, respectively [3].

By contrast, Cavallari and coworkers published a pharmacogenetic model for warfarin dose prediction in Hispanics, where the *VKORC1* -1639G>A polymorphism alone explained 30% of the variability in warfarin dose and *CYP2C9* genotypes explained approximately 9% of the overall variance [6]. Their final model including genotypes, age, body surface area, and history of venous thromboembolism explained 56% of the interpatient variability in warfarin dose requirements of 50 Hispanics (89% of Mexican descent).

In previous reports, *CYP2C9* status by itself accounts for approximately 3.2–22% of the interindividual variability in warfarin dose requirements in various populations, with the greatest contribution in Caucasian populations due to higher variant allele frequencies [3,5,20–24]; whereas, the *VKORC1* promoter status (i.e., A/B haplotype) independently determines 14–34% of such variance to be the primary genetic factor contributing to warfarin sensitivity, irrespective of the population [15,20,25–29]. Frequency distributions of the most common *VKORC1* and *CYP2C9* polymorphisms vary by ethnogeographic origin. Together, combinatorial *CYP2C9* and *VKORC1* genotypes explain up to 45% of warfarin response variability in European [5,15,21,30–37] and 30% in African populations [25,28]. We speculate that the estimated partial R^2 values from *CYP2C9* (9.5%) and *VKORC1* (24.5%) contributions to our multiple regression model can be partly explained by the high degree of admixture in the Puerto Rican population [11]. In this regard, the Amerindian ancestry might account for a relatively larger than expected contribution of *VKORC1*

-1639G>A polymorphism to the interindividual variability in warfarin dose requirements among Puerto Ricans, while their African ancestry could explain the observed lower contribution from common *CYP2C9* polymorphisms.

Despite the observed contribution of these common *VKORC1* and *CYP2C9* polymorphisms to the interpatient variability, there might be other genetic factors (e.g., *CYP4F2*, *EPHX1*, *CALU* and *GGCX* etc) or unknown variants in the coding regions of the *VKORC1* and *CYP2C9* genes that may account for additional dose variability in the admixed Puerto Rican population. However, we will need a larger learning sample to identify true associations, with unknown genetic variants having a relatively small effect on effective dose. Our dosing algorithm also includes nongenetic covariates that have previously been reported as independent predictors of warfarin dose in other patient populations, such as age, thromboembolic diseases (i.e., PE) and cotreatment with amiodarone [1–5].

Surprisingly, in our model, PE indication lowers the individual's dose requirements, while venous thromboembolism has commonly been associated with a dose increment in other models [1,4,6]. We believe that a diagnostic for PE among patients enrolled in this study might reflect the presence of other risk factors such as increasing age, heart failure and acute medical illness. Both older age and heart failure have been suggested as predictors of an increased response to warfarin, leading to a dose reduction. Further analyses are warranted in order to draw valid conclusions on this matter.

We were not able to account for two subjects who showed extremely high actual warfarin daily doses (>10 mg/day). They did not have the rare *VKORC1* missense variants associated with warfarin resistance that were interrogated with the assay kit (i.e., 172A>G [p.R58E], 85G>T [p.V29L], 121G>T [p.A41S], 134T>C [p.V45A], 1331G>A [p.V66M] or 3487T>G [L128R]). Although outliers have already been reported by other early studies with no definitive explanation [2,38], we speculate that medication noncompliance might be associated with these cases.

One of the study limitations is that it does not address whether a pharmacogenetically predicted dose of warfarin translates into better clinical end points, such as a reduction in the time to dose stabilization, fewer out-of-range INRs and/or a reduced incidence of bleeding episodes. Therefore, further studies need to be conducted in order to determine the clinical utility and benefits of incorporating genotyping into a predictive model for Puerto Rican patients, particularly for those who require low doses, which was the subcohort for whom dose estimates based on our pharmacogenetic algorithm differed significantly from those based on clinical approaches. Three large trials are currently under way to investigate whether using genetic data will significantly improve outcomes – COAG and GIFT in the USA and EU-PACT in Europe [39–41].

Our study also has other limitations. The population included in this study represents the typical population that is treated with warfarin (i.e., the elderly). Moreover, the majority of recruited patients were male. Therefore, additional studies need to be performed with respect to the use of the algorithm in women, children and younger adults. However, more than half of patients in the validation cohort are women (n = 35). Finally, patients in the derivation cohort were recruited from a single center (VACHS), which, along with the retrospective nature of the study and the inability to control for medication noncompliance in the derivation cohort, may raise concern about applicability and generalizability of results.

Like any data-driven modeling approach, algorithms might reflect unique features within data collected from their corresponding derivation cohorts, rather than a causal relationship between variables and effective dose of warfarin. In this regard, the observed tendency of the IWPC algorithm to underestimate in our study cohort, particularly for those patients

within the intermediate dose range (i.e., 30.5% of the 118 patients in this subgroup), may be partially explained by the high percentage of males, which translated to higher average stable dose of warfarin. Indeed, the average weekly dose of our cohort was higher than that in the IWPC study cohort (32 vs 28 mg/week, respectively).

Finally, our model is based on a combination of genetic and clinical variables (i.e., dose-adjusted INR at day 3), which are not readily available for the initiation of therapy and hence may preclude its use for determining an initial dose. Since the turnaround time of genetic test results usually takes 1–2 days on average, patients would have to be started on warfarin (initial dose) without that information. However, the genetic information can yet be useful in refining the effective warfarin maintenance dose when patients are in need for dose adjustments.

Indeed, the International Warfarin Dose Refinement Collaboration project demonstrated that pharmacogenetic knowledge still adds valuable information for improving dose predictions by deriving a pharmacogenetic dose refinement model ($R^2 \approx 60\%$ after internal validation; $n = 1213$) that can be used on days 4–5 after initiating warfarin therapy [8]. Notably, this pharmacogenetic-guided dose revision model is currently used in the COAG, GIFT and EU-PACT trials [17,39–41].

On the other hand, Horne and coworkers used clinical, INR measurement and genotyping data from 2022 patients at treatment days 6–11 to develop and validate an accurate warfarin dosing algorithm based on dosing history [42]. Results also showed that their 14-variable pharmacogenetic algorithm resulted in significant improvements in the prediction of maintenance warfarin dose compared with an algorithm including clinical factors alone.

Likewise, Michaud and coworkers used an innovative strategy combining *CYP2C9* and *VKORC1* genotyping and phenotypic measurements (e.g., dose-adjusted INR at day 4 and S:R-warfarin racemic mixture ratio at 14 h post-dose), as well as covariates such as body surface area and age, to explain dose requirements of warfarin in 132 hospitalized, heavily medicated patients [43]. Comparatively, the dose-adjusted INR at day 4 in the Michaud model explained 31% of dose variability observed at day 14 [43], whereas the natural logarithm of INR variable included in the Lenzini and coworkers model was associated with a 12% reduction effect on the maintenance warfarin dose [8].

Conclusion

In conclusion, using data from Puerto Rican patients at the VACHS San Juan and Hartford Hospital, we developed and validated a customized pharmacogenetic-guided warfarin dosing algorithm that uses combinatorial genotyping information from common variants in two candidate genes (*VKORC1* and *CYP2C9*) plus INR response, clinical and demographic variables to predict the effective warfarin daily dose in Puerto Ricans. This pharmacogenetic algorithm predicts the effective dose of warfarin better than the clinically-driven approaches (i.e., a fixed-dose approach and a clinical algorithm built from the same data set but excluding genotyping information) and closer to the required stable dose than those derived from two previously published pharmacogenetic algorithms [2,8]. Our pharmacogenetic algorithm produced significantly better ‘ideal dose’ estimates, with the greatest benefit seen in patients ultimately requiring 3 mg or less of warfarin per day and within the intermediate range (3–7 mg/day).

Future perspective

At this point, we envisage an opportunity for using our customized pharmacogenetic-based algorithm to predict not only the effective maintenance doses (mg/day) in Puerto Rican

patients receiving warfarin at the VACHS (mainly in those at the highest risk of poor control), but also to predict the individualized initial doses for patients commencing anticoagulation therapy by combining our pharmacogenetic model with an estimated accumulation index that is based on differences in warfarin clearance due to *CYP2C9* genotypes, according to the formulas by Avery and coworkers [44]. We also plan to use this pharmacogenetic algorithm, along with relevant data from previous studies, to build a web-based decision-support tool that can be further incorporated into the VACHS hospital's electronic medical record system to perform warfarin initial and maintenance dose calculations automatically. This clinician-oriented, web-based resource will enable clinicians to receive genotyping results and guidance on treating patients with warfarin in real-time in order to facilitate their transition to a DNA-guided personalized prescribing model.

Because the predictive power of a pharmacogenetic algorithm for warfarin dose estimation seems to be highly sensitive to within-population diversity, we do not anticipate our model can be extrapolated to other Hispanic populations. As previously suggested by others, it is necessary to recognize pharmacogenetic differences not only across different ethnic groups but also within a given ethnicity [45,46].

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Executive summary

Background

- Warfarin is the standard of care for oral anticoagulation in thromboembolic disorders. *CYP2C9* and *VKORC1* genotypes, in addition to known nongenetic covariates, account for approximately 50–65% of warfarin dose variability in different populations; however, this information is currently lacking for admixed Puerto Ricans.

Patients & methods

- We conducted a pharmacogenetic study to test the association between combinatorial *CYP2C9* and *VKORC1* genotypes, International Normalized Ratio (INR) measurements and warfarin dosing in 175 warfarin-treated Puerto Rican patients, from which 163 patients were analyzed based on complete data acquisition.

Results

- A total of 69.9% of participants (114) were carriers of at least one functional polymorphism on either *VKORC1* or *CYP2C9*, subdivided as follows: 42% single, 23% double, 4% triple and 1% quadruple carriers. Among all patients, 26.4% had at least one polymorphism in *CYP2C9*, whereas 60.1% were carriers of the *VKORC1* -1639G>A variant.
- A novel DNA-guided warfarin dosing algorithm for Puerto Ricans that included age, *CYP2C9* and *VKORC1* genotypes, dose-adjusted INR at day 3, amiodarone and pulmonary embolism as independent predictors was developed.
- The model explained more than two-thirds of the observed variance in the therapeutic warfarin dose in Puerto Ricans ($R^2 = 67.8\%$; $p = 0.033$; standard error of estimate: 0.23 mg/day; mean absolute error: 0.79 mg/day).
- Clinical validity of the model was assessed using a validation cohort of 55 Puerto Rican patients from the Brownstone Clinic in Hartford, CT, USA ($R^2 = 51\%$).

Discussion & conclusion

- Our algorithm produced significantly better ‘ideal dose’ estimates than clinical nomograms, or even two previously published pharmacogenetic algorithms by the International Warfarin Pharmacogenomics Consortium and International Warfarin Dose Refinement Collaboration projects, with the greatest benefit seen in patients ultimately requiring 3 mg/day and within the intermediate range (3–7 mg/day).
- Our findings provide the basis for planning prospective pharmacogenetic studies to demonstrate the clinical utility of genotyping warfarin-treated Puerto Rican patients.

Figure 1. DNA-guided warfarin dosing algorithm in Puerto Rican patients (Veterans Affairs Caribbean Healthcare System model)

The model was developed using a multiple regression analysis in 163 individuals of the derivation cohort. The solid 'identity' line illustrates perfect prediction. Two outliers, excluded from the final regression model, are depicted as open red circles on the graph. MAE: Mean absolute error; SEE: Standard error of estimate.

Figure 2. Validation of the generated Puerto Rican pharmacogenetic algorithm as compared to two publicly available algorithms in an independent sample of 55 Puerto Ricans from the Brownstone Clinic in Hartford, CT, USA
(A) Puerto Rican model, **(B)** International Warfarin Pharmacogenomics Consortium, **(C)** Lenzini *et al.* model [8]. The solid line illustrates perfect prediction in this validation cohort. MAE: Mean absolute error.

Table 1

Descriptive clinical (nongenetic) and demographic characteristics of the study population of warfarin-treated patients from the Veterans Affairs Caribbean Healthcare System, San Juan (PR, USA; derivation cohort) and the independent sample of warfarin-treated Puerto Ricans from the Brownstone Clinic in Hartford (CT, USA; validation cohort).

Characteristics	Derivation cohort (n = 163)			Validation cohort (n = 55)
	Total (n = 163; 100%)	Noncarriers (n = 49; 30.1%)	Carriers (n = 114; 69.9%)	
Stable warfarin dose (mg/day)				
Mean (± SD)	4.5 (1.71)	5.5 (1.83)	4.1 (1.48)	5.0 (2)
Range	1.4–11.0	1.6–11.0	1.4–10.0	1.5–10.0
Age (years)				
Mean (± SD)	67.9 (9.0)	69.0 (7.4)	67.0 (9.5)	60.7 (14.4)
Range	31–90	55–85	31–90	23–90
Weight (lbs)				
Mean (± SD)	186.0 (33.9)	185.0 (31.1)	187.0 (35.2)	179.0 (41.9)
Range	112–350	132–298	112–350	114.8–277
Height (cm)				
Mean (± SD)	67.4 (2.5)	67.2 (2.5)	67.5 (2.6)	62.7 (3.6)
Range	62–76	63–73	62–76	53–69
Indications				
Atrial fibrillation $\dot{\bar{r}}$ /flutter, n (%)	120 (73.6)	35 (71.4)	85 (74.5)	33 (60)
Pulmonary embolism, n (%)	9 (5.5)	4 (8.2)	5 (4.4)	8 (14.5)
Other indication, n (%)	34 (21)	10 (20)	24 (21)	14 (25.5)
Smoker, n (%)	12 (7.4)	2 (4.1)	10 (8.8)	NA
Comedications				
Amiodarone users, n (%)	16 (9.8)	5 (10.2)	11 (9.7)	2 (3.6)
Azoles users, n (%)	33 (20.2)	10 (20.4)	23 (20.2)	NA
Statins users, n (%)	87 (53.4)	24 (48.9)	63 (55.3)	NA
Race				
Whites, n (%)	146 (89.6)	43 (87.8)	103 (90.4)	51 (92.7)
Blacks, n (%)	17 (10.4)	6 (12.2)	11 (9.6)	4 (7.3)
INR/dose at day 3				
Mean (± SD)	0.64 (0.39)	0.56 (0.39)	0.68 (0.39)	0.65 (0.53)
Range	0.17–2.27	0.17–2.23	0.24–2.27	0.14–2.47
Target INR				
Mean (± SD)	2.5 (0.5)	2.5 (0.5)	2.5 (0.5)	2.5 (0.5)
Range	2–3.5	2–3.5	2–3.5	2–3.5
Gender				
Male, n (%)	162 (99.4)	49 (100)	113 (99.1)	20 (36)
Females, n (%)	1 (0.6)	0 (0)	1 (0.9)	35 (64)

Mean refers to arithmetic mean. All comparisons of the mean values (or percentages) between carriers and noncarriers were not significant, except for the stable warfarin dose variable ($p = 0.022$). p -values for the difference between the carriers and noncarriers were calculated with the use of the two-tailed Mann–Whitney U/Wilcoxon's rank-sum test (for warfarin dose, age, height, weight, target INR, INR/dose at third day) and χ^2 or Fisher's exact test (for comedications, smoking status, race, gender and comorbidities). Comedications (brand name in parenthesis when used): amiodarone; statins: simvastatin, rosuvastatin (Crestor®; AstraZeneca, DE, USA), fluvastatin (Lescol®; Novartis Pharmaceuticals Co., NJ, USA) and pravastatin; azoles: fluconazole (Diflucan®; Pfizer Inc., NY, USA), itraconazole and ketoconazole.

[†]Chronic, paroxysmal or postoperative included.

INR: International Normalized Ratio; NA: Information not available; SD: Standard deviation.

Table 2

Allele and genotype frequency distributions of *CYP2C9* and *VKORC1* -1639G>A polymorphisms in 163 Puerto Rican patients treated with warfarin at the Veterans Affairs Caribbean Healthcare System (PR, USA).

Genotype/alleles	n; % (95% CI)	χ^2 test
VKORC1 -1639G>A		
GG	68; 41.7 (40.9–42.5)	0.6
GA	75; 46 (45.2–46.8)	
AA	20; 12.3 (11.8–12.8)	
G	211; 64.7 (64.2–65.2)	
A	115; 35.3 (34.8–35.8)	
CYP2C9		
*1/*1	120; 73.6 (72.9–74.3)	3.09
*1/*2	24; 14.7 (14.2–15.2)	
*1/*3	8; 5 (4.6–5.2)	
*1/*5	2; 1.2 (1.0–1.4)	
*2/*2	2; 1.2 (1.0–1.4)	
*2/*3	6; 3.7 (3.4–4.0)	
*2/*5	1; 0.6 (0.56–0.64)	
*1	274; 84 (83.6–84.4)	
*2	35; 10.7 (10.4–11.0)	
*3	14; 4.3 (4.1–4.5)	
*5	3; 1 (0.87–0.93)	

Data expressed as percentage (95% CI). χ^2 test result is also included. $\chi^2 < 3.84$ meets the Hardy–Weinberg equilibrium. Only those SNPs detected in the analyzed samples from Puerto Ricans are reported in this table. SNPs assigned by the HILOMet PhyzioType™ system [9–10] as noncalls were excluded.

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Table 3

Summary of attributes of the pharmacogenetic equation for warfarin dosing prediction in Puerto Ricans using the derivation cohort from the Veterans Affairs Caribbean Healthcare System (PR, USA).

Variables [†]	Partial regression coefficient	SE	Adjusted R ² after entry	Effect on warfarin dose (%) [‡]	p-value
<i>VKORC1</i> :					
– VKAA	–0.463	0.066	0.245	–21	<0.0001
– VKGA	–0.153	0.042			
<i>CYP2C9</i>					
	–0.174	0.034	0.340	–17	0.003
Age (years), per decade					
	–0.0086	0.002	0.404	–9	0.010
PE					
	–0.272	0.091	0.474	–27	0.015
Amiodarone [§]					
	–0.276	0.107	0.486	–28	0.030
Dose-adjusted INR [¶]					
	–0.569	0.055	0.678	–14	<0.0001

Constant value of the model equation is 2.602 (SE: 0.158).

[†] Variables are listed in the order they were incorporated into the model using stepwise regression analysis.

[‡] Effect on the estimates of the effective dose is calculated per number of variant alleles (*CYP2C9* and *VKORC1*), per decade (age) and per 0.25-unit increase in the dose-adjusted INR response at day 3.

[§] Patients who are taking amiodarone concomitantly.

[¶] INR over dose at day 3.

INR: International Normalized Ratio; PE: Pulmonary embolism; SE: Standard error.

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Table 4
Comparison of models' predictability in the low-, intermediate- and high-dose groups of the derivation cohort.

Prediction model	IWPC-derived PGx algorithm	Lenzini and coworkers model	Clinically based algorithm [†]	Fixed-dose approach [‡]	Puerto Rican algorithm
Low doses (< 3 mg/day)					
MAE (95% CI), mg/day	1.22 (1.18–1.26)	0.78 (0.62–0.94)	1.54 (1.50–1.58)	2.59 (2.53–2.65)	0.70 (0.54–0.86)
R ² (%)	20.1	34.2	49.0	–	48.4
p-value [‡]	NS	NS	0.0162	0.0009	–
Intermediate doses (>3 and <7 mg/day)					
MAE (95% CI), mg/day	0.99 (0.94–1.04)	1.18 (1.04–1.32)	0.89 (0.83–0.95)	0.88 (0.83–0.93)	0.61 (0.52–0.70)
R ² (%)	3.51	15.1	21.9	–	36.8
p-value [‡]	0.0021	0.001	0.0001	0.0002	–
High doses (> 7 mg/day)					
MAE (95% CI), mg/day	3.74 (3.64–3.84)	2.97 (2.28–3.66)	6.11 (5.99–6.23)	3.20 (3.11–3.29)	2.35 (1.58–3.12)
R ² (%)	1.76	6.8	0.34	–	4.17
p-value [§]	NS	NS	NS	NS	–

Predicted warfarin daily doses (mg/day) with the Puerto Rican model, the Lenzini and coworkers pharmacogenetic refinement model [8], the IWPC pharmacogenetic-guided algorithm [2], and the clinical-based and fixed-dose approaches as compared with the actual doses of warfarin for the therapeutic effect in patients requiring low (< 3 mg/day) intermediate (>3 and <7 mg/day), or high (> 7 mg/day) exposure. Data correspond to the study 'derivation' cohort of 163 Puerto Rican patients at the Veterans Affairs Caribbean Healthcare System-affiliated anticoagulation clinic. The 95% CIs of the estimates of MAEs were calculated. R² is the coefficient of determination.

[†]This algorithm is the same model developed in Puerto Ricans but excluding genotypes.

[‡]The fixed-dose was set as 5 mg of warfarin per day.

[§]p-values for the comparison of proportions of 'ideal dose' estimates using the Puerto Rican pharmacogenetic algorithm versus the clinically based, Lenzini's, IWPC-derived algorithms and the fixed-dose approach, as derived with the use of McNemar's test of paired proportions.

IWPC: International Warfarin Pharmacogenomics Consortium; MAE: Mean absolute error; NS: Not significant differences (p > 0.05); PGx: Pharmacogenomics.