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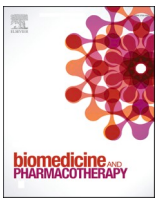

New insights into the role of *VKORC1* polymorphisms for optimal warfarin dose selection in Caribbean Hispanic patients through an external validation of a population PK/PD model

Karine Rodríguez-Fernández [a], Gledys Reynaldo-Fernández [b], Stephanie Reyes-González [c], Camila de las Barreras [b], Leyanis Rodríguez-Vera [d], Cornelis Vlaar [c], Jean-Christophe M. Monbaliu [e], Torsten Stelzer [c,f], Jorge Duconge [c,*], Victor Mangas-Sanjuan [a,g]

[a] *Department of Pharmacy and Pharmaceutical Technology and Parasitology, University of Valencia, Valencia, Spain*
[b] *Institute of Pharmacy and Foods, University of Havana, Havana 11300, Cuba*
[c] *Department of Pharmaceutical Sciences, School of Pharmacy, University of Puerto Rico - Medical Sciences Campus, San Juan 00936, PR, USA*
[d] *Center for Pharmacometrics and System Pharmacology at Lake Nona (Orlando), Department of Pharmaceutics, College of Pharmacy, University of Florida, Orlando, FL 32827, USA*
[e] *Center for Integrated Technology and Organic Synthesis, MolSys Research Unit, University of Liège, B-4000 Liège (Sart Tilman), Liège, Belgium*
[f] *Crystallization Design Institute, Molecular Sciences Research Center, University of Puerto Rico, San Juan 00926, PR, USA*
[g] *Interuniversity Research Institute for Molecular Recognition and Technological Development, Polytechnic University of Valencia-University of Valencia, Valencia, Spain*

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A B S T R A C T

Warfarin, an oral anticoagulant, has been used for decades to prevent thromboembolic events. The complex interplay between *CYP2C9* and *VKORC1* genotypes on warfarin PK and PD properties is not fully understood in special sub-groups of patients. This study aimed to externally validate a population pharmacokinetic/pharmacodynamic (PK/PD) model for the effect of warfarin on international normalized ratio (INR) and to evaluate optimal dosing strategies based on the selected covariates in Caribbean Hispanic patients. INR, and *CYP2C9* and *VKORC1* genotypes from 138 patients were used to develop a population PK/PD model in NONMEM. The structural definition of a previously published PD model for INR was implemented. A numerical evaluation of the parameter-covariate relationship was performed. Simulations were conducted to determine optimal dosing strategies for each genotype combinations, focusing on achieving therapeutic INR levels. Findings revealed elevated IC_{50} for G/G, G/A, and A/A *VKORC1* haplotypes (11.76, 10.49, and 9.22 mg/L, respectively), in this population compared to previous reports. The model-guided dosing analysis recommended daily warfarin doses of 3–5 mg for most genotypes to maintain desired INR levels, although subjects with combination of *CYP2C9* and *VKORC1* genotypes * 2/* 2-, * 2/* 3- and * 2/* 5-A/A would require only 1 mg daily. This research underscores the potential of population PK/PD modeling to inform personalized warfarin dosing in populations typically underrepresented in clinical studies, potentially leading to improved treatment outcomes and patient safety. By integrating genetic factors and clinical data, this approach could pave the way for more effective and tailored anticoagulation therapy in diverse patient groups.

Abbreviations: PK, pharmacokinetics; PD, pharmacodynamics; PK/PD, pharmacokinetic/pharmacodynamic; INR, international normalized ratio; CYP2C9, cytochrome P4502C9 gene; VKORC1, vitamin K epoxide reductase complex subunit 1 gene; VACHS, Veteran Affairs Caribbean Healthcare System; CI, confidence intervals; OFV, objective function value; IIV, inter-individual variability; RUV, residual unexplained variability; GOF, goodness of fit; NPDE, normalized prediction distribution errors; pc-VPC, prediction-corrected visual predictive checks; SAEM, Stochastic Approximation of the Expectation Maximization; IMP, Importance Sampling Estimation; F, bioavailability fraction; CL, clearance; V, volume of distribution; MTT, mean transit time; I_{max}, maximum inhibitory effect; IC_{50}, concentration resulting in 50% of I_{max}; DV, dependent variable; PRED, population prediction; K-PD, kinetic-pharmacodynamic model; MIPD, model-informed precision dosing; GGCX, γ-glutamyl carboxylase.

* Correspondence to: Medical Sciences Campus, Pharmaceutical Sciences Dept./ Pharmacogenomics Lab B-214, PO Box 365067, San Juan, PR 00936-5067, USA.
E-mail address: jorge.duconge@upr.edu (J. Duconge).

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1. Introduction

Warfarin is an oral anticoagulant that has been used for over six decades to prevent thromboembolic events, such as stroke and deep vein thrombosis [1]. It works by inhibiting the synthesis of vitamin K-dependent clotting factors in the liver, thus reducing the production of blood clots [2]. Despite its widespread use, warfarin therapy is associated with several challenges, including a narrow therapeutic window, a high risk of bleeding, and significant interpatient variability in response [3,4]. The management of warfarin therapy involves maintaining a stable therapeutic international normalized ratio (INR) range, which is typically between 2.0 and 3.0 for most indications [5]. However, achieving and maintaining this target range can be challenging due to the wide interpatient variability in warfarin exposure and response. This variability can be attributed to various factors, including age, sex, body weight, diet, comorbidities, and concomitant medications, as well as genetic variations [4].

The impact of genetic polymorphisms on warfarin response has been extensively studied, particularly for two relevant pharmacogenes: the *CYP2C9* gene encodes the cytochrome P450 isoform 2C9, a member of the cytochrome P450 superfamily of enzymes, and the *VKORC1* gene encodes the catalytic subunit 1 of the vitamin K epoxide reductase complex enzyme [3,4]. CYP2C9 is responsible for the metabolism of S-warfarin, the more active enantiomer of warfarin, and genetic polymorphisms in this gene have been associated with altered warfarin clearance (CL) and higher INR values [4,6,7]. VKORC1 is responsible for the reduction of inactive vitamin K 2,3-epoxide to active vitamin K in the endoplasmic reticulum membrane and, therefore, is involved in the synthesis of vitamin K-dependent clotting factors [6]. Genetic polymorphisms affecting this gene have been associated with reduced sensitivity to warfarin [8,9]. Given the impact of genetic polymorphisms on warfarin response, the use of a pharmacogenetic-driven algorithm has been recommended to guide warfarin dosing in clinical setting, particularly in patients starting warfarin therapy [6,10–12]. The results of genetic testing for *CYP2C9* and *VKORC1* polymorphisms can be used to inform initial warfarin dosing and subsequent dose adjustments, leading to more effective and personalized therapy [6,11–14].

In recent years, population pharmacokinetic (PK) and pharmacokinetic/pharmacodynamic (PK/PD) models that incorporate genetic information have been developed to predict optimal warfarin doses for individual patients, improving therapeutic efficacy and reducing the risk of adverse events and thromboembolic events [7,15–19]. The models use a two-step approach: first, a suitable starting dose is estimated based on patient factors that have been previously identified as predictors for dose individualization, which is referred to as a priori individualization. Second, once the treatment has started, the dosing can be further personalized based on feedback observations from the patient, which is referred to as a posteriori individualization [7,15,20].

However, the development of a reliable and accurate PK/PD model that incorporates genetic information can be challenging due to the complex interplay between genetic and non-genetic factors affecting warfarin dosing. Furthermore, the predictive performance and comparability of different PK/PD models that incorporate genetic information have not been extensively evaluated. External validation studies are necessary to assess the accuracy and reliability of these models in different populations, allowing for more personalized and effective warfarin dosing. While PK/PD modeling has shown promise in predicting the optimal warfarin dose for individual patients, most of the studies have been conducted in Caucasian populations, with few studies focusing on non-Caucasian populations. Furthermore, there is limited data on the application of PK/PD models incorporating *CYP2C9* and *VKORC1* genotypes information in Caribbean Hispanic patients.

In this study, we aimed to (i) externally validate a population PK/PD model of INR after concomitant administration of warfarin in clinical practice, and (ii) evaluate optimal dosing strategies of warfarin in Caribbean Hispanic patients based on the selected covariates.

2. Materials and methods

2.1. Ethics approval

This is a secondary analysis of a previous pharmacogenetic study of warfarin in Puerto Rican patients (IRB approval #A4070109). Proper safeguards against any potential violation of privacy and/or breach of confidentiality will be ensured. Authorization to use the data for the purpose stated in this project was previously obtained from individual patients by an informed consent process. Accordingly, the study was conducted following Helsinki's declaration for human subject protection in clinical surveys.

2.2. Study design

Data for analyses were available from elderly patients, mostly males, followed upon at the anticoagulation clinic in the Veteran Affairs Caribbean Healthcare System (VACHS) at San Juan, PR. The patients received long-term warfarin anticoagulant therapy for different thromboembolic disorders in total weekly doses ranged from 7 to 82 mg, depending on the 24- or 48-hour dosing interval. Also, they carried eight different *CYP2C9* alleles (*1/*2, *1/*3, *1/*5, *1/*8, *2/*2, *2/*3, *2/*5) and three *VKORC1* genotypes (G/A, G/G, A/A). Detailed information was recorded, including demographics and clinical response, evaluated longitudinally using the assessment tool INR.

2.3. Base population PK/PD model

Due to the absence of longitudinal PK information of warfarin in the current study, a previously published population PK model was implemented to retrieve the structural PK parameter estimates for each subgroup of *CYP2C9* genotype [20]. Given the absence of experimental PK values in the recruited patients, inter-individual variability (IIV) was not associated to the PK parameters.

The structural definition of a previously published PD model for INR was implemented, which relies on an indirect response model coupled to a series of transit compartments to account for the delay between warfarin exposure and INR levels [7,17]. The effect of warfarin concentration was incorporated using a sigmoid response function based on the typical longitudinal PK levels for each *CYP2C9* genotype. IIV associated to the PK/PD model parameters was modeled exponentially preventing negative values for the individual estimates, and residual unexplained variability (RUV) was described with a proportional model. The population PD parameters were re-estimated as part of the development of the base PD model from the experimental information (INR) available in the recruited patients. The significance of the non-diagonal elements of the Ω variance-covariance matrix and subject specific RUV were also evaluated.

2.4. Final population PK/PD model

A numerical evaluation of the parameter-covariate relationship was performed manually in a univariate testing. A decrease in 6.63 units (p value <0.01) of the objective function value (OFV) provided by NONMEM®. Covariates evaluated included: body weight, age, *CPY2C9* and *VKORC1* genotypes, race, diabetes mellitus and smoking status. For categorical covariates the relative size of the different categories had to be larger than 5% to be considered for covariate testing. Covariates were also investigated for co-linearity. If two covariates had a correlation coefficient $> |0.6|$ then one of the two covariates was excluded from testing.

Assessment of model adequacy was influenced by convergence stability, biological plausibility, and parsimony. Additional evaluation of standard goodness-of-fit (GOF) plots together with the normalized prediction distribution errors (NPDE) plots was conducted [21].

Model evaluation of the selected models was performed through

prediction-corrected visual predictive checks (pc-VPC) [22] with 1000 datasets obtained by Monte Carlo simulation using the final parameter estimates for both fixed and random effects. Each simulated dataset has the study design features (covariates, dosing times, PK sampling times) identical to those in the analysis dataset. For each simulated dataset, the 2.5th, 50th, and 97.5th percentiles of the simulated concentrations in each bin were calculated. Then, the 95% prediction intervals of the above-described percentiles were calculated and displayed graphically together with corresponding percentiles computed from raw data.

2.5. Optimal dosing regimen evaluation

A simulation-based analysis using a Monte Carlo approach (n = 10,000) was conducted assuming log-normal distribution of PD parameters and different combinations of statistically significant covariates. A multiple dose regimen of daily oral administration of 1, 3, 5, 7, and 10 mg of warfarin to achieve steady-state concentrations were assumed. Individual INR levels were computed at 23 h post-dose of the 10th administration cycle (PK steady-state conditions) of warfarin. Dosing regimen selection was established with the goal of achieving the highest probability of INR levels within the therapeutic range (2.0–3.0).

2.6. Data analysis

All data analyses were performed based on the population approach with the software NONMEM® (v7.5, Icon Development Solutions, Ellicott City, MD). The population parameters were estimated using the Stochastic Approximation of the Expectation Maximization and the Importance Sampling Estimation (SAEM) and the Importance Sampling Estimation (IMP) method.

For graphical and statistical analysis, the R® software v4.2.1 was used (R Foundation for Statistical Computing, Vienna, Austria). The pc-VPC were performed using PsN version 5.0.0 [23].

3. Results

The modelling dataset consisted of 1033 INR observations from 138 patients with Table 1 presenting subject demographics. Fig. 1 shows the individual INR (PD) profiles available. Distributions of continuous covariates at baseline are displayed in Supplementary Fig. S1.

Table 1
Summary of patients' characteristics.

Demographics	Value
N	138
Median age, years (range)	68 (31–90)
Median body weight, kg (range)	83 (51–159)
Race, n (%), self-reported	
White Hispanics	33 (24)
Black Hispanics	26 (19)
Admixed	37 (27)
Others or not reported	42 (30)
Smoking status, n (%)	
Yes	59 (43)
No	79 (57)
type 2 DM status, n (%)	
Yes	39 (28)
No	72 (57)
CYP2C9 genotypes, n (%)	
** 1/* 1* (wild type)	98 (71)
** 1/* 2*	21 (15)
** 1/* 3*	7 (5.1)
** 1/* 5*	1 (0.72)
** 1/* 8*	2 (1.44)
** 2/* 2*	2 (1.44)
** 2/* 3*	6 (4.34)
** 2/* 5*	1 (0.72)
VKORC1 haplotypes, n (%)	
G/A	62 (45)
G/G	57 (41.3)
A/A	19 (13.7)

INR, international normalized ratio; DM, diabetes mellitus.

3.1. Base population PK/PD model

A previously developed one-compartment model with first-order oral absorption and *CYP2C9* genotype effect on CL was considered for generating longitudinal PK profiles across different *CYP2C9* genotypes [20]. Since the population PK models were developed from oral administration of warfarin, the bioavailability fraction (F) is unknown, and the estimates of CL and V represent the apparent estimates.

Parameter estimates of the base population PK/PD model are summarized in Supplementary Table S1 and the corresponding GOF, NPDE and VPC are shown in Supplementary Fig. S2. The structural definition of the base PK/PD, which incorporates two transit compartment chains with three compartments each to account for the delay between exposure and INR response, can satisfy the overall INR trend with no appreciable systematic bias that suggest model inadequacies. System-related PD parameters (MTT_1, and MTT_2) were fixed to the values reported by Hamberg et al., 2010 (MTT_1: 27.2 h or 1.13 d, and MTT_2: 110.9 h or 4.62 d). A typical INR at baseline (INR_{base} =1.86) was estimated and the maximum INR was set to 20 as previously reported [7, 17]. An inhibitory sigmoid E_{max} model of warfarin on the zero-order synthesis rate constants for each chain was assumed. The model assumes a complete (100%) inhibition of warfarin (I_{max}) to the vitamin K epoxide reductase, as previously reported [7,17,24], and the IC_{50} was estimated (15.4 mg/L). This parametrization helps to enhance the model stability. Due to the lack of intensive PD sampling, inter-individual variability was only incorporated on INR_{base} (25%) and IC_{50} (35%) parameters.

3.2. Final population PK/PD model

The final population PK/PD model incorporates *CYP2C9* on CL and body weight on V, as previously reported [20] and for the pharmacodynamic parameters, *VKORC1* polymorphisms as statistically significant

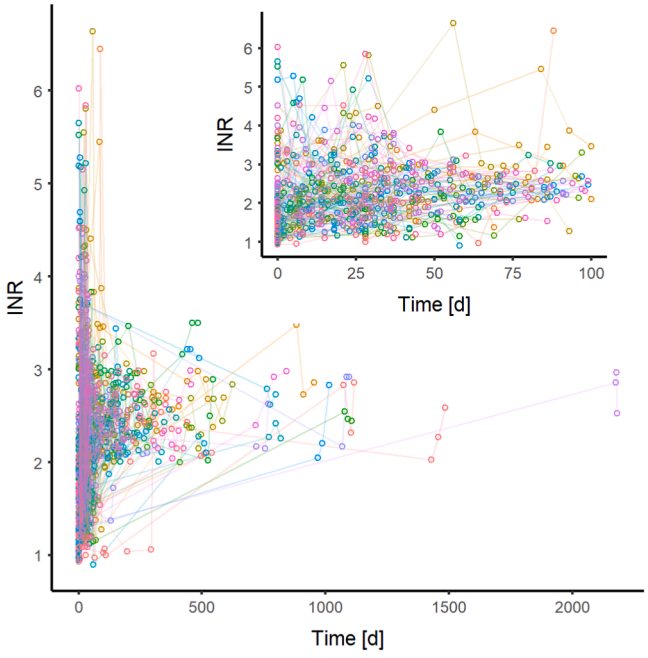

Fig. 1. Experimental raw data of INR observations vs time for all subjects included in the study (N = 138). The open circles represent the measured INRs in each patient and each color line represents an individual time profile of INR variations. INR: international normalized ratio.

covariate on INR_{base} and IC_{50} ($p < 0.01$, $\Delta OFV = -11.8$), respectively (Table 2). Other covariates, binary or (normalized) continuous variables, were investigated during the modeling process, but no statistical improvement ($p < 0.01$) after their inclusion was observed.

Supplementary Fig. S3 depicts the eta-distribution values of INR_{base} (ETA1) and IC_{50} (ETA2) across the different covariates available from the base population PK/PD model. Based on the GOF and NPDE plots (Fig. S4A), no systematic bias was observed and a slight improvement in the DV vs PRED plot is detected after the inclusion of the covariate effects. Individual observed vs predicted longitudinal INR profiles are shown in Supplementary Fig. S4B. The examination of the pc-VPC (Fig. 2) suggests a reasonable agreement between the observed data and model predictions for the median (50th percentile) and the variability (2.5th and 97.5th percentiles). The impact of *VKORC1* haplotypes on IC_{50} was modelled by accounting for the effects of individual alleles (G and A), which provides a more robust estimation in uncommon genotypes. The gamma parameter (1.47) of the sigmoid-Emax function was estimated, which is in accordance with other published values [7,15]. Warfarin IC_{50} for G/G, G/A, and A/A were 11.76, 10.49, and 9.22 mg/L, respectively, which are higher than the reported ranges [7,15,17,25]. The INR_{base} for the G/A, G/G, and A/A *VKORC1* haplotypes were 1.78, 1.84, and 2.18, respectively. After the covariate analysis, the IIV on INR_{base} and IC_{50} was reduced to 23% and 34%, respectively. Supplementary Fig. S5 depicts the eta-distribution values of INR_{base} (ETA1) and IC_{50} (ETA2) across the different covariates available from the final population PK/PD model.

3.3. Optimal dosing regimen evaluation

Based on the PD model developed, including the significant covariates on PK (*CYP2C9* genotypes on CL) and PD (*VKORC1* haplotypes on baseline and IC_{50}), we aimed to optimize the oral dosing strategy of warfarin in Caribbean Hispanic patients to achieve the optimal benefit/risk ratio. Fig. 3 represents the simulated INR levels across the dosing regimens tested. Table 3 summarizes the optimal dosing regimen selected for each combination of *CYP2C9* genotypes and *VKORC1* haplotypes.

4. Discussion

Currently, treatment with warfarin in the Caribbean Hispanic population generates certain therapeutic gaps since there are no clinical guidelines that evaluate the impact of different *CYP2C9* and *VKORC1* genotypes on routine INR measures (efficacy surrogate endpoint) in this population. Consequently, patients show sub-optimal clinical response rates, according to individual INR values. To address this, we have adapted a population PK/PD model of warfarin from individual data and dose records over 2 years in Caribbean Hispanic patients from a local anticoagulation clinic to optimize the dosing regimens in this population. In this paper, a simulation-based analysis to calculate the probability of guaranteeing therapeutic INR levels (2−3) for different daily regimens of warfarin was performed. The simulations accommodated different patient's genotypes resulting from combinatorial *CYP2C9* and *VKORC1* polymorphisms.

A relevant aspect of this work is that it provides external validation of previously published PK and PD models. The combination of both has made it possible to establish a PK/PD model capable of collecting behavior in the Hispanic Caribbean population, which is different from that used in previous studies. Longitudinal PK model predictions between the structural PK definition of Hamberg *et al.* [7,17] and Reyes-González *et al.* [20] can be found in Fig. 4, where no relevant differences were observed across both PK model structures. This highlights the importance of adapting published structural models to predict the behavior of anticoagulant therapy and corroborates their predictive capacity at the structural level.

Due to the paucity of longitudinal profiles with a high number of INR samples, the maturation times (MTT_1 and MTT_2) of each of the transit-compartment chains were fixed to published values. Although some authors propose a K-PD structure [7], whereby the INR response is not governed by warfarin concentrations, in this article we have generated the PK profiles from the pharmacogenetic information available in each patient to predict the change in response over time as a consequence of warfarin concentrations. Despite the limitation of not having observed concentrations of warfarin and the fact that all patients with the same genetic profile present the same longitudinal profile of warfarin, we believe that this strategy makes it possible to partly mitigate the excessive IIV of warfarin observed in clinical practice.

One of the most surprising results of this study is the IC_{50} values obtained for each of the *VKORC1* haplotypes studied in the Caribbean Hispanic population, which were clearly higher (9.22–11.76 mg/L) than those earlier reported in other populations (1.56–3.11 mg/L) [7,17]. This is consistent with the therapeutic gaps observed in this underrepresented population and suggests increased resistance of Caribbean Hispanic patients to warfarin, who may require more intensive dosing regimens to achieve similar target INR responses. This phenomenon may be the consequence of a longer longitudinal evaluation than in previous studies (>2000 d), as well as a slightly higher distribution of G/G and G/A polymorphisms that may be partially affecting the point estimate. The underlying cause for these increased IC_{50} values remains unclear. However, we also speculate it might in part be linked to the presence of the *NQO1** 2 allele (g .559 C>T, p. P187S) that has been previously associated with warfarin resistance in Hispanics [26,27]. The *NQO1* gene encodes a NAP(H)-dependent quinone oxide reductase enzyme, responsible for catalyzing the reduction of quinones, including vitamin K, into hydroquinone. This mechanism could potentially serve as an alternate vitamin K recycling pathway to VKORC1 in carriers of the haplotype A, who show a lower *VKORC1* gene expression. Nevertheless, this hypothesis needs further validation.

On the other hand, when taking into account the INR values at baseline (INR_{base}) across different *VKORC1* haplotypes, we observed higher measures in A/A carriers (2.18) compared to those in patients with haplotypes containing the G allele (1.78 and 1.84, respectively). This could be explained by a more prolonged prothrombin time and lower levels of functional (active) prothrombin-dependent coagulation factors in carriers of the *VKORC1*-A allele due to their reduced expression of the hepatic VKORC1 enzyme, which plays a pivotal role in the vitamin K cycle in the liver [28]. Vitamin K dihydroquinone is oxidized to vitamin K epoxide during this process and γ-glutamyl carboxylase (GGCX) carboxylates the various hypofunctional coagulation factors

Table 2
Final parameter estimates of the final population pharmacokinetic-pharmacodynamic model of warfarin in Caribbean Hispanic patients.

		Population PK Model Estimates		Bootstrap Results	
Fixed-Effect		Value	Shrinkage (%)	Median	95%CI
MTT_1 (d)		1.13 FIX		1.13 FIX	
MTT_2 (d)		4.62 FIX		4.62 FIX	
Baseline					
	G/A	1.78		1.76	1.48–1.91
	G/G	1.84		1.85	1.53–2.05
	A/A	2.18		2.14	1.97–2.23
IC_{50}					
	G	5.88		5.91	5.61–6.37
	A	4.61		4.63	4.41–4.98
γ		1.47		1.48	1.27–1.61
Inter-individual variability					
Baseline (%)		23	13	24	19–30
IC_{50} (%)		34	44	34	28–39
Residual unexplained variability					
Proportional (%)		27	6	26	22–31

MTT: mean transit time; IC_{50}: concentration resulting in 50% of I_{max}.

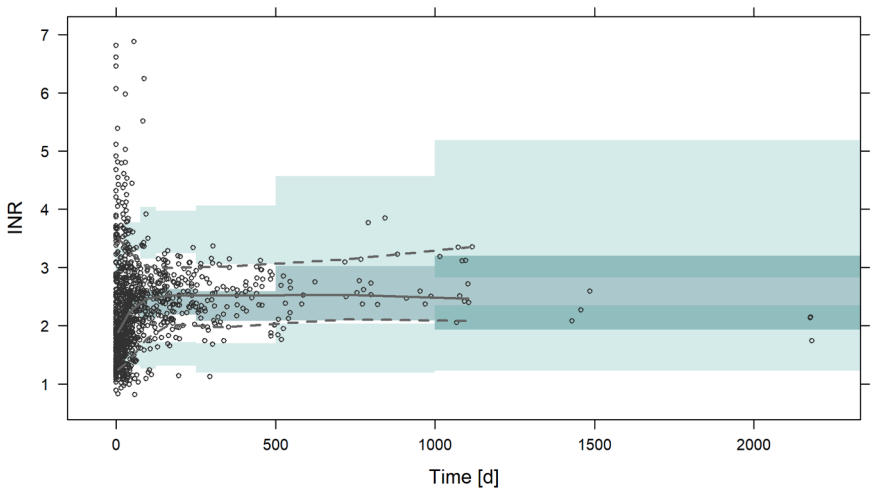

Fig. 2. Prediction-corrected visual predictive check of the final population pharmacodynamic model of INR after warfarin administration. Grey lines represent the median of 2.5th, 50th and 97.5th percentiles of the experimental INR observations. Green shaded areas encompass the 95% confidence intervals of prediction interval at 2.5th, 50th and 97.5th percentiles for the simulated INR data (n = 1000). Empty grey dots represent the experimental INR observations. INR: international normalized ratio.

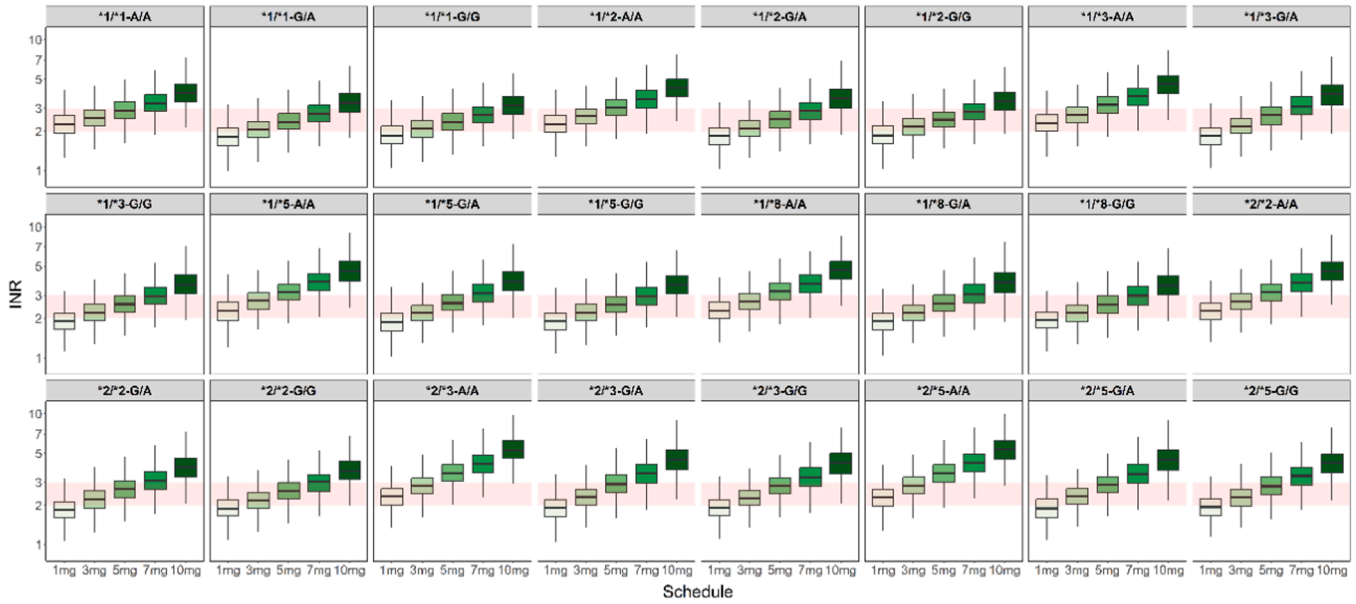

Fig. 3. Stochastic simulations (n = 10,000) of INR levels using the final population PK/PD model assuming different daily dosing regimens for each sub-population of CYP2C9 and VKORC1 polymorphisms. The red band represents the therapeutic INR interval (2−3). INR: international normalized ratio.

involved in the clotting cascade including prothrombin (FII). VKORC1 is responsible for the reduction of vitamin K epoxide back to vitamin K1 and vitamin K dihydroquinone, which is the rate-limiting step in vitamin K recycling. Therefore, even in the absence of any competitive inhibition by warfarin (baseline), reduced VKORC1 levels in carriers of the group A haplotype will deplete the formation of vitamin K1 dihydroquinone. Since vitamin K1 dihydroquinone is the essential cofactor to GGCX, further post-translational activation of hypofunctional coagulation factors is critically compromised in this situation. As a result, the prothrombin time will be prolonged, and the INR level will rise accordingly. This would open the door to hypothesize that *VKORC1* polymorphisms not only affect the potency of warfarin but also influence basal INR levels, partially explaining the excessive inter-individual variability in INR_{base}.

A model-informed optimal dosing regimen selection has been conducted based on the probability of achieving therapeutic INR levels in a virtual population using the fixed and random parameters from the final population PK/PD model. The different sub-populations considered are the result of the combination of *CYP2C9* and *VKORC1* polymorphisms. Overall, the predicted probability in all scenarios reaches therapeutic INR levels in at least 60% of the patients. Previous authors stated that due to the moderate inter-individual variability and residual error and independently of the structural definition of the PK/PD model, probabilities less than 70% are not expected for warfarin dose selection [7, 29]. In this regard, 3–5 mg of daily warfarin would achieve therapeutic INR levels in most of the scenarios considered. The sub-group of patients with * 2/* 2-, * 2/* 3- and * 2/* 5-A/A would require only 1 mg daily of warfarin to achieve therapeutic INR levels at steady-state conditions.

The absence of tailored clinical guidelines considering the impact of distinct *CYP2C9* and *VKORC1* genotypes on routine INR measures in Caribbean Hispanics has led to therapeutic gaps, contributing to suboptimal clinical response rates within this population. Through simulation-based analyses encompassing various patient genotypes resulting from *CYP2C9* and *VKORC1* polymorphisms, this study delved

Table 3
Model-informed dosing regimen selection of daily warfarin in patients with combinatorial *CYP2C9* and *VKORC1* polymorphisms.

Type of patient	Schedule	Probability
* 1/* 1-A/A	3 mg/d	67
* 1/* 1-G/A	5 mg/d	66.7
* 1/* 1-G/G	5 mg/d	66.2
* 1/* 2-A/A	3 mg/d	64.7
* 1/* 2-G/A	5 mg/d	67.3
* 1/* 2-G/G	5 mg/d	69.2
* 1/* 3-A/A	3 mg/d	64.6
* 1/* 3-G/A	3 mg/d	60.7
* 1/* 3-G/G	5 mg/d	65.9
* 1/* 5-A/A	3 mg/d	60.6
* 1/* 5-G/A	5 mg/d	63.6
* 1/* 5-G/G	5 mg/d	65.2
* 1/* 8-A/A	3 mg/d	63.5
* 1/* 8-G/A	5 mg/d	62.4
* 1/* 8-G/G	5 mg/d	63.6
* 2/* 2-A/A	1 mg/d	60.8
* 2/* 2-G/A	5 mg/d	62.5
* 2/* 2-G/G	5 mg/d	63.5
* 2/* 3-A/A	1 mg/d	64.3
* 2/* 3-G/A	3 mg/d	63
* 2/* 3-G/G	3 mg/d	64.9
* 2/* 5-A/A	1 mg/d	63.2
* 2/* 5-G/A	3 mg/d	67.3
* 2/* 5-G/G	3 mg/d	62.4

into optimizing dosing regimens to ensure therapeutic INR levels. Our findings support the significance and clinical relevance of using a population PK/PD approach in elucidating the role of genetic polymorphisms (e.g., *CYP2C9* and *VKORC1* haplotypes) for the optimal design of warfarin dosing schemes in Caribbean Hispanic patients. The simulations provided a comprehensive understanding of how a broader range of *CYP2C9* variants and, specifically, the *VKORC1*-1636 A allele influence the PK and PD of warfarin, as well as baseline INR measures, within this diverse population and shed light on potential underlying mechanisms linked to increased IC_{50} values.

The participants in our study are mainly elderly men, which is a limitation of the study as sex differences in warfarin PK have been suggested in previous studies [30,31]. However, the genotypes included as covariates in the PK/PD analyses were based on the *CPY2C9* and *VKORC1* polymorphisms, which are not sex-linked variants and are therefore unlikely to represent significant sex bias.

5. Conclusions

By performing an external validation of the PK/PD model, we can confidently extrapolate findings from the model to real-world patient scenarios, enabling tailored and precise warfarin dosing recommendations for Caribbean Hispanic individuals. Therefore, this model-informed precision dosing (MIPD) approach is expected to minimize the risk of adverse events linked to inaccurate warfarin dosing due to genetic differences at individual level and enhances therapeutic outcomes. This strategy will ultimately foster safer and more effective clinical management of personalized anticoagulation therapy in this specific patient subgroup, which is often underrepresented in clinical studies. In conclusion, by adapting this MIPD strategy to the Caribbean Hispanic population's unique characteristics, this research underscores the predictive capacity of the PK/PD modeling approach in guiding anticoagulant therapy.

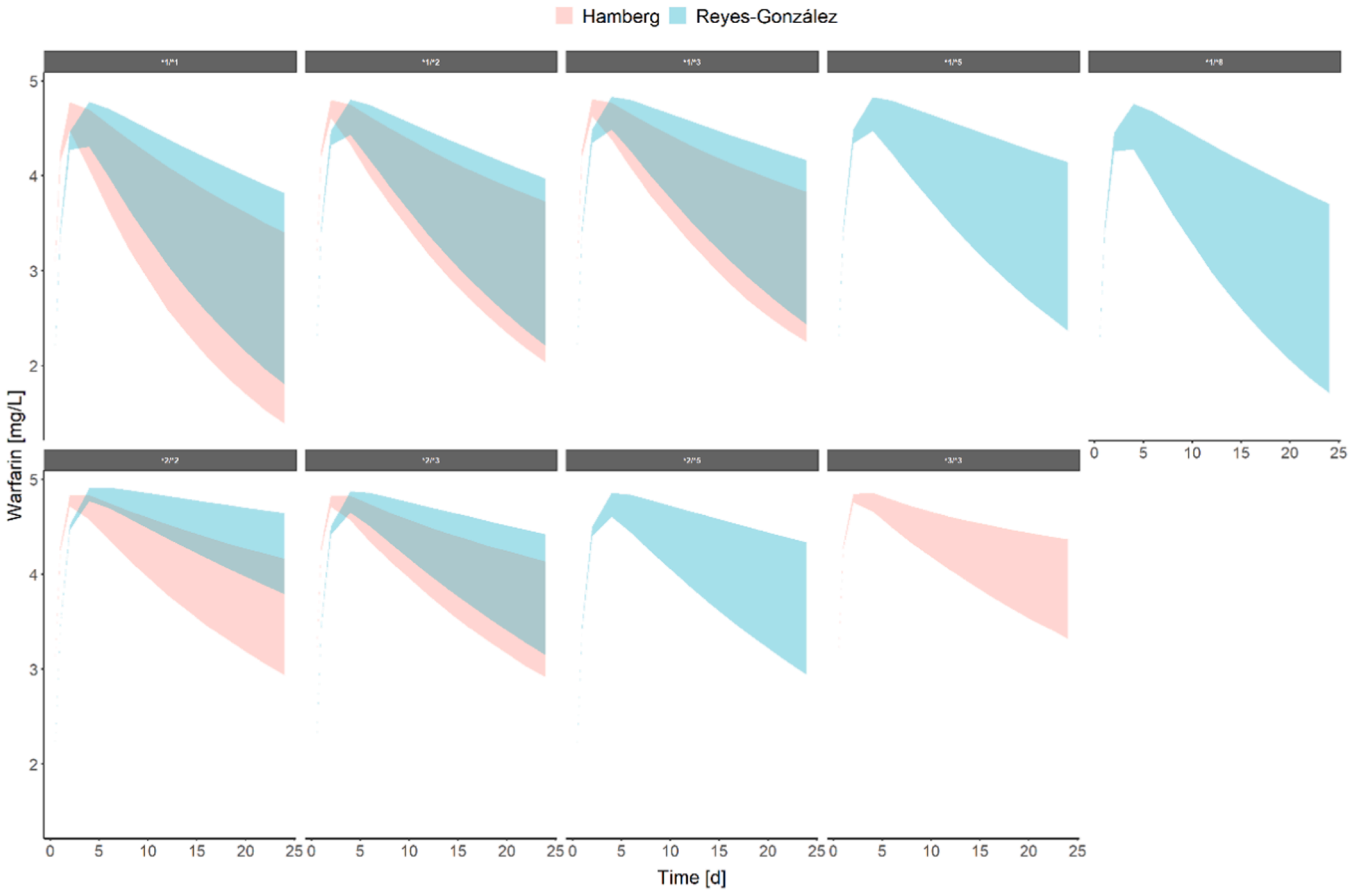

Fig. 4. Comparison of the longitudinal PK model predictions between the structural PK definition of Hamberg et al. (2007, 2010) and Reyes-González et al. (2020).

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CRediT authorship contribution statement

Rodríguez-Fernández Karine: Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Reynaldo-Fernández Gledys:** Data curation, Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Reyes-González Stephanie:** Data curation, Investigation. **de las Barreras Alonso Camila de las Mercedes:** Data curation, Formal analysis, Investigation. **Rodríguez-Vera Leyanis:** Data curation, Methodology. **Vlaar Cornelis:** Conceptualization, Funding acquisition, Resources. **Monbaliu Jean-Christophe M.:** Conceptualization, Funding acquisition, Resources. **Stelzer Torsten:** Conceptualization, Funding acquisition, Resources. **Duconge Jorge:** Conceptualization, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Mangas-Sanjuan Victor:** Formal analysis, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2023.115977.

References

[1] J. Ansell, J. Hirsh, E. Hylek, A. Jacobson, M. Crowther, G. Palareti, Pharmacology and management of the vitamin K antagonists: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition), Chest 133 (6 Suppl) (2008) 160s–198s, https://doi.org/10.1378/chest.08-0670.

[2] R. Wallin, S.M. Hutson, Warfarin and the vitamin K-dependent gamma-carboxylation system, Trends Mol. Med. 10 (7) (2004) 299–302, https://doi.org/10.1016/j.molmed.2004.05.003.

[3] R. Liu, X. Li, W. Zhang, H.H. Zhou, Comparison of nine statistical model based warfarin pharmacogenetic dosing algorithms using the racially diverse international warfarin pharmacogenetic consortium cohort database, PloS One 10 (8) (2015), e0135784, https://doi.org/10.1371/journal.pone.0135784.

[4] I.G. Asiimwe, M. Pirmohamed, Ethnic diversity and warfarin pharmacogenomics, Front. Pharmacol. 13 (2022), 866058, https://doi.org/10.3389/fphar.2022.866058.

[5] K. Huynh, Milestone 6: Birth of the INR, Nat. Rev. Cardiol. (2017), https://doi.org/10.1038/nrcardio.2017.176.

[6] J.A. Johnson, K.E. Caudle, L. Gong, M. Whirl-Carrillo, C.M. Stein, S.A. Scott, M. T. Lee, B.F. Gage, S.E. Kimmel, M.A. Perera, J.L. Anderson, M. Pirmohamed, T. E. Klein, N.A. Limdi, L.H. Cavallari, M. Wadelius, Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Pharmacogenetics-Guided Warfarin Dosing: 2017 Update, Clin. Pharmacol. Ther. 102 (3) (2017) 397–404, https://doi.org/10.1002/cpt.668.

[7] A.K. Hamberg, M. Wadelius, J.D. Lindh, M.L. Dahl, R. Padrini, P. Deloukas, A. Rane, E.N. Jonsson, A pharmacometric model describing the relationship between warfarin dose and INR response with respect to variations in CYP2C9, VKORC1, and age, Clin. Pharmacol. Ther. 87 (6) (2010) 727–734, https://doi.org/10.1038/clpt.2010.37.

[8] International Warfarin Pharmacogenetics Consortium, N.A. Limdi, M. Wadelius, L. Cavallari, N. Eriksson, D.C. Crawford, M.-T.M. Lee, C.-H. Chen, A. Motsinger-Reif, H. Sagreiya, N. Liu, A.H.B. Wu, B.F. Gage, A. Jorgensen, M. Pirmohamed, J.-G. Shin, G. Suarez-Kurtz, S.E. Kimmel, J.A. Johnson, T.E. Klein, M.J. Wagner, Warfarin pharmacogenetics: a single VKORC1 polymorphism is predictive of dose across 3 racial groups, Blood 115 (18) (2010) 3827–3834, https://doi.org/10.1182/blood-2009-12-255992.

[9] H.Y. Yuan, J.J. Chen, M.T. Lee, J.C. Wung, Y.F. Chen, M.J. Charng, M.J. Lu, C. R. Hung, C.Y. Wei, C.H. Chen, J.Y. Wu, Y.T. Chen, A novel functional VKORC1 promoter polymorphism is associated with inter-individual and inter-ethnic differences in warfarin sensitivity, Hum. Mol. Genet. 14 (13) (2005) 1745–1751, https://doi.org/10.1093/hmg/ddi180.

[10] International Warfarin Pharmacogenetics Consortium, T.E. Klein, R.B. Altman, N. Eriksson, B.F. Gage, S.E. Kimmel, M.T. Lee, N.A. Limdi, D. Page, D.M. Roden, M. J. Wagner, M.D. Caldwell, J.A. Johnson, Estimation of the warfarin dose with clinical and pharmacogenetic data, N. Engl. J. Med. 360 (8) (2009) 753–764, https://doi.org/10.1056/NEJMoa0809329.

[11] M. Pirmohamed, G. Burnside, N. Eriksson, A.L. Jorgensen, C.H. Toh, T. Nicholson, P. Kesteven, C. Christersson, B. Wahlström, C. Stafberg, J.E. Zhang, J.B. Leathart, H. Kohnke, A.H. Maitland-van der Zee, P.R. Williamson, A.K. Daly, P. Avery, F. Kamali, M. Wadelius, A Randomized Trial of Genotype-Guided Dosing of Warfarin, N. Engl. J. Med. 369 (24) (2013) 2294–2303, https://doi.org/10.1056/NEJMoa1311386.

[12] P.J. Avery, A. Jorgensen, A.K. Hamberg, M. Wadelius, M. Pirmohamed, F. Kamali, A proposal for an individualized pharmacogenetics-based warfarin initiation dose regimen for patients commencing anticoagulation therapy, Clin. Pharmacol. Ther. 90 (5) (2011) 701–706, https://doi.org/10.1038/clpt.2011.186.

[13] N.S. Ferder, C.S. Eby, E. Deych, J.K. Harris, P.M. Ridker, P.E. Milligan, S. Z. Goldhaber, C.R. King, T. Giri, H.L. McLeod, R.J. Glynn, B.F. Gage, Ability of VKORC1 and CYP2C9 to predict therapeutic warfarin dose during the initial weeks of therapy, J. Thromb. Haemost.: JTH 8 (1) (2010) 95–100, https://doi.org/10.1111/j.1538-7836.2009.03677.x.

[14] U.S. Foods and Drug Administration (FDA), Coumadin (warfarin sodium) tablets label – FDA. Full prescribing information. ⟨https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/009218s107lbl.pdf⟩, 2011 (Accessed 5 August 2023).

[15] A.K. Hamberg, M. Wadelius, L.E. Friberg, T.T. Biss, F. Kamali, E.N. Jonsson, Characterizing variability in warfarin dose requirements in children using modelling and simulation, Br. J. Clin. Pharmacol. 78 (1) (2014) 158–169, https://doi.org/10.1111/bcp.12308.

[16] A.K. Hamberg, L.E. Friberg, K. Hanséus, B.M. Ekman-Joelsson, J. Sunnegårdh, A. Jonzon, B. Lundell, E.N. Jonsson, M. Wadelius, Warfarin dose prediction in children using pharmacometric bridging–comparison with published pharmacogenetic dosing algorithms, Eur. J. Clin. Pharmacol. 69 (6) (2013) 1275–1283, https://doi.org/10.1007/s00228-012-1466-4.

[17] A.K. Hamberg, M.L. Dahl, M. Barban, M.G. Scordo, M. Wadelius, V. Pengo, R. Padrini, E.N. Jonsson, A PK-PD model for predicting the impact of age, CYP2C9, and VKORC1 genotype on individualization of warfarin therapy, Clin. Pharmacol. Ther. 81 (4) (2007) 529–538, https://doi.org/10.1038/sj.clpt.6100084.

[18] U. Falkenhagen, J. Knöchel, C. Kloft, W. Huisinga, Deriving mechanism-based pharmacodynamic models by reducing quantitative systems pharmacology models: an application to warfarin, CPT: Pharmacomet. Syst. Pharmacol. 12 (4) (2023) 432–443, https://doi.org/10.1002/psp4.12903.

[19] J. Deng, V. Vozmediano, M. Rodriguez, L.H. Cavallari, S. Schmidt, Genotype-guided dosing of warfarin through modeling and simulation, Eur. J. Pharm. Sci. Off. J. Eur. Fed. Pharm. Sci. 109s (2017) S9–s14, https://doi.org/10.1016/j.ejps.2017.05.017.

[20] S. Reyes-González, C. de Las Barreras, G. Reynaldo, L. Rodríguez-Vera, C. Vlaar, V. L. Mejias, J.M. Monbaliu, T. Stelzer, V. Mangas, J. Duconge, Genotype-driven pharmacokinetic simulations of warfarin levels in Puerto Ricans, Drug Metab. Pers. Ther. (2020), https://doi.org/10.1515/dmdi-2020-0135.

[21] E. Comets, K. Brendel, F. Mentré, Computing normalised prediction distribution errors to evaluate nonlinear mixed-effect models: the npde add-on package for R, Comput. Methods Prog. Biomed. 90 (2) (2008) 154–166, https://doi.org/10.1016/j.cmpb.2007.12.002.

[22] M. Bergstrand, A.C. Hooker, J.E. Wallin, M.O. Karlsson, Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models, AAPS J. 13 (2) (2011) 143–151, https://doi.org/10.1208/s12248-011-9255-z.

[23] L. Lindbom, P. Pihlgren, E.N. Jonsson, PsN-Toolkit–a collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM, Comput. Methods Prog. Biomed. 79 (3) (2005) 241–257, https://doi.org/10.1016/j.cmpb.2005.04.005.

[24] E.M. Hylek, S. Regan, A.S. Go, R.A. Hughes, D.E. Singer, S.J. Skates, Clinical predictors of prolonged delay in return of the international normalized ratio to within the therapeutic range after excessive anticoagulation with warfarin, Ann. Intern. Med. 135 (6) (2001) 393–400, https://doi.org/10.7326/0003-4819-135-6-200109180-00008.

[25] S.M. Saffian, S.B. Duffull, R.L. Roberts, R.C. Tait, L. Black, K.A. Lund, A. H. Thomson, D.F. Wright, Influence of Genotype on Warfarin Maintenance Dose Predictions Produced Using a Bayesian Dose Individualization Tool, Ther. Drug Monit. 38 (6) (2016) 677–683, https://doi.org/10.1097/ftd.0000000000000347.

[26] D.F. Hernandez-Suarez, K. Claudio-Campos, J.E. Mirabal-Arroyo, B.A. Torres-Hernández, A. López-Candales, K. Melin, J. Duconge, Potential of a pharmacogenetic-guided algorithm to predict optimal warfarin dosing in a high-risk Hispanic patient: role of a novel NQO1*2 Polymorphism, J. Invest. Med. High. Impact Case Rep. 4 (4) (2016), 2324709616682049, https://doi.org/10.1177/2324709616682049.

[27] N. El Rouby, L. Rodrigues Marcatto, K. Claudio, L. Camargo Tavares, H. Steiner, M. R. Botton, S.A. Lubitz, E.N. Fallon, K. Yee, J. Kaye, S.A. Scott, J. Karnes, P. Caleb Junior de Lima Santos, J. Duconge, L.H. Cavallari, Multi-site Investigation of Genetic Determinants of Warfarin Dose Variability in Latinos, Clin. Transl. Sci. 14 (1) (2021) 268–276, https://doi.org/10.1111/cts.12854.

[28] PharmGKB website, Warfarin Pathway Pharmacodynamics. ⟨https://www.pharmgkb.org/pathway/PA145011114⟩, (Accessed 5 August 2023).

[29] PAGE. Abstracts of the Annual Meeting of the Population Approach Group in Europe. ISSN 1871–6032 Reference: PAGE 31 (2023) Abstr 10711 ⟨[]⟨www.page-meeting.org/?abstract=10711⟩].

[30] J. Hirsh, J. Dalen, D.R. Anderson, et al., Oral anticoagulants: mechanism of action, clinical effectiveness, and optimal therapeutic range, Chest 119 (1_suppl) (2001) 8S–21S.

[31] A.E. Rettie, L.C. Wienkers, F.J. Gonzalez, W.F. Trager, K.R. Korzekwa, Impaired (S)-warfarin metabolism catalysed by the R144C allelic variant of CYP2C9, Pharmacogenetics 4 (1) (1994) 39–42.