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Abbreviations:

CYP: cytochrome P450
DDI: drug-drug interaction
TMDD: target mediated drug disposition
PK: pharmacokinetic
PD: pharmacodynamic
CL: clearance
NLME: nonlinear mixed effect
IRB: institutional review board
EBE: empirical bayes estimate
IIV: inter-individual variability
IOV: inter-occasion variability
RUV: residual unexplained variability
EM: expectation-maximization
BQL: below the quantification limit
IMP: importance sampling
RSE: relative standard error
SIR: sampling importance resampling
VPC: visual predictive check
CI: confidence interval
TRT: treatment
LLOQ: lower limit of quantification
CL_Flu: fluconazole effect on clearance
CL_Rif: rifampin effect on clearance
INR: international normalized ratio

LC/MS: liquid chromatography/mass spectrometry

VKORC1: vitamin K epoxide reductase complex subunit 1
Abstract

The objective of this study is to characterize the impact of CYP2C9 genotype on warfarin drug-drug interactions when warfarin is taken together with fluconazole, a cytochrome P450 (CYP) inhibitor, or rifampin, a CYP inducer with a nonlinear mixed effect modeling approach. A target mediated drug disposition model with a urine compartment was necessary to characterize both S-warfarin and R-warfarin plasma and urine pharmacokinetic profiles sufficiently. Following the administration of fluconazole, our study found subjects with CYP2C9 *2 or *3 alleles experience smaller changes in S-warfarin CL compared with subjects without these alleles (69.5%, 64.8%, 59.7% and 47.8% decrease in subjects with CYP2C9 *1/*1, *1/*3, *2/*3 and *3/*3 respectively). Whereas, following the administration of rifampin, subjects with CYP2C9 *2/*3 or CYP2C9 *3/*3 experience larger changes in S-warfarin CL compared with subjects with at least one copy of CYP2C9 *1 or *1B (115%, 111%, 119%, 198% and 193% increase in subjects with CYP2C9 *1/*1, *1B/*1B, *1/*3, *2/*3 and *3/*3 respectively). The results suggest different dose adjustments are potentially required for patients with different CYP2C9 genotypes if warfarin is administered together with CYP inhibitors or inducers.
Significance Statement

The present study found a target mediated drug disposition model is needed to sufficiently characterize the clinical pharmacokinetic profiles of warfarin racemates under different co-treatments in subjects with various CYP2C9 genotypes, following a single dose of warfarin administration. The study also found S-warfarin, the pharmacologically more active ingredient in warfarin, exhibits CYP2C9 genotype-dependent drug-drug interactions, which indicates the dose of warfarin may need to be adjusted differently in subjects with different CYP2C9 genotypes in the presence of drug-drug interactions.
Introduction

Although the use of new direct oral anticoagulants has increased recently, warfarin, a vitamin K antagonist, continues to be one of the most extensively used oral anticoagulants worldwide (Barnes et al., 2015; Mak et al., 2019). However, despite being highly effective in preventing stroke and other thromboembolic events in patients with atrial fibrillation (Takahashi and Echizen, 2001; Hart et al., 2007), warfarin is notorious for its unpredictable pharmacokinetic (PK) and pharmacodynamic (PD) behaviors, narrow therapeutic index and high between-subject variability (Ufer, 2005; Hamberg et al., 2007).

Warfarin is administered orally as a racemic mixture of R- and S-warfarin, in a 1:1 molar ratio. Following oral administration, warfarin enantiomers undergo rapid absorption and are almost completely bioavailable (Ufer, 2005). Although both enantiomers possess pharmacological activity, S-warfarin is much more potent than R-warfarin (Breckenridge et al., 1974; O'Reilly, 1974). Warfarin is eliminated primarily through hepatic metabolism with negligible urinary excretion (Lewis et al., 1974; Ufer, 2005). Various cytochrome P450 (CYP) enzymes are involved in the elimination of R- and S-warfarin to form multiple monohydroxylated metabolites. S-warfarin is metabolized mainly through CYP2C9, whereas R-warfarin is metabolized through various CYP isoforms, such as CYP1A2, CYP2C19 and CYP3A4 (Rettie et al., 1992; Ufer, 2005; Rettie and Tai, 2006).

CYP2C9 is susceptible to substantial genetic polymorphisms with 15% of Caucasians carrying at least one functionally impaired allele of CYP2C9 variants *2 (Arg144Cys) or *3 (Ile359Leu), which have been shown to be closely related to the reduced catalytic activity of CYP2C9 (Flora et al., 2017). Since CYP2C9 is highly associated with the elimination of pharmacologically more active S-warfarin (Ufer, 2005), subjects with reduced CYP2C9 metabolic status, attributable to CYP2C9 *2 or *3 alleles, are subject to higher drug exposure and greater risk of dose-related toxicity. Indeed, studies have reported the CYP2C9 genotype-dependent exposure of S-warfarin (Flora et al., 2017; Xue et al., 2017) as well as the association between CYP2C9 genotype and the risk of warfarin induced toxicity (Kawai et al., 2014).

Additionally, the CYP2C9 regulatory polymorphism *1B (-3089G>A and -2663delTG) has been shown to be significantly associated with determining the maintenance dose of phenytoin because of its effect on phenytoin CYP2C9 auto-induction (Chaudhry et al., 2010). Although,
CYP2C9 *1B has been shown to have little impact on the dose of warfarin in various populations (Veenstra et al., 2005; Chaudhry et al., 2010), its impact on the clearance (CL) of warfarin following the administration of CYP inducers is unknown.

Considerable information has been curated regarding warfarin metabolism, pharmacogenetics, and drug-drug interactions (DDIs) and that information has been incorporated into several warfarin dosing algorithms (Gage et al., 2008; Finkelman et al., 2011; Kimmel et al., 2013; Asiimwe et al., 2021). Nonetheless, warfarin dosing remains challenging and a personalized medicine approach is not yet realized. Additional complications continue to be uncovered and a recent case report highlights the need for further investigations on the gene-DDIs of warfarin (Salem et al., 2021).

We previously reported the impact of CYP2C9 genotypes on the PK or warfarin parent compounds and metabolites. (Flora et al., 2017). The present study is a comprehensive model-based analysis of the impact of CYP2C9 genotype on warfarin drug-drug interactions (DDIs) when warfarin is administered together with CYP inhibitors and inducers. This manuscript is the first of a companion pair (Cheng et al., concurrently published) that extends the analysis using a rigorous nonlinear mixed effect (NLME) model-based analysis that incorporates a target mediated drug disposition (TMDD) model for warfarin. The scope of this paper is a model-based analysis of the impact of CYP2C9 genotype on the DDIs of warfarin’s (R) and (S) enantiomers following administration of the racemic mixture. Built upon the models developed for S- and R-warfarin in this study, the companion paper reports the model-based analysis of 10 warfarin metabolites, which contributes to the mechanistic understanding of CYP2C9 genotype on the DDIs of warfarin enantiomers (Cheng et al., concurrently published).
Methods

Study Population

Study subjects were selected based on their CYP2C9 genotypes from a pharmacogenetics registry (Flora et al., 2017). The CYP2C9 genotyping were performed by the University of Minnesota Genomics Center following the isolation of subjects’ DNA. The genotypes of CYP2C9 *2 (rs1799853) and *3 (rs1057910) were determined using a Taqman probe-based allele determination assays as previously described (Flora et al., 2017). The CYP2C9 *1B genotype was characterized by -3089G>A (rs12782374) and -2663delTG (rs71486745) using assays described in a previous study (Chaudhry et al., 2010). All the genotyping assays were ordered from Applied Biosystems (Foster City, California). It is worth mentioning that although *1B genotypes may occur with multiple CYP2C9 genotype background, the study presented here only involved *1B subjects with a wild-type CYP2C9 background (*1/*1).

Written informed consent was required for subject enrollment. Subjects were eligible for enrollment if they were 18-60 years old, agreed to avoid the use of known CYP2C9 or CYP3A4 substrates, inhibitors, inducers or activators, avoid the ingestion of grapefruit or grapefruit related products, and avoid taking herbal medications or supplements from one week before the beginning of the study period to the end of the study period. Female subjects were eligible for enrollment only if they agreed to avoid conception during the study period. Smokers, subjects with abnormal renal/hepatic functions or abnormal capacity of blood coagulation, and subjects with an allergy to study drugs (warfarin, fluconazole and rifampin) were excluded.

Study Design

The study was an open-label, multi-phase and cross-over clinical pharmacogenetic study approved by the University of Minnesota’s Institutional Review Board (IRB). The study design diagram is shown in Figure 1. Twenty-nine healthy subjects with CYP2C9 *1/*1 (n=8), CYP2C9 *1B/*1B (n=5), CYP2C9 *1/*3 (n=9), CYP2C9 *2/*3 (n=3) and CYP2C9 *3/*3 (n=4) were enrolled in the study. The number of subjects enrolled for each CYP2C9 genotype was determined to detect a 20% difference in S-warfarin 7-hydroxylation between subjects with CYP2C9 *1/*1 and *1/*3 and achieve 80% statistical power (P<0.05) (Kumar et al., 2008). Each subject went through three treatment periods during which warfarin was administered alone, with fluconazole or with rifampin. For the first treatment period of study, each subject was
administered a single 10 mg oral dose of warfarin (Jantoven; Upsher-Smith Laboratories, Maple Grove, Minnesota) after an overnight fast. Seven-mL blood samples were collected prior to the dose and at 2 hours (hr), 6 hr, 1 day (d), 2 d, 3 d, 4 d, 5 d, 6 d, 7 d, 9 d, 11 d for all subjects. Additional blood samples were collected at 13 d for subjects with CYP2C9 *1/*3, CYP2C9 *2/*3 and CYP2C9 *3/*3 and at 15 d for subjects with CYP2C9 *2/*3 and CYP2C9 *3/*3 as the half-life was expected to be longer in these subjects. Urine samples were collected over a 24-hour period on days 1, 4, 7 and 10 following warfarin administration. Each subject underwent a 7-day washout before entering the second treatment period of study. For the second treatment period, subjects were randomized to receive either 400 mg fluconazole or 300 mg rifampin orally once per day for 7 consecutive days as pretreatment to allow the fluconazole/rifampin interaction capacity to reach steady state. After pretreatment, a 10 mg oral dose of warfarin was administered followed by the same blood and urine sampling scheme as the first treatment period. The administration of fluconazole or rifampin was continued until the end of sampling. Another 7-day washout period was required before entering the third treatment period. The design of the third period was the same as the second period with subjects crossing over to the alternative interacting drug. S-warfarin and R-warfarin concentrations in blood and urine samples were analyzed by liquid chromatography/mass spectrometry (LC/MS) as previously described (Miller et al., 2009; Flora et al., 2017).

**PK Modeling**

Dose-dependent changes in the volume of distribution have been observed in several preclinical and clinical studies with warfarin (Takada and Levy, 1979; Takada and Levy, 1980; King et al., 1995). To explain this unusual PK behavior exhibited by warfarin, Levy et al. proposed a complex PK phenomenon termed target mediated drug disposition (TMDD) for the first time in 1994 (Levy, 1994) and successfully characterized warfarin clinical PK profiles with a TMDD model in 2003 (Levy et al., 2003). With the rapid development of therapeutic biologics in the early 2000s, the TMDD model has been widely used to explain the unusual PK nonlinearity in monoclonal antibodies (Luu et al., 2012; Vexler et al., 2013; Zheng et al., 2014). In addition, several studies published recently readdressed the importance of the application of the TMDD models in small molecule drugs as well (Yamazaki et al., 2013; An et al., 2015; An, 2017).
The PK models used for fitting both S- and R-warfarin PK profiles are adapted from the TMDD model proposed for warfarin by Levy et al (Levy et al., 2003; Bach et al., 2019) (Figure 2). The model is described by equations (1-6) as shown below.

\[
\frac{dA_{\text{depot}}}{dt} = -K_a \times A_{\text{depot}} \quad (1)
\]

\[
\frac{dA_{\text{cent}}}{dt} = K_a \times A_{\text{depot}} - CL \times \frac{A_{\text{cent}}}{V_C} - K_{on} \times A_{\text{cent}} \times A_R + K_{off} \times A_{DR} \times V_C - CL_D \times \left( \frac{A_{\text{cent}}}{V_C} - \frac{A_{\text{periph}}}{V_P} \right) \quad (2)
\]

\[
\frac{dA_R}{dt} = -K_{on} \times \frac{A_{\text{cent}}}{V_C} \times A_R + K_{off} \times A_{DR} \quad (3)
\]

\[
\frac{dA_{DR}}{dt} = K_{on} \times \frac{A_{\text{cent}}}{V_C} \times A_R - K_{off} \times A_{DR} \quad (4)
\]

\[
\frac{dA_{\text{periph}}}{dt} = CL_D \times \left( \frac{A_{\text{cent}}}{V_C} - \frac{A_{\text{periph}}}{V_P} \right) \quad (5)
\]

\[
\frac{dA_{\text{urine}}}{dt} = CL_r \times \frac{A_{\text{cent}}}{V_C} \quad (6)
\]

where \( A_{\text{depot}}, A_{\text{cent}}, A_{\text{periph}} \) and \( A_{\text{urine}} \) represent amounts in depot, central, peripheral and urine compartments, respectively. \( A_R \) and \( A_{DR} \) represent concentrations in receptor and drug-receptor complex compartments, respectively. The definitions for other parameters are provided in Table 1 and 2.

The S- and R-warfarin data sets were modeled independently. For each enantiomer, plasma data from all three treatment periods were fit simultaneously. Equations (1-5) were used to estimate the parameters of each parent drug in plasma. A sequential modeling approach was applied for plasma and urine data. Once an adequate model with drug interaction parameters for plasma concentrations was determined, the Empirical Bayes Estimates (EBE) of individual PK parameters were exported and merged into the data set. Equation (6) was added and the drug amounts from the 12 urine collections (4 collection times per treatment period) were fitted to estimate the renal CL (CLR) portion of total CL. The bioavailability for each parent compound was assumed to be 1 for each dose during the study.

For the first treatment period, baseline plasma concentrations for S- and R-warfarin in central and peripheral compartments were assumed to be 0 given no detectable baseline warfarin concentrations at the beginning of first period. Baseline level of receptor compartment (R) was
parameterized as $R_{BL}$ for estimation and baseline level of drug-receptor complex compartment (DR) was set as 0.

For the second and third treatment periods, warfarin concentrations were still occasionally measured after the 7-day washout period. The system was reinitialized at the beginning of subsequent treatments but baseline concentrations for S- and R- warfarin in central and peripheral compartments were parameterized as BL and $BL_P$ for estimation. Assuming a steady state at baseline for R (receptor) and DR (drug-receptor complex) compartments, equations (7-8) could be written as shown below.

\[
A_R + A_{DR} = R_{BL} \quad (7)
\]

\[
K_{on} \times \frac{A_{cent}}{V_c} \times A_R - K_{off} \times A_{DR} = 0 \quad (8)
\]

Given baseline concentration in central compartment is parameterized as BL, equation (8) could be written as equation (9).

\[
K_{on} \times BL \times A_R - K_{off} \times A_{DR} = 0 \quad (9)
\]

With equation (7) and equation (9), $A_R$ and $A_{DR}$ baseline levels could be solved as shown by equations (10-11).

\[
A_R = \frac{K_{off} \times R_{BL}}{K_{on} \times BL + K_{off}} \quad (10)
\]

\[
A_{DR} = \frac{K_{on} \times BL \times R_{BL}}{K_{on} \times BL + K_{off}} \quad (11)
\]

Equations (10-11) were used for calculating baseline levels of R and DR compartments for study periods with fluconazole and rifampin (periods 2 and 3, respectively).

The covariate effects of $CYP2C9$ genotypes and co-treatments were added on PK parameters using equation (12) and equation (13), respectively as shown below.

\[
TVP = TVP_{ref} \times P_{Geno} \quad (12)
\]

\[
TVP = TVP_{ref} \times P_{TRT} \quad (13)
\]

where ($TVP$: typical values of parameters; $TVP_{ref}$: typical values of parameters in reference groups; $P_{Geno} i$: $CYP2C9$ genotype effect on parameters ($i = 1, 2, 3, 4, 5$ represent $CYP2C9$ genotypes).
*1/*1. *1B/*1B, *1/*3, *2/*3, *3/*3, respectively); \( P_{TRT} \): co-treatment effect on parameters (TRT: Flu: fluconazole, Rif: rifampin))

If an association between \( P_{TRT} \) and \( CYP2C9 \) genotypes was detected visually, \( CYP2C9 \) genotypes were added as a covariate on \( P_{TRT} \) using equation (14).

\[
P_{TRT} = P_{TRT \_Geno \ i} \tag{14}
\]

where \((P_{TRT \_Geno \ i}: \) co-treatment effect on parameters for subjects with genotype \( i \) (\( i = 1, 2, 3, 4, 5 \) represent \( CYP2C9 \) \*1/*1. *1B/*1B, *1/*3, *2/*3, *3/*3, respectively))

A covariate introducing a 3.84 decrease in objective function values (OFVs) with one degree of freedom at an \( \alpha \) level of 0.05 is considered to be statistically significant.

During model development, \( K_a \) and \( BLP \) were found to be estimated with inadequate precision. Since the warfarin is generally considered to be rapidly absorbed with almost complete bioavailability (Ufer, 2005), the bioavailability of warfarin was assumed to be 100\% and the \( K_a \) of both S- and R- warfarin were arbitrarily fixed as 2 hr\(^{-1}\). \( BLP \) were determined to fix as the closest positive integer value to the estimated values, which is 1 \( \mu \)g/L.

All the inter-individual variabilities (IIVs) were parameterized as log-normal distributions, as was inter-occasion variability (IOV) on \( R_{BL} \). Residual unexplained variabilities (RUVs) were parameterized as proportional errors. All the IIVs and IOVs are assumed to be independent during plasma PK modeling so no off diagonal elements were estimated. In contrast, full omega matrices were estimated during urine PK modeling. MU-referencing is used for improving the efficiency of expectation-maximization (EM) based optimization methods in NONMEM (Bauer, 2019). Fixed 1\% IIVs were assumed for unwanted IIV terms to facilitate the optimization efficiency of EM based methods (Chigutsa et al., 2017). Due to the existence of plasma concentrations below the quantification limit (BQL) in R-warfarin PK data, the M3 method (Ahn et al., 2008; Bergstrand and Karlsson, 2009) suggested by Stuart Beal was utilized for fitting R-warfarin plasma PK profiles. All the modeling codes are provided in the supplementary materials (R- and S-warfarin plasma and urine PK model NONMEM codes).

**Model Evaluation**

The model fitting was evaluated by standard diagnostic plots and visual prediction checks (VPCs) with 200 simulations. The precision of parameter estimations was assessed by relative standard
error (RSE) in the output and 95% confidence intervals (CIs) generated following sampling importance resampling (SIR) procedures (Dosne et al., 2016).

Model-based Analysis on S-warfarin CL

Following the model development, the typical values of the effect of fluconazole and rifampin on S-warfarin CL (CL_Flu and CL_Rif) in subjects with different CYP2C9 genotypes were exported. The percent changes in CL of S- and R-warfarin following the administration of warfarin together with fluconazole or rifampin is calculated using equation (15) as shown below.

$$\% \text{ changes in CL} = |CL_{TRT} - 100\%|$$ (15)

where ($| CL_{TRT} - 100\% |$: absolute difference between co-treatment effects on CL and 100% (TRT: Flu: fluconazole, Rif: rifampin))

The 95% CIs were constructed with the RSE estimated from the covariance step by assuming a symmetrical normal distribution. The typical values and constructed 95% CIs were then plotted and compared.

Software

All the model fittings were performed using the EM-based algorithm, Importance Sampling (IMP) with interaction, using MU-referencing and “AUTO=1” option, within NONMEM 7.4 (ICON Development Solutions, Ellicott City, Maryland) (Bauer, 2015). SIR and VPCs were performed with Perl-speaks-NONMEM (PsN 4.9.0, Uppsala, Sweden) within Pirana (Keizer et al., 2011). Plots were generated with R 3.6.3 (The R Foundation for Statistical Computing) and Rstudio 1.1.453 (Rstudio, Inc., Boston, Massachusetts).
Results

Data Summary

The demographic information for subjects involved in the study are provided in the supplementary materials (Table S1). Data were available from 29 subjects that provided 957 S-warfarin plasma concentrations, all of which were above the lower limit of quantification (LLOQ, 0.67 ng/mL for S-warfarin). Those blood samples also provided 940 R-warfarin plasma concentrations. Of the 921 non-baseline R-warfarin plasma concentrations, 24 measurements (2.6%) were below the LLOQ (0.67 ng/mL for R-warfarin). 258 and 266 urine amount measurements were included in S- and R-warfarin urine PK model development, respectively. Not all subjects participated in three study periods; six subjects only participated in two study periods and one subject only participated in one study period. These subjects were included in the analysis.

S-warfarin and R-warfarin plasma and urine PK profiles for subjects with different CYP2C9 genotypes stratified by co-treatments are plotted in Figure 3. The S-warfarin PK profiles in both plasma and urine under warfarin only treatment (Figure 3 (A) and (B) left) clearly demonstrate CYP2C9 genotype-dependent drug elimination. In contrast, R-warfarin plasma and urine PK profiles under warfarin only treatment indicate the elimination of R-warfarin is independent of CYP2C9 genotypes (Figure 3 (C) and (D) left). Comparing S- and R-warfarin PK profiles under different co-treatments, the elimination appears to be slower and faster after the administration of fluconazole and rifampin, respectively.

S-warfarin Model Parameters

The S-warfarin plasma PK model was able to converge after the inclusion of CYP2C9 genotypes and co-treatments as covariates on CL. A TMDD model with a peripheral compartment (equations (1)-(5)) was able to simultaneously characterize S-warfarin plasma PK profiles in the three treatment periods. Initially, the estimates for absorption rate constant (Ka) and the baseline concentration in the peripheral compartment (BLp) were estimated with inadequate precision (large %RSE). These parameters were then fixed as biologically plausible values as suggested in the methods section. In addition, the estimations of Kon and Koff exhibited a high degree of correlation and were initially estimated with poor precision. Literature reported Koff for racemic
warfarin (Levy et al., 2003) was then fixed in the model, which enabled a precise estimation of \( K_{on} \).

Subsequent visual inspections of the fluconazole effect on CL (CL_Flu) vs \( CYP2C9 \) genotype plot (Figure S2 left) demonstrated the \( CYP2C9 \) genotype-dependent changes in CL following the administration of fluconazole, with subjects possessing the \( CYP2C9 *2 \) or *3 variants exhibiting smaller percentage changes. In contrast, visual inspection of the rifampin effect on CL (CL_Rif) vs \( CYP2C9 \) genotypes (Figure S2 right) demonstrated \( CYP2C9 \) genotype-dependent changes of CL following the administration of rifampin, with subjects possessing \( CYP2C9 *2 \) or *3 variants exhibiting larger percentage changes. Thus, \( CYP2C9 \) genotype was added as a covariate to CL_Flu and CL_Rif. Further visual inspections of the central volume of distribution (\( V_C \)), association rate constant (\( K_{on} \)) and baseline receptor level (\( R_{BL} \)) vs \( CYP2C9 \) genotype relationships showed that subjects with \( CYP2C9 *2/*3 \) exhibit lower \( V_C \) and subjects with \( CYP2C9 *2/*3 \) and \( CYP2C9 *3/*3 \) exhibit lower \( K_{on} \) and higher \( R_{BL} \). These covariate effects were then added as fractions for estimation. The inclusion of IOV on \( R_{BL} \) significantly decreased the objective function value (OFV, -56.739).

The EBE of individual PK parameters of S-warfarin were exported to the data set following the development of the plasma PK model. The urine PK model (equation (6)) for S-warfarin was developed subsequently with S-warfarin urine PK data. The final parameter estimations for S-warfarin are shown in Table 1.

**R-warfarin Model Parameters**

Similar to the S-warfarin plasma PK model, the R-warfarin plasma PK model was able to converge after the inclusion of co-treatments as a covariate on CL. A TMDD model with a peripheral compartment (equations (1)-(5)) was able to sufficiently characterize the R-warfarin plasma PK profiles under different co-treatments simultaneously. The model parameters \( K_a, BL_p \) and \( K_{off} \) were fixed as described for the S-warfarin PK model to avoid inadequate precision in model parameter estimations.

Visual inspection of model parameter vs \( CYP2C9 \) genotype relationships found subjects with \( CYP2C9 *2/*3 \) and \( CYP2C9 *3/*3 \) tended to have a lower and higher \( V_C \), respectively. Subjects with \( CYP2C9 *1/*3 \), \( CYP2C9 *2/*3 \) and \( CYP2C9 *3/*3 \) tend to have a lower \( R_{BL} \) and subjects with \( CYP2C9 *2/*3 \) tend to have a higher CL_Rif. These covariate effects were added as a
fractional multiplier for estimations. The inclusion of IOV on R_{BL} significantly decreased the OFV (-63.796).

The EBE of individual PK parameters of R-warfarin were exported to the data set following the development of the plasma PK model. Afterwards, a urine PK model (equation (6)) for R-warfarin was developed subsequently with R-warfarin urine PK data. The final parameter estimations for R-warfarin are shown in Table 2.

*Model Evaluations*

The visual prediction checks (VPCs) for S-warfarin plasma and urine PK profiles and R-warfarin plasma and urine PK profiles stratified by both CYP2C9 genotype and co-treatments are shown in Figure 4 and 5. In general, the VPCs suggested all the models developed were able to explain the PK observations reasonably well. The relative standard error (RSE) generated with covariance step and 95% CIs assessed by SIR suggested the model parameters were estimated with reasonable precisions (Table 1-2).

Standard diagnostic plots (Figures S3-S6: S-warfarin; Figure S8-S11: R-warfarin) stratified by either CYP2C9 genotype or co-treatments and individual PK profile fittings (Figure S7: S-warfarin; Figure S12: R-warfarin) provide insufficient evidence to reject the models.

*CYP2C9 Genotype-dependent DDIs Exhibited by S-warfarin*

The parameter estimations from our model demonstrate the existence of the CYP2C9 genotype-dependent changes in S-warfarin CL following the administration of fluconazole and rifampin (Figure 6). The percentage inhibition in S-warfarin CL following the administration of fluconazole is largest in subjects with CYP2C9 *1/*1, followed by subjects with CYP2C9 *1/*3, CYP2C9 *2/*3 and CYP2C9 *3/*3. In contrast, the percentage induction in S-warfarin CL following the administration of rifampin is much smaller in subjects with at least one copy of CYP2C9 *1 or *1B (*1/*1, *1B/*1B, *1/*3) than subjects without CYP2C9 *1 or *1B (*2/*3, *3/*3).
Discussion

Numerous studies have been conducted to investigate the PK of warfarin since its introduction into clinical practice in the 1950s (Wen and Lee, 2013). Although CYP2C9 genotype-dependent CL of S-warfarin has been shown in many studies (Hamberg et al., 2007; Gong et al., 2011; Flora et al., 2017; Xue et al., 2017), few have investigated the impact of the CYP2C9 genotypes on warfarin DDIs. Taking advantage of PK data collected from a well-designed clinical warfarin DDI study, our study performed comprehensive population PK analysis on both S- and R-warfarin in plasma and urine, either administered alone or together with different co-medications. Our study confirmed the existence of CYP2C9 genotype-dependent CL of S-warfarin, but not R-warfarin. More importantly, our study supports the existence of CYP2C9 genotype-dependent DDIs of S-warfarin, the major active component in warfarin, when warfarin is administered with either fluconazole or rifampin. The study results indicate subjects with different CYP2C9 genotypes potentially require different warfarin dose adjustments when warfarin is administered together with CYP inhibitors or inducers.

One of the obvious characteristics of small molecule drugs exhibiting TMDD is the dose-dependent changes in apparent volume of distribution. This is caused by the saturation of the high affinity, low capacity binding sites at relatively high doses rather than low doses (An, 2017; Bach et al., 2019). This phenomenon was first reported by Dr. Gerhard Levy based on extensive preclinical studies conducted on warfarin PK (Takada and Levy, 1979; Takada and Levy, 1980). In fact, the term target mediated drug disposition (TMDD) was first proposed by Dr. Levy in 1994 to explain the nonlinear PK behavior exhibited by small molecule drugs like warfarin (Levy, 1994). In spite of the relatively high prevalence of applying the TMDD models in characterizing the PK of large molecules, its usefulness in modeling small molecule compounds has gained recognition only recently (An, 2017). Indeed, with a linear compartmental PK model, we failed to fit either S- or R-warfarin plasma PK profiles under different co-treatments simultaneously (Figure S1, Table S2). Interestingly, adequate fitting can be achieved with linear compartmental models if the PK profiles in each treatment period are fitted separately. However, a higher volume of distribution was estimated when warfarin is administered together with rifampin, and unrealistically long terminal half-lives were estimated. To some extent, this is consistent with the dose-dependent changes in volume of distribution shown by an early warfarin clinical PK study, in which a higher volume of distribution is shown for subjects with lower...
doses (King et al., 1995). We suspected that when either a low dose of warfarin is administered or warfarin is cleared faster following the co-administration of a CYP inducer, the unsaturation of the high affinity, low capacity binding sites causes a higher apparent volume of distribution to be estimated. Additionally, a prolonged terminal phase was commonly observed for small molecule drugs exhibiting TMDD (An et al., 2015). The back-extrapolation to the intercept of the prolonged terminal phase normally converges to the same concentration regardless of dose (An, 2017; Bach et al., 2019). This is because the high affinity binding between drugs and binding sites makes the dissociation between them extremely slow, which becomes the rate-limiting step for drug elimination when drug concentration in the plasma is low (Bach et al., 2019).

Although the phenomenon of TMDD for certain small molecule drugs dates back many years, the application of TMDD models in modeling warfarin PK is rare (Levy, 1994; Mager and Jusko, 2001). This is not surprising given the difficulties in study design to enable observation of the TMDD type of PK behavior in small molecule drugs like warfarin. Although the unsaturation of binding sites at relatively low doses causes a higher volume of distribution to be estimated, following repeated low doses, the binding sites are generally saturated, which leads to observations of linear PK (An, 2017; Bach et al., 2019). Thus, a single dose study with different dosage levels is normally required to fit a TMDD model adequately. In addition, to capture the prolonged terminal phase, a relatively long follow up time is also required. Given many studies were conducted with patients taking warfarin on a regular basis or relatively short follow up time following single dose of administration (Hamberg et al., 2007; Xue et al., 2017), it is not surprising that linear compartmental models are still widely used for modeling warfarin PK in these studies. Additionally, TMDD models are known to be overparametrized and are difficult to converge (Gibiansky et al., 2008). Indeed, a full TMDD model was tested initially. However, several model parameters such as $K_{on}$ and $K_{off}$ were highly correlated and cannot be estimated with adequate precisions. Thus, $K_{off}$ values of warfarin were fixed to the literature reported value (Levy et al., 2003) and only $K_{on}$ were estimated. While this approach is sufficient to overcome the difficulties we encountered, several approximation methods of TMDD, such as Quasi-equilibrium, could be used if reliable $K_{off}$ values are not available (Mager and Krzyzanski, 2005; Gibiansky et al., 2008). During the model development, $K_a$ and $B_L$ were estimated with inadequate precision and were also fixed in the model as 2 hr$^{-1}$ and 1 µg/L, respectively. Similar
values of $K_a$ were used for developing other S- and R-warfarin PK models (Hamberg et al., 2007; Xue et al., 2017). BL$_P$ were determined to fix as the closest positive integer value to the estimated values, which is 1 ug/L. Given the initial concentration of both S- and R-warfarin can reach as high as 1000 ug/L and the scale of $V_C$ and $V_P$ are similar, a 1 ug/L BL$_P$ is highly unlikely to be impactful (Table 1).

Polypharmacy is more prevalent in older individuals (Maher et al., 2014). A better understanding in CL changes of warfarin, especially when warfarin is administered together with either CYP inhibitors or inducers, is critical to adjust warfarin doses rationally for patients under polypharmacy. Interestingly, our study shows subjects with $CYP2C9$ *2 or *3 alleles experience a smaller and larger percentage of CL changes for S-warfarin following the administration of fluconazole and rifampin, respectively. Since S-warfarin is the more active enantiomer in the racemate, smaller dose adjustments should be made for subjects with $CYP2C9$ *2 or *3 variants, when they take warfarin together with fluconazole. In contrast, larger dosing adjustments should be made for these subjects when they take warfarin together with rifampin. It is also worth mentioning that both fluconazole and rifampin are non-specific CYP inhibitors and inducers, respectively. The differences in the percentage of fluconazole inhibition or rifampin induction in S-warfarin CL for patients with different $CYP2C9$ genotypes might indicate certain CYP enzymes involved in warfarin elimination are potentially more inhibitable or inducible than others. The warfarin metabolic profile changes following the administration of CYP inhibitors or inducers, in subjects with different $CYP2C9$ genotypes, are evaluated in our companion study, where the PK profiles of 10 warfarin metabolites under different treatment conditions are modeled on the basis of the parent compound models presented here (Cheng et al., concurrently published). The elucidation of metabolic profile changes of warfarin, following the administration of non-specific CYP inhibitors or inducers, is not only useful in gaining more mechanistic insights behind the $CYP2C9$ genotype-dependent DDIs exhibited by S-warfarin, but also valuable to inform the DDIs of other drugs which undergo similar metabolic pathways.

Although the impact of $CYP2C9$ alone on warfarin therapeutic outcomes, such as international normalized ratio (INR), was well recognized (Ufer, 2005), the impact of $CYP2C9$ on warfarin DDIs in the context of therapeutic outcomes has rarely been investigated. Hamberg et al. developed a PK-PD model for warfarin (Hamberg et al., 2007) and demonstrated the EC$_{50}$ of INR responses to S-warfarin concentrations vary across different vitamin K epoxide reductase
complex subunit 1 (VKORC1) genotypes (GG: 4.61 mg/L, GA: 3.20 mg/L, AA: 2.20 mg/L) and the IIV of PD parameters are relatively high. It is likely the PD variability of warfarin overwhelms the PK variability of warfarin, which causes the PK variability of warfarin less of a concern when monitoring INR. Nevertheless, many studies suggested CYP2C9 and VKORC1 genetic polymorphisms together can account for up to 30% of total variability in warfarin doses, in which VKORC1 and CYP2C9 genetic polymorphism alone can account for 25% and 9% respectively (Limdi et al., 2008; Fung et al., 2012). Furthermore, a recent clinical case report showed that a subject with CYP2C9 *3/*3 and VKORC1 GA mutations required a larger magnitude of warfarin dose adjustments while warfarin was treated together with rifampin (Salem et al., 2021), the conclusion of the case report is consistent with our findings to some extent. Thus, the impact of CYP2C9 genotype-dependent DDIs on the therapeutic outcomes of warfarin is ambiguous, which may warrant further investigations given the narrow therapeutic index of warfarin. Although the dosing of warfarin remains challenging, substantial progress has been made using model-informed approach. For example, Wright et al proposed warfarin dose individualization under a Bayesian framework which allow the maintenance of the steady state INR 65% to 80% of time within the therapeutic index of warfarin when more than 3 INR measurements are available (Wright and Duffull, 2013). The incorporation of TMDD mechanism presented in this study may provide more mechanistic insights about warfarin dispositions and further improves the warfarin dosing in scenarios such as warfarin treatment initialization and discontinuation.

Limitations were noted in the present study. For example, several covariate effects, such as the CYP2C9 effects on $K_{on}$, $V_C$ and $R_{BL}$, are lacking mechanistic basis although they are statistically significantly. Indeed, the original objective of this clinical study is to characterize and quantify the CYP2C9 genotype-dependent DDIs of warfarin. Based on the purpose of the study, small number of subjects (n=29) were enrolled and limited number of covariates were collected. During the model development, we found the PK variabilities cannot be fully accounted by the effect of CYP2C9 and drug interactions on CL. This is anticipated given the PK of warfarin is notoriously known to be impacted by many factors, such as diet and drug transporter functions, that were not collected in the current study (Ovesen et al., 1988; Bi et al., 2018). Thus, although lacking mechanistic basis, these covariate effects were still decided to be added to account for some observed PK variability so that the CYP2C9 genotype-dependent DDIs of warfarin can be
better characterized. However, future clinical studies with more covariates collected may be warranted to validate these covariate effects.

In summary, we conducted a comprehensive NLME PK analysis to evaluate the impact of \textit{CYP2C9} genotypes on both S- and R-warfarin DDIs. Our study found subjects with different \textit{CYP2C9} genotypes experience differences in S-warfarin CL changes following the administration of CYP inhibitors or inducers, indicating \textit{CYP2C9} genotype-dependent warfarin dose adjustments are potentially required. In the future, connecting with literature reported PD models, the PK models presented in this study are potentially useful in informing dose adjustments on the basis of therapeutic outcome predictions. Thus, the models presented in this study may serve as a valuable tool for optimizing warfarin dosing adjustments in a polypharmacy setting.
Author Contributions

Participated in research design: Cheng, Flora, Rettie, Brundage, Tracy
Conducted experiments: Flora, Rettie, Tracy
Performed data analysis: Cheng, Brundage
Wrote or contributed to the writing of the manuscript: Cheng, Flora, Rettie, Brundage, Tracy
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Cheng S, Flora DR, Tracy TS, Rettie AE and Brundage RC (concurrently published) Pharmacokinetic Modeling of Warfarin II – Model-based Analysis of Warfarin Metabolites following Warfarin Administered either Alone or Together with Fluconazole or Rifampin.


**Footnotes**

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Figure legends

Figure 1. Study Design Diagram. Each subject went through 3 study periods (upper dark-blue section). Period 1 (red box), single 10mg dose of warfarin. Periods 2 and 3 in crossover (yellow box), each subject was pretreated with either 400mg fluconazole or 300mg rifampin once daily for 7 consecutive days, followed by a single 10mg dose of warfarin and continuous treatment with either 400mg fluconazole or 300mg rifampin once daily through the sampling phase. Notes: q.d.: Once daily.

Figure 2. PK model structure for S- and R-warfarin. Notes: Periph: peripheral; Cent: central; R: receptors; DR: drug-receptor complexes.

Figure 3. PK profiles for S-warfarin in plasma (A) and urine (B) and R-warfarin in plasma (C) and urine (D). All the PK profiles are stratified by co-treatments. Colors represent different CYP2C9 genotypes as shown in figure legends. Plots are on log scales. Points represent mean and error bars represent 95% confidence intervals.

Figure 4. Visual prediction checks (VPCs) for S-warfarin PK profiles in plasma (A) and urine (B). Blue dots represent the observations. Red solid lines represent the medians of model predicted concentrations. The upper and lower red dashed lines represent the 10th and 90th percentiles of the model predicted concentrations, respectively. The figure is stratified by genotypes and co-treatments. The black dashed lines represent the lower limit of quantification (LLOQ) for S-warfarin (0.67 ng/mL). No observations were collected from CYP2C9 *1B/*1B subjects and treated with warfarin plus fluconazole. Note: Warf: Warfarin; Flu: Fluconazole; Rif: Rifampin; *1/*1: CYP2C9 *1/*1; *1B/*1B: CYP2C9 *1B/*1B; *1/*3: CYP2C9 *1/*3; *2/*3: CYP2C9 *2/*3; *3/*3: CYP2C9 *3/*3.

Figure 5. Visual prediction checks (VPCs) for R-warfarin PK profiles in plasma (A) and urine (B). Blue dots represent the observations. Red solid lines represent the medians of model predicted concentrations. The upper and lower red dashed lines represent the 10th and 90th percentiles of the model predicted concentrations, respectively. The figure is stratified by genotypes and co-treatments. The black dashed lines represent the lower limit of quantification (LLOQ) for R-warfarin (0.67 ng/mL). No observations were collected from CYP2C9 *1B/*1B subjects and treated with warfarin plus fluconazole. Note: Warf: Warfarin; Flu: Fluconazole; Rif: Rifampin; *1/*1: CYP2C9 *1/*1; *1B/*1B: CYP2C9 *1B/*1B; *1/*3: CYP2C9 *1/*3; *2/*3: CYP2C9 *2/*3; *3/*3: CYP2C9 *3/*3.
Figure 6. Genotype-dependent CL changes of S-warfarin following the administration of fluconazole (A) and rifampin (B). The dots and error bars represent the typical values and 95% confidence intervals (CIs), respectively. The 95% CIs are constructed with relative standard error (RSE) as shown in Table 1 assuming a symmetric normal distribution.
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Definitions</th>
<th>Estimates (RSE)</th>
<th>SIR medians (95% CIs)</th>
<th>HV/IOV Estimates (RSE)</th>
<th>HV/IOV SIR medians (95% CIs)</th>
<th>Shrinkage</th>
<th>Units</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_a$</td>
<td>Absorption rate constant</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>/hour</td>
<td>Fixed</td>
</tr>
<tr>
<td>CL</td>
<td>Clearance for subjects with CYP2C9 *1/*1 when warfarin is administered alone</td>
<td>0.260 (8%)</td>
<td>0.261 (0.221, 0.301)</td>
<td>22.9% (19%)</td>
<td>22.7% (17.5%, 27.8%)</td>
<td>1%</td>
<td>L/hour</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>$V_C$</td>
<td>Central compartment volume of distribution for subjects with CYP2C9 *1/*1, *1B/*1B, *1/*3 and *3/*3</td>
<td>5.00 (8%)</td>
<td>5.01 (4.42, 5.64)</td>
<td>21.4% (12%)</td>
<td>21.3% (15.8%, 26.1%)</td>
<td></td>
<td>L</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>$CL_D$</td>
<td>Distribution clearance</td>
<td>1.25 (18%)</td>
<td>1.23 (0.94, 1.56)</td>
<td>21.7% (13%)</td>
<td>22.3% (5.8%, 34.8%)</td>
<td>6%</td>
<td>L/hour</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>$V_P$</td>
<td>Peripheral compartment volume of distribution</td>
<td>3.81 (8%)</td>
<td>3.80 (3.32, 4.24)</td>
<td>12.9% (54%)</td>
<td>13.5% (4.5%, 20.8%)</td>
<td></td>
<td>L</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>$K_{on}$</td>
<td>Association rate constant between drug and receptor for subjects with CYP2C9 *1/*1, *1B/*1B and *1/*3</td>
<td>0.00494 (10%)</td>
<td>0.00500 (0.00402, 0.00590)</td>
<td>28.9% (44%)</td>
<td>30.5% (11.9%, 46.9%)</td>
<td></td>
<td>µg/(µg*hour)</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>$K_{off}$</td>
<td>Dissociation rate constant for drug-receptor complex</td>
<td>0.0405</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>µg/L</td>
<td>Fixed (Levy et al., 2003)</td>
</tr>
<tr>
<td>$R_{0,s}$</td>
<td>Baseline receptor level for subjects with CYP2C9 *1/*1, *1B/*1B and *1/*3</td>
<td>182 (10%)</td>
<td>181 (155, 212)</td>
<td>18.9% (26%) (IV)</td>
<td>19.6% (8.7%, 26.7%)</td>
<td></td>
<td>µg/L</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>$R_{0,a}$</td>
<td>Renal clearance</td>
<td>0.00369 (5%)</td>
<td>0.00368 (0.00337, 0.00401)</td>
<td>20.1% (19%)</td>
<td>22.5% (15.5%, 29.2%)</td>
<td></td>
<td>µg/L</td>
<td>Estimated by urine model</td>
</tr>
<tr>
<td>BL_P2</td>
<td>Period 2 baseline concentration in central compartment</td>
<td>3.65 (18%)</td>
<td>3.66 (2.53, 4.96)</td>
<td>100% (15%)</td>
<td>108% (74%, 153%)</td>
<td></td>
<td>µg/L</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>BL_P3</td>
<td>Period 3 baseline concentration in central compartment</td>
<td>3.85 (29%)</td>
<td>3.89 (2.18, 5.97)</td>
<td>174% (40%)</td>
<td>172% (93%, 154%)</td>
<td></td>
<td>µg/L</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>BL_P2</td>
<td>Period 2 baseline concentration in peripheral compartment</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>µg/L</td>
<td>Fixed</td>
</tr>
<tr>
<td>BL_P3</td>
<td>Period 3 baseline concentration in peripheral compartment</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>µg/L</td>
<td>Fixed</td>
</tr>
<tr>
<td>CLGeno2</td>
<td>% CL for subjects with CYP2C9 *1B/*1B (reference CYP2C9 *1/*1)</td>
<td>88.5% (13%)</td>
<td>88.8% (69.0%, 112.0%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLGeno3</td>
<td>% CL for subjects with CYP2C9 *1/*3 (reference CYP2C9 *1/*1)</td>
<td>60.7% (11%)</td>
<td>61.0% (49.0%, 75.6%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLGeno4</td>
<td>% CL for subjects with CYP2C9 *1/*3 (reference CYP2C9 *1/*1)</td>
<td>27.3% (16%)</td>
<td>27.8% (19.6%, 35.8%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLGeno5</td>
<td>% CL for subjects with CYP2C9 *1/*3 (reference CYP2C9 *1/*1)</td>
<td>21.5% (14%)</td>
<td>21.7% (16.3%, 27.4%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Summary of population PK parameter estimations for S-warfarin

This article has not been copyedited and formatted. The final version may differ from this version.
<table>
<thead>
<tr>
<th>Genotype</th>
<th>% of CL when administered with fluconazole for subjects with CYP2C9</th>
<th>% of CL when administered with rifampin for subjects with CYP2C9</th>
<th>% of CL when administered with fluconazole for subjects with CYP2C9</th>
<th>% of CL when administered with rifampin for subjects with CYP2C9</th>
<th>Estimated by plasma model</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL_Flu_Geno1</td>
<td>30.5% (5%)</td>
<td>12.5% (41%)</td>
<td>25%</td>
<td>Estimated by plasma model</td>
<td></td>
</tr>
<tr>
<td>CL_Flu_Geno3</td>
<td>35.2% (5%)</td>
<td>12.7% (8.2%, 18.4%)</td>
<td>25%</td>
<td>Estimated by plasma model</td>
<td></td>
</tr>
<tr>
<td>CL_Flu_Geno4</td>
<td>40.3% (8%)</td>
<td>11.0% (20%)</td>
<td>12.5% (4%)</td>
<td>11.3% (8.5%, 14.2%)</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>CL_Flu_Geno5</td>
<td>52.2% (7%)</td>
<td>40.4% (34.8%, 46.5%)</td>
<td>12.7% (8.2%, 18.4%)</td>
<td>Estimated by plasma model</td>
<td></td>
</tr>
<tr>
<td>CL_Rif_Geno1</td>
<td>215% (4%)</td>
<td>215% (198%, 232%)</td>
<td>211% (5%)</td>
<td>212% (190%, 233%)</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>CL_Rif_Geno3</td>
<td>219% (4%)</td>
<td>218% (203%, 235%)</td>
<td>219% (4%)</td>
<td>218% (203%, 235%)</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>CL_Rif_Geno4</td>
<td>298% (8%)</td>
<td>299% (253%, 347%)</td>
<td>297% (8%)</td>
<td>298% (254%, 348%)</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>CL_Rif_Geno5</td>
<td>293% (6%)</td>
<td>294% (265%, 330%)</td>
<td>293% (6%)</td>
<td>294% (265%, 330%)</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>Vc_Geno4</td>
<td>55.6% (23%)</td>
<td>83.7% (26%)</td>
<td>251% (29%)</td>
<td>255% (152%, 385%)</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>Kon_Geno4</td>
<td>86.3% (49.1%, 131.3%)</td>
<td>51.8% (30%)</td>
<td>83.7% (26%)</td>
<td>86.3% (49.1%, 131.3%)</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>Kon_Geno5</td>
<td>54.0% (29.9%, 83.1%)</td>
<td>451% (29%)</td>
<td>54.0% (29.9%, 83.1%)</td>
<td>451% (29%)</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>RBL_Geno4</td>
<td>189% (17%)</td>
<td>193% (134%, 258%)</td>
<td>189% (17%)</td>
<td>193% (134%, 258%)</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>CLR_Flu</td>
<td>85.4% (7.8%, 93.2%)</td>
<td>10% (5%)</td>
<td>85.4% (7.8%, 93.2%)</td>
<td>Estimated by plasma model</td>
<td></td>
</tr>
<tr>
<td>CLR_Rif</td>
<td>132% (117%, 148%)</td>
<td>23.4 (25%)</td>
<td>132% (117%, 148%)</td>
<td>Estimated by urine model</td>
<td></td>
</tr>
<tr>
<td>σwarf_RUV</td>
<td>7.40% (5%)</td>
<td>7.40% (5%)</td>
<td>7.40% (5%)</td>
<td>Estimated by plasma model</td>
<td></td>
</tr>
<tr>
<td>σwarf_Flu</td>
<td>7.43% (6.84%, 7.95%)</td>
<td>7.43% (6.84%, 7.95%)</td>
<td>7.43% (6.84%, 7.95%)</td>
<td>Estimated by plasma model</td>
<td></td>
</tr>
<tr>
<td>σwarf_Rif</td>
<td>9.20% (5%)</td>
<td>9.20% (5%)</td>
<td>9.20% (5%)</td>
<td>Estimated by plasma model</td>
<td></td>
</tr>
<tr>
<td>σwarf_U</td>
<td>26.0% (8%)</td>
<td>26.0% (8%)</td>
<td>26.0% (8%)</td>
<td>Estimated by urine model</td>
<td></td>
</tr>
<tr>
<td>σwarf_Flu</td>
<td>27.8% (24.1%, 31.7%)</td>
<td>27.8% (24.1%, 31.7%)</td>
<td>27.8% (24.1%, 31.7%)</td>
<td>Estimated by urine model</td>
<td></td>
</tr>
<tr>
<td>σwarf_Rif</td>
<td>31.4% (22.4%, 31.9%)</td>
<td>31.4% (22.4%, 31.9%)</td>
<td>31.4% (22.4%, 31.9%)</td>
<td>Estimated by urine model</td>
<td></td>
</tr>
<tr>
<td>σwarf_U</td>
<td>26.0% (10%)</td>
<td>26.0% (10%)</td>
<td>26.0% (10%)</td>
<td>Estimated by urine model</td>
<td></td>
</tr>
</tbody>
</table>
Notes: IIV: inter-individual variability; IOV: inter-occasion variability; RUV: residual unexplained variability; RSE: relative standard error; CI: confidence interval; IIV and IOV terms are expressed as CV% ($\sqrt{e^\sigma - 1}$); RUV terms are expressed as CV% ($\sqrt{\sigma^2}$); SIR: sampling importance resampling.
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Definitions</th>
<th>Estimates (RSE)</th>
<th>SIR medians (95% CIs)</th>
<th>HV/IOV Estimates (RSE)</th>
<th>HV/IOV SIR medians (95% CIs)</th>
<th>Shrinkage</th>
<th>Units</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ka</td>
<td>Absorption rate constant</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CL</td>
<td>Clearance when warfarin is administered alone</td>
<td>0.119 (5%)</td>
<td>0.119 (0.108, 0.131)</td>
<td>28.3% (12%)</td>
<td>29.1% (23.1%, 35.1%)</td>
<td>0%</td>
<td>L/hour</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>VC</td>
<td>Central compartment volume of distribution for subjects with CYP2C9 *1/*1, *1B/*1B and *1/*3.</td>
<td>3.18 (8%)</td>
<td>3.19 (2.75, 3.65)</td>
<td>35.1% (11%)</td>
<td>38.1% (30.1%, 45.1%)</td>
<td>3%</td>
<td>L</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>CLD</td>
<td>Distribution clearance</td>
<td>2.49 (2%)</td>
<td>2.46 (2.36, 2.56)</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>VP</td>
<td>Peripheral compartment volume of distribution</td>
<td>4.79 (1%)</td>
<td>4.79 (4.65, 4.93)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kon</td>
<td>Association rate constant between drug and receptor</td>
<td>0.00137 (10%)</td>
<td>0.00139 (0.00116, 0.00163)</td>
<td>23.1% (64%)</td>
<td>29.1% (10.1%, 46.1%)</td>
<td>5%</td>
<td>L/(µg*hour)</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>Koff</td>
<td>Dissociation rate constant for drug-receptor complex</td>
<td>0.0405</td>
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</tr>
<tr>
<td>RBL</td>
<td>Baseline receptor level for subjects with CYP2C9 *1/*1, *1B/*1B..</td>
<td>188 (13%)</td>
<td>188 (154, 230)</td>
<td>15.6% (7%) (DIV)</td>
<td>21.4% (7.1%, 36.1%)</td>
<td>36.0% (23%) (IOV)</td>
<td>µg/L</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>CLR</td>
<td>Renal clearance</td>
<td>0.0043 (5%)</td>
<td>0.0043 (0.00396, 0.00480)</td>
<td>24.8% (18%)</td>
<td>27.4% (20.2%, 34.8%)</td>
<td>15%</td>
<td>L/hour</td>
<td>Estimated by urine model</td>
</tr>
<tr>
<td>BL_P2</td>
<td>Period 2 baseline concentration in central compartment</td>
<td>2.75 (23%)</td>
<td>2.82 (1.84, 4.01)</td>
<td>100% (33%)</td>
<td>121% (77%, 203%)</td>
<td>5%</td>
<td>µg/L</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>BL_P3</td>
<td>Period 3 baseline concentration in central compartment</td>
<td>1.97 (13%)</td>
<td>2.03 (1.57, 2.57)</td>
<td>29.3% (64%)</td>
<td>39.1% (20.1%, 63.1%)</td>
<td>3%</td>
<td>µg/L</td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>BL_P2</td>
<td>Period 2 baseline concentration in peripheral compartment</td>
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</tr>
<tr>
<td>BL_P3</td>
<td>Period 3 baseline concentration in peripheral compartment</td>
<td>1</td>
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<td></td>
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</tr>
<tr>
<td>CL_Fia</td>
<td>% of CL when administered with fluconazole</td>
<td>51.3% (4%)</td>
<td>51.1% (48.1%, 55.1%)</td>
<td>18.3% (13%)</td>
<td>19.1% (15.1%, 23.1%)</td>
<td>5%</td>
<td>µg/L</td>
<td>Fixed</td>
</tr>
<tr>
<td>CL_Rif</td>
<td>% of CL when administered with rifampin for subjects with CYP2C9 *2/*3, *1B/*1R, *2/*2 and *3/*3.</td>
<td>268% (3%)</td>
<td>268% (254%, 282%)</td>
<td>13.5% (14%)</td>
<td>14.1% (11.1%, 17.1%)</td>
<td>3%</td>
<td></td>
<td>Estimated by plasma model</td>
</tr>
<tr>
<td>CL_Rif_Geno4</td>
<td>% of CL when administered with rifampin for subjects with CYP2C9 *2/*3.</td>
<td>377% (10%)</td>
<td>380% (316%, 451%)</td>
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</tr>
<tr>
<td>VC_Geno4</td>
<td>% VC for subjects with CYP2C9 *2/*3 (reference *1/*1, *1B/*1R, *1/*3)</td>
<td>71.1% (22%)</td>
<td>73.1% (48.1%, 104.1%)</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Genotype</td>
<td>%Vc</td>
<td>%Vc for subjects with CYP2C9 *3/*3 (reference *1/*1, *1B/*1B, *1/*3)</td>
<td>169% (19%)</td>
<td>175% (121%, 230%)</td>
<td>Estimated by plasma model</td>
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<td>---------------------------------------------------------------</td>
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</tr>
<tr>
<td>Geno3</td>
<td>%Rac</td>
<td>%Rac for subjects with CYP2C9 *1/*3 (reference *1/*1, *1B/*1B)</td>
<td>47.9% (17%)</td>
<td>49.1% (36.1%, 65.1%)</td>
<td>Estimated by plasma model</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Geno4</td>
<td>%Rac</td>
<td>%Rac for subjects with CYP2C9 *2/*3 (reference *1/*1, *1B/*1B)</td>
<td>50.6% (22%)</td>
<td>52.1% (31.1%, 74.1%)</td>
<td>Estimated by plasma model</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geno5</td>
<td>%Rac</td>
<td>%Rac for subjects with CYP2C9 *3/*3 (reference *1/*1, *1B/*1B)</td>
<td>21.0% (22%)</td>
<td>21.1% (14.1%, 30.1%)</td>
<td>Estimated by plasma model</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Geno3</td>
<td>%CLR_Flu</td>
<td>% of CLR when administered with fluconazole</td>
<td>75.2% (5%)</td>
<td>75.6% (67.8%, 83.1%)</td>
<td>Estimated by urine model</td>
<td></td>
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</tr>
<tr>
<td>Geno4</td>
<td>%CLR_Rif</td>
<td>% of CLR when administered with rifampin</td>
<td>143.5% (8%)</td>
<td>143.5% (123%, 165%)</td>
<td>Estimated by urine model</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Geno5</td>
<td>%CLR_Flu</td>
<td>% of CLR when administered with fluconazole</td>
<td>7.37% (4%)</td>
<td>7.39% (6.82%, 8.05%)</td>
<td>Estimated by plasma model</td>
<td></td>
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<tr>
<td>Geno5</td>
<td>%CLR_Rif</td>
<td>% of CLR when administered with rifampin</td>
<td>6.26% (5%)</td>
<td>6.29% (5.33%, 6.88%)</td>
<td>Estimated by plasma model</td>
<td></td>
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<tr>
<td>Geno3</td>
<td>%σwarf_Flu</td>
<td>RUV for warfarin + fluconazole period plasma</td>
<td>8.60% (5%)</td>
<td>8.59% (7.85%, 9.39%)</td>
<td>Estimated by plasma model</td>
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<tr>
<td>Geno4</td>
<td>%σwarf_Rif</td>
<td>RUV for warfarin + rifampin period plasma</td>
<td>25.7% (10%)</td>
<td>27.4% (24.1%, 30.7%)</td>
<td>Estimated by urine model</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Geno5</td>
<td>%σwarf_Flu</td>
<td>RUV for warfarin + fluconazole period urine</td>
<td>31.1% (10%)</td>
<td>31.1% (26.9%, 36.3%)</td>
<td>Estimated by urine model</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Geno5</td>
<td>%σwarf_Rif</td>
<td>RUV for warfarin + rifampin period urine</td>
<td>26.8% (11%)</td>
<td>27.8% (23.4%, 33.0%)</td>
<td>Estimated by urine model</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Notes: IIV: inter-individual variability; IOV: inter-occasion variability; RUV: residual unexplained variability; RSE: relative standard error; CI: confidence interval; IIV and IOV terms are expressed as CV% (\(\sqrt{e^{\sigma^2} - 1}\)); RUV terms are expressed as CV% (\(\sqrt{\sigma^2}\)); SIR: sampling importance resampling.
Figure 1

29 healthy volunteers
- CYP2C9 *1/*1 = 8
- CYP2C9 *1/*1B = 5
- CYP2C9 *1/*3 = 9
- CYP2C9 *2/*3 = 3
- CYP2C9 *5/*5 = 4

Period 1
- 10mg Warfarin single oral dose
- 400mg Flu oral q.d.
- 300mg Rif oral q.d.
- 10mg Warfarin single oral dose

Period 2
- 10mg Warfarin single oral dose
- 400mg Flu oral q.d.
- 300mg Rif oral q.d.

Period 3
- 10mg Warfarin single oral dose
- 400mg Flu oral q.d.
- 300mg Rif oral q.d.

Sampling Phase 13-15 days
- Washout Phase 7 days
- Period 1 terminated

Warfarin 10mg

Flu: Fluconazole
Rif: Rifampin
Figure 2
Figure 3
Figure 4

A

<table>
<thead>
<tr>
<th>Warf <em>1</em>/1</th>
<th>Warf <em>1B</em>/1B</th>
<th>Warf <em>1</em>/3</th>
<th>Warf <em>2</em>/3</th>
<th>Warf <em>3</em>/3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma 5-oxo-S-warfarin Concentration (ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warf + Flu <em>1</em>/1</td>
<td>Warf + Flu <em>1</em>/3</td>
<td>Warf + Flu <em>2</em>/3</td>
<td>Warf + Flu <em>3</em>/3</td>
<td>Warf + Rif <em>1</em>/1</td>
</tr>
<tr>
<td>Warf + Rif <em>1B</em>/1B</td>
<td>Warf + Rif <em>1</em>/3</td>
<td>Warf + Rif <em>2</em>/3</td>
<td>Warf + Rif <em>3</em>/3</td>
<td></td>
</tr>
<tr>
<td>Time (hours)</td>
<td>0 100 200 300 400</td>
<td>0 100 200 300 400</td>
<td>0 100 200 300 400</td>
<td>0 100 200 300 400</td>
</tr>
</tbody>
</table>

B

<table>
<thead>
<tr>
<th>Warf <em>1</em>/1</th>
<th>Warf <em>1B</em>/1B</th>
<th>Warf <em>1</em>/3</th>
<th>Warf <em>2</em>/3</th>
<th>Warf <em>3</em>/3</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-warfarin Urinary Amount (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warf + Flu <em>1</em>/1</td>
<td>Warf + Flu <em>1</em>/3</td>
<td>Warf + Flu <em>2</em>/3</td>
<td>Warf + Flu <em>3</em>/3</td>
<td>Warf + Rif <em>1</em>/1</td>
</tr>
<tr>
<td>Warf + Rif <em>1B</em>/1B</td>
<td>Warf + Rif <em>1</em>/3</td>
<td>Warf + Rif <em>2</em>/3</td>
<td>Warf + Rif <em>3</em>/3</td>
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<tr>
<td>Time (hours)</td>
<td>0 100 200 300 400</td>
<td>0 100 200 300 400</td>
<td>0 100 200 300 400</td>
<td>0 100 200 300 400</td>
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</tbody>
</table>
Figure 5

A

B
Figure 6

A

B

% S-warfarin CL Decrease

% S-warfarin CL Increase

Genotype

Genotype