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The influence of genotype on warfarin maintenance dose predictions produced using a Bayesian dose individualisation tool

Shamin M Saffian, BPharm, PgCertPharm[1,2,] Stephen B Duffull, PhD[1], Rebecca L Roberts, PhD[3], R Campbell Tait, MBChB[4], Leanne Black, MSc[5], Kirstin A Lund, MBChB[4], Alison H Thomson, PhD[5,6], Daniel F B Wright, PhD[1]

[1]School of Pharmacy, University of Otago, Dunedin, New Zealand
[2]Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Malaysia
[3]Department of Surgical Sciences, University of Otago, Dunedin, New Zealand
[4]Department of Haematology, Royal Infirmary, Glasgow, Scotland
[5]Pharmacy Department, Glasgow Royal Infirmary, Glasgow, Scotland
[6]Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, Scotland

Corresponding author
Shamin M Saffian
School of Pharmacy, University of Otago
PO Box 56, Dunedin 9054, New Zealand
Phone: +64 3 479 5045
Fax: +64 3 4797034
Email: shamin.mohdsaffian@otago.ac.nz

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Background

A previously established Bayesian dosing tool for warfarin was found to produce biased maintenance dose predictions. In the following study we aimed to: (1) determine if the biased warfarin dose predictions previously observed could be replicated in a new cohort of patients from two different clinical settings; (2) explore the influence of *CYP2C9* and *VKORC1* genotype on the predictive performance of the Bayesian dosing tool; and (3) determine if the prior population used to develop the kinetic-pharmacodynamic (KPD) model underpinning the Bayesian dosing tool was sufficiently different from the test (posterior) population to account for the biased dose predictions.

Methods

The warfarin maintenance doses for 140 patients were predicted using the dosing tool and compared to the observed maintenance dose. The impact of genotype was assessed by predicting maintenance doses with prior parameter values known to be altered by genetic variability (e.g. EC_{50} for *VKORC1* genotype). The prior population was evaluated by fitting the published kinetic-pharmacodynamic model, which underpins the Bayesian tool, to the observed data using NONMEM and comparing the model parameter estimates to published values.

Results

The Bayesian tool produced positively biased dose predictions in the new cohort of patients (mean prediction error [95% CI]; 0.32 mg/day [0.14, 0.5]). The bias was only observed in patients requiring ≥7 mg/day. The direction and magnitude of the observed bias was not influenced by genotype. The prior model provided a good fit to our data, suggesting that the bias was not caused by different prior and posterior populations.

Conclusions

Maintenance doses for patients requiring ≥7 mg/day were over-predicted. The bias was not due to the influence of genotype nor was it related to differences between the prior and posterior populations. There is a need for a more mechanistic model that captures warfarin dose-response relationship at higher warfarin doses.

INTRODUCTION

Warfarin is an anticoagulant used to treat and prevent blood clots in patients with deep vein thrombosis, pulmonary embolism and atrial fibrillation. It is a difficult drug to dose accurately and safely due to a narrow therapeutic range and a delay in the time course of anticoagulant response relative to dosing. Furthermore, the maintenance dose required to achieve therapeutic anticoagulation varies by upwards of 15 fold between patients.[1, 2] Anticoagulation is monitored during warfarin therapy using the international normalised ratio (INR). The target INR for most indications, including atrial fibrillation, is approximately 2.5 with a suggested therapeutic range of 2-3. Treatment is considered successful if the time that patients spend within the therapeutic range (TTR) is maximised. Conventional, heuristic, dosing methods have been found to result in a TTR of only 40-60%.[3-6]

There have been a large number of publications exploring strategies to improve INR control and to predict warfarin dose requirements. A variety of nomograms, computerised decision-support tools and Bayesian prediction tools have been proposed to aid dosing decisions in the clinic.[7-10] In the past ten years, more than 30 warfarin dosing algorithms have been published.[11, 12] These algorithms are developed by regressing patient characteristics, including age, body size, ethnicity, concomitant drug use and genetic variability in warfarin metabolism (via the cytochrome P-450 enzyme *CYP2C9*) or vitamin K recycling (via vitamin K epoxide reductase, *VKORC1*)[1, 12-14], against maintenance dose requirements. The algorithms therefore provide a means of rapidly predicting warfarin dosing requirements prior to the initiation of therapy.[1, 13, 15, 16] To date however, clinical trials have not consistently demonstrated that dose prediction using warfarin algorithms improves anticoagulant control or patient outcomes compared to other methods.[17-23] For this reason, neither the American College of Chest Physicians (ACCP)[24] nor the British Committee for Standards in Haematology (BCSH)[25] consensus guideline advocate the use of dose prediction tools or genotyping prior to the initiation of therapy.

Bayesian forecasting methods for warfarin may provide a means of improving INR control without prior knowledge of *CYP2C9* or *VKORC1* genotype.[26] In limited studies to date, Bayesian methods show the potential to increase the TTR to 80% or more.[26-29] In addition, Bayesian algorithms can easily be integrated with decision support tools for use in the clinic (see Hamberg et al. 2015[30] for an example).

We have shown previously that a Bayesian forecasting method can produce unbiased warfarin maintenance dose predictions on average.[11, 26] However, in a *post-hoc* analysis we found that doses for patients with *VKORC1* (-1639 G>A) GG genotype were positively biased by about 1 mg/day on average, suggesting a possible influence of genotype on predictive performance. Of note, this effect was evident only in those patients taking doses greater

than about 7 mg daily. In the following study we aimed to: (1) determine if the previously observed biased warfarin dose predictions could be replicated in a new cohort of patients from two different clinical settings; (2) explore the influence of *CYP2C9* and *VKORC1* genotype on the predictive performance of the Bayesian dosing tool; and (3) determine if the prior population used to develop the kinetic-pharmacodynamic (KPD) model underpinning the Bayesian dosing tool was sufficiently different from the test (posterior) population to account for the biased dose predictions.

METHODS

Patient data

The data consisted of warfarin dose and INR values from patients initiating warfarin therapy for any indication. INR values were recorded from the start of therapy until a stable INR had been achieved. A stable INR was defined as the second INR within 20% of the INR target (2.5 in most cases) as described elsewhere.[11] Patients were genotyped for *VKORC1* (–1639 G>A rs9923231) and *CYP2C9 *1*, *2* (430C>T, rs1799853) and *3* (1075A>C, rs1057910) as described elsewhere.[11, 31] Ethical approval was obtained from the Lower South Regional Ethics Committee, New Zealand (LRS/10/11/056) and West of Scotland Research Ethics Service, Glasgow, Scotland. All participating patients gave written informed consent.

Data from a total of 153 patients were available for analysis and came from two centers;

  i.   Dataset I - Dunedin Hospital, Dunedin, New Zealand[11] (n=55)

  ii.   Dataset II – Glasgow Royal Infirmary, Glasgow, Scotland[32] (n=98)

Maintenance dose predictions

Details of the development of the Bayesian dosing tool (TCIWorks) have been published previously.[33] The underpinning model that describes the warfarin dose and anticoagulant response was a KPD model developed by Hamberg et al.[34] Briefly, a KPD model is a pharmacokinetic-pharmacodynamic model where the pharmacokinetic parameters are estimated solely from the pharmacodynamic data. This means that no warfarin concentration data are required to predict the INR response. The KPD model developed by Hamberg et al[30] consists of two transit compartment chains with three compartments in each chain to describe the time course of INR response. An Emax model was used to describe the link between pharmacokinetics and pharmacodynamics. The KPD model also included a covariate model for *VKORC1* on EC50 (concentration of s-warfarin at 50 % of maximum drug effect)

and age and $CYP2C9$ on clearance parameters, however, genotype was not included as a covariate in the Bayesian dosing tool (see Wright and Duffull[33] for details).

The full dosing history for each patient from initiation to a stable INR, age, and all available INR observations up to the final dose change were entered into the Bayesian dosing tool. The Bayesian dosing tool was then used to predict the daily dose required to achieve the observed stable INR for each patient. The patients were assumed to be taking their prescribed dose at 6 pm and INR was assumed to be sampled at 10 am. The effects of concomitant drugs on warfarin INR response would be captured by the INR measurement itself and therefore was not accounted for *a priori*.

The predictive performance of the Bayesian dosing tool

The predicted maintenance doses were compared to the observed maintenance doses using measures of bias (mean prediction error [MPE]) and mean squared error [MSE] or root mean squared error [$RMSE$]. MPE, MSE, and $RMSE$ were calculated as follows:

$$MPE = \frac{1}{N}\sum_{i=1}^{N} PE_i, \tag{1}$$

$$MSE = \frac{1}{N}\sum_{i=1}^{N} (PE_i)^2, \tag{2}$$

$$RMSE = \sqrt{MSE}, \tag{3}$$

where MPE is the mean prediction error, N is the number of patients, PE_i is the prediction error (predicted minus observed maintenance dose) of the ith individual. No statistical bias was concluded if the 95% confidence interval (CI) of the MPE included zero. MSE is the average of the sum of squared differences between predicted minus observed maintenance dose, and $RMSE$ is the square root of MSE. $RMSE$ provides an estimate of the variability of the prediction errors given in approximately the same units of the data (mg/day).

We stratified the dataset by observed maintenance dose into two groups; those who required ≥ 7 mg/day and those who required < 7 mg/day. A cut off of 7 mg/day has been used in other studies[1,35] to categorize patients requiring a "high" daily dose and therefore was used here. MPE and $RMSE$ were assessed as above for each dose group.

The impact of genotype on the predictive performance of the Bayesian dosing tool

The influence of genotype on warfarin dose predictions was assessed by stratifying the study cohort into different $VKORC1$ and $CYP2C9$ genotypes and measuring MPE and $RMSE$, as above, for each genotype group.

We also tested the influence of *VKORC1* genotype on Bayesian dose predictions by altering the mean prior parameter values of EC_{50} to reflect the observed genotype in each patient. This is intended as a diagnostic aid to understand a possible source of bias and not how the Bayesian tool would normally be used. The prior parameter values was normally set to the wild-type (GG) value for all patients. For the purposes of this analysis the mean EC_{50} was changed manually for each patient prior to predicting the maintenance dose. The parameter values chosen corresponded to the observed genotype effect reported in the published model by Hamberg et al[34], i.e. an EC_{50} of 4.01 mg/L for those with *VKORC1* GG genotype, 3.01 mg/L for individuals with *VKORC1* GA genotype, and 1.92 mg/L for the *VKORC1* AA genotype. The initial parameter estimates of *CYP2C9* were set to the wild-type (*1*1) and only tested further if there was any significant improvement in bias was observed by changing the *VKORC1* prior parameter estimates.

The impact of the prior population on predictive performance of the Bayesian dosing tool

We explored the hypothesis that the bias may be caused by inherent differences in warfarin dose-response within our study population compared to the population used to develop the KPD model by Hamberg et al.[34] To address this, we fitted the published KPD model to our dataset using a nonlinear mixed effects modelling methodology in NONMEM (version 7.2 (Icon Inc. [PA, USA]). Initial parameter estimates were the values reported by Hamberg et al.[34] A non-parametric bootstrap was carried out to assess the precision of the parameter estimates. A total of 1000 bootstrap datasets were generated by randomly sampling the original dataset and simulated from the model to obtain the 95% CI of each parameter estimate. The mean and 95% CI of the estimated model parameters from our study cohort were compared to the values from the published KPD model and considered not significantly different if the means were within 20% of the published values and confidence intervals overlapped with the published 95% CI.

RESULTS

Thirteen of the original 153 patients were excluded from the analysis; 12 who were not genotyped and 1 who did not reach a stable INR. The demographic details of the remaining 140 patients are summarised in Table 1. The distributions of both genotypes were consistent with the Hardy-Weinberg equilibrium (p>0.05). Median (range) age was 62 years (23-87) and 65 years (15-85) for datasets I and II, respectively.

The predictive performance of the Bayesian dosing tool

A summary of the *MPE* and *RMSE* results for each dataset analyzed separately and combined is presented in Table 2. The Bayesian dosing tool, on average, produced positively biased dose predictions (*MPE* mg/day [95%

CI]; 0.32 [0.14, 0.50]). When analysed separately, dose predictions for both the Dunedin (dataset I) and Glasgow datasets (dataset II) were also both positively biased (*MPE* mg/day [95% CI]; 0.53 [0.11, 0.94] and 0.22 [0.04, 0.39] respectively).

The average prediction error for patients requiring ≥7 mg/day was +1.15 mg/day which represents an average over-prediction of approximately 18%, while the average prediction error for patients requiring <7 mg/day was +0.18 mg/day. *RMSE* values for patients requiring <7 mg/day and ≥7 mg/day were 0.94 mg/day and 1.90 mg/day, respectively.

The impact of genotype on the predictive performance of the Bayesian dosing tool

Dose predictions for patients with the *VKORC1* GG genotype were found to be positively biased but dose predictions for patients with *VKORC1* AG and AA were unbiased (see Table 2). Similarly, dose predictions for patients with *CYP2C9* *1*1 were positively biased in the combined dataset.

To explore the influence of genotype further, we compared the *MPE* for patients with *VKORC1* GG genotype who had *CYP2C9* *1*1 and those who were not *CYP2C9* *1*1 and found a positive bias for both combinations. However, when we grouped patients with *CYP2C9* *1*1 patients who were not *VKORC1* GG, we found that dose predictions were not biased. This suggests that the source of the bias is more likely to be *VKORC1* genotype.

Visual plots of the observed versus prediction maintenance dose according to *VKORC1* and *CYP2C9* are presented in Figures 1 and 2, respectively.

The Bayesian dose predictions conducted using individual values of EC_{50} for each patient, corresponding to their observed genotype, were found to produce positively biased results overall (see Table 3). It is noteworthy that those subjects with reduced EC_{50} values (i.e. *VKORC1* AG and AA genotypes) had unbiased dose predictions. The *MPE* for the *VKORC1* GG genotype group remained the same since the wild-type values were used previously.

The impact of the prior population on the predictive performance of the Bayesian dosing tool

The results of the estimated parameters and bootstrap runs using the model to estimate into a new population are summarized in Table 4. 93.8% of the bootstrap runs minimized successfully. Several mean parameter estimates were above the 20% criterion, however, mean parameter estimates for the more important parameters, such as EC_{50} for G allele and EC_{50} for A allele and between subject variability for EC_{50}, were within 20%. The 95% CI for

all the parameters estimated from the test population overlapped with the published model, except the population

value for MTT1 (mean transit time). This parameter is not considered likely to be a cause of bias in predictions.

DISCUSSION

In this study, we have explored the possible causes of bias in warfarin maintenance dose predictions with a

Bayesian dosing tool. A previously reported over-prediction of warfarin maintenance dose requirements was

successfully replicated using two datasets from different clinical centres. Furthermore, dose predictions were

carried out in the Bayesian system using all available INR measurements for each patient which should provide

the most accurate estimate of the patient's response. The biased warfarin dose predictions were only observed in

patients requiring ≥7 mg/day.

Several publications on the application of Bayesian methodologies to warfarin dose individualisation have been

reported previously.[9, 10, 27, 36-38] A summary of previous Bayesian dosing tools for warfarin and how they differ

from the dosing tool in our study have also been reviewed.[26, 33] Vadher et al[9] compared the observed dose to the

predicted maintenance dose and found that their Bayesian dosing tool was negatively biased on average. A scatter

plot of the observed dose versus predicted dose reported by Vadher et al[9] also showed biased predictions in

patients requiring higher daily doses, although in a different direction (under-prediction in higher dose patients).

Other publications on Bayesian dosing tools for warfarin did not analyse the predictive performance of the dosing

tool in a way that would allow comparison with our work.

Two possible explanations for the bias were explored in this study. First, we explored the hypothesis that the bias

may be due to the influence of *VKORC1* or *CYP2C9* genotype. We focused primarily on *VKORC1* since it was

identified as a candidate in previous work[11] but in the present study it was found to have a relatively minor

influence on dose predictions. In addition, the direction of dose prediction error (i.e. over or under-predicted dose)

in each patient requiring ≥ 7 mg/day was the same despite changing the EC_{50} priors to the values as per published

prior model. This suggests that differences in EC_{50} prior parameter estimates (and hence influence of *VKORC1*)

does not have significant influence on the overall dose predictions and therefore does not explain the observed

bias. Furthermore, the initial apparent association of bias and *VKORC1* GG may be explained by a higher

proportion of patients with *VKORC1* GG in this dose category and therefore, the initial link between *VKORC1*

GG and bias was more likely to be an association rather than a cause. We also explored the possibility that the

population used to derive the prior population parameter estimate for the KPD model had a different dose-response

to our study population which would mean that the model could not be extrapolated to our study population. Our results suggest that the prior and posterior populations were not sufficiently different to be a plausible cause of the observed bias.

As noted above, the bias in warfarin dose predictions was evident only for those patients taking ≥ 7 mg/day. The reason for this is not currently clear. Since we have found that it is not likely to be the parameter values used as the basis of the prior, then this suggests it could be the structure of the prior model. We know the coagulation network is exceedingly complex.[39] We therefore feel it is plausible that the application of a single E_{max} model when used across a large range of dose-response values (as seen here) may not be sufficiently flexible to account for the inherent feedback and feedforward mechanisms of the coagulation network, which may be more apparent in those patients taking higher than average warfarin doses. A further exploration of this hypothesis is beyond the scope of the current work.

Although an accurate method for predicting the maintenance dose of warfarin intuitively should improve clinical outcomes, this can only be proven in clinical trials. Importantly, whether the observed bias in warfarin dose predictions might result in altered clinical outcomes cannot be explored in this study. Exploring a non-parametric approach to population modelling and Bayesian forecasting was beyond the scope of the current study.

CONCLUSION

In two patient cohorts, the Bayesian dosing tool resulted in positively biased warfarin dose predictions. Warfarin doses were over-predicted for patients requiring ≥ 7 mg/day by 1.15 mg/day on average. The bias was not found to be due to the influence of *VKORC1* genotype, nor to differences in the prior and posterior populations. We conclude that there is a need for a more mechanistic dose-response model for warfarin that is capable of capturing the complexities of the coagulation network at higher warfarin doses.

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Legend to figures

Fig. 1 Scatter plot of the observed versus predicted maintenance dose according to *VKORC1*(-1639 G>A, rs9923231). The solid line is a line of identity. Filled symbols represent dataset I (Dunedin, New Zealand) and open symbols represent dataset II (Glasgow, Scotland)

Fig. 2 Scatter plot of the observed versus predicted maintenance dose for patients according to *CYP2C9*. The solid line is a line of identity. Filled symbols represent dataset I (Dunedin, New Zealand) and open symbols represent dataset II (Glasgow, Scotland)

Table 1

Patient characteristics and a summary of maintenance dose and stable INR

	Dataset I (n = 46)	Dataset II (n = 94)	Combined dataset (n =140)
Age (years)	62 (23-87)	65 (15-85)	64 (15-87)
Male/Female (number of patients)	19/27	48/46	67/73
Time to reach first stable INR (days)	38 (11-118)	24 (9-64)	28 (9-118)
Number of INR observations to reach stable INR	11 (6-21)	9 (6-24)	10 (6-24)
Stable INR value	2.4 (2 – 3.1)	2.3 (2-3)	2.4 (2-3.1)
Dose at stable INR (mg/day)	5 (1.5 – 11)	4 (0.75 - 10)	4.5 (0.75-11)
Interquartile range [Q1 – Q3]	[4-7]	[3-5.4]	[3-5.57]
CYP2C9 genotype (number (%) of patients)			
*1*1	28 (61%)	60 (64%)	88 (63%)
*1*2	8 (17%)	19 (20%)	27 (19%)
*1*3	9 (20%)	10 (11%)	19 (14%)
*2*2	-	2 (2%)	2 (1%)
*2*3	-	2 (2%)	2 (1%)
*3*3	1 (2%)	1 (1%)	2 (1%)
VKORC1 (-1639 G>A, rs9923231) genotype (number (%) of patients)			
GG	23 (50%)	45 (48%)	68 (49%)
AG	18 (39%)	38 (40%)	56 (40%)
AA	5 (11%)	11 (12%)	16 (11%)

Values are expressed as median (range) unless specified otherwise
Dataset I = Dunedin, New Zealand
Dataset II = Glasgow, Scotland

Table 2

A summary of average bias (mean prediction error (95% CI lower, upper) and root mean squared error of the dose predictions according to genotype.

		All genotypes	VKORC1			CYP2C9					
			GG	AG	AA	*1*1	*1*2	*1*3	*2*2	*2*3	*3*3
Dataset I (n=46)	MPE	0.53	1.13	-0.05	-0.13	0.46	1.06	0.29	-	-	0.4
	(95% CI)	(0.11, 0.94)	(0.48, 1.77)	(-0.67, 0.56)	(-0.68, 0.43)	(-0.13, 1.05)	(-0.43, 2.56)	(-0.35, 0.93)			(NA)
	RMSE	1.52	1.85	1.21	0.42	1.56	1.98	0.83	-	-	0.40
Dataset II (n=94)	MPE	0.22	0.23	0.23	0.08	0.32	-0.1	0.29	0.25	-0.18	0
	(95% CI)	(0.04, 0.39)	(-0.05, 0.52)	(-0.03, 0.5)	(-0.37, 0.54)	(0.07, 0.57)	(-0.37, 1.7)	(-0.21, 0.79)	(NA)	(NA)	(NA)
	RMSE	0.88	0.97	0.84	0.65	1.00	0.55	0.72	0.79	0.80	0.00
Combined dataset	MPE	0.32	0.53	0.14	0.02	0.36	0.24	0.29	-0.5	-0.18	0.39
	(95% CI)	(0.14, 0.5)	(0.21, 0.86)	(-0.11, 0.4)	(-0.28, 0.32)	(0.12, 0.61)	(-0.22, 0.71)	(-0.07, 0.65)	(-9.3, 9.8)	(-10, 9.7)	(-2.3, 2.7)
	RMSE	1.13	1.34	0.97	0.59	1.21	1.17	0.78	0.79	0.80	0.28

MPE mean prediction error (mg/day), *CI* confidence interval, *RMSE* root mean squared error (mg/day), Dataset I = Dunedin, New Zealand, Dataset II = Glasgow, Scotland, NA not available.

1 **Table 3**
2 A summary of mean prediction error (*MPE*) and root mean squared error (*RMSE*) using the individual's
3 genotype to determine the prior value of EC_{50} used in the population model
4

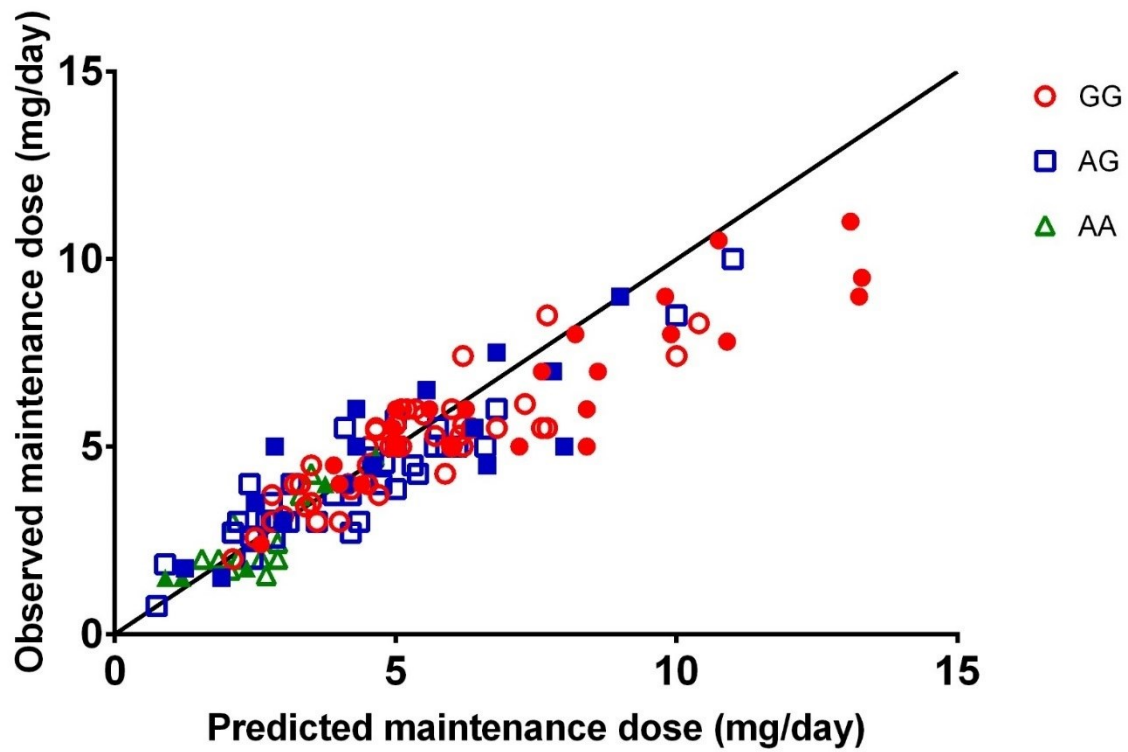| *VKORC1* (-1639 G>A) genotype	*MPE* (mg/day) [95% CI]	*RMSE*
All data	0.28 (0.09, 0.46)	1.14
GG	0.53 (0.21, 0.86)	1.34
AG	0.08 (-0.18, 0.35)	1.01
AA	-0.11 (-0.46, 0.23)	0.65

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8 **Table 4**

9 Parameter estimates from the published prior kinetic-pharmacodynamic (KPD) model (as a comparison) and
10 bootstrap resampling

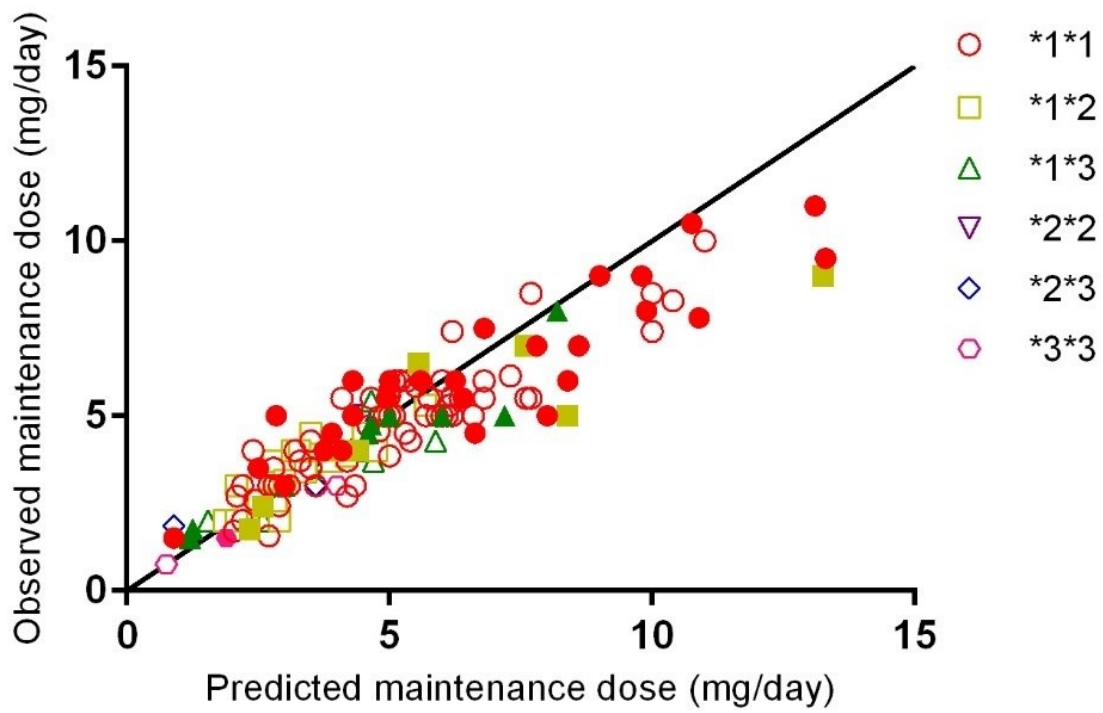| Parameter	Published prior KPD model [95% CI]	Bootstrap mean estimate [95% CI]
$EC_{50}G$ (mg/L)	2.05 [1.64, 2.46]	2.24 [1.32, 3.64]
$EC_{50}A$ (mg/L)	0.96 [0.78, 1.14]	1.10 [0.63, 1.82]
MTT_1 (hours)	28.6 [27.25, 29.95]	34.92 [29.25, 45.50]
MTT_2 (hours)	118.30	64.86 [1.43, 140]
Proportional residual error (ε_{INR}) CV %	20.00	14.70 [13.50, 15.90]
γ	1.15 [1.05, 1.25]	1.51 [1.14, 2.01]
BSV EC_{50} (CV %)	34 [29.93, 38.07]	29.73 [25.78, 33.98]
BSV K_{10} (CV %)	58.90	63.24 [35.29, 97]
BSV CL (CV %)	29.83 [17.9, 41.76]	44.18 [21.48, 67.72]
BSV V (CV %)	23.23 [10.17, 36.31]	49.46 [15.01, 79.96]

11 $EC_{50}G$ and $EC_{50}A$ concentration of s-warfarin at 50 % of maximum drug effect for G and A allele, MTT mean
12 transit time, CV coefficient of variation, γ Hill coefficient, BSV between subject variability, K_{10} first-order
13 elimination rate constant, CL clearance, V volume of distribution of *s*-warfarin
14
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Figure 1

19

20   **Figure 2**