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Factors influencing pharmacokinetics of warfarin in African–Americans: implications for pharmacogenetic dosing algorithms

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Abstract

Aim: This study attempted to identify predictors of *S*-warfarin clearance (CL[S]) and to make a pharmacokinetic evaluation of genotype-based dosing algorithms in African–Americans.

Methods: Using plasma *S*-warfarin concentration (Cp[S]) at a steady state and eight SNPs previously shown to influence warfarin dose in African–Americans, CL(S) and its predictors were estimated by population pharmacokinetic analysis in 60 African–Americans. The time courses of Cp(S) following either the loading dose or maintenance dose were simulated using the population pharmacokinetic estimates.

Results: *CYP2C9*8* and body surface area or body weight were predictors of CL(S) (–30 and –5% per –0.1 m²/–10 kg reduction in CL[S], respectively) in African–Americans. Simulations of Cp(S) showed that Cp(S) at steady state was 1.4-times higher in patients with *CYP2C9*8* than in those with *CYP2C9*1/*1*, irrespective of the algorithm for loading dose or maintenance dose.

Conclusion: African–Americans possess independent predictors of CL(S), possibly leading to a prediction error of any dosing algorithm that excludes African-specific variant(s).

Keywords

African–American; *CYP2C9*8*; genotype; pharmacokinetics; warfarin

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Authors' contributions

R Nagai, M Ohara and M Hibiya analyzed the data. R Nagai, M Ohara and H Takahashi interpreted the data and wrote the manuscript. H Takahashi, LH Cavallari, MA Perera and EA Nutescu designed the study and obtained funding. K Drozda and W Hernandez performed sample and data collection. SR Patel and N Kaneko performed the experiments.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Considerable interindividual and interpopulation variability has been observed in the maintenance dose (MD) of warfarin [1,2]. Genetic polymorphisms of *VKORC1* and *CYP2C9* have been established as major determinants of this variability, especially in Caucasians [3]. Accordingly, pharmacogenetic-based algorithms, for example, the International Warfarin Pharmacogenetics Consortium (IWPC) and the warfarindosing.org [4-6] are currently available for estimating the initial dose of warfarin. However, the performance of these algorithms, based mainly on data from Caucasian patients, is lower for African-Americans [1], partly because of the lack of African-specific variants predictive of warfarin requirements [7]. A number of previous studies have searched for genetic polymorphisms that influence warfarin dose in African-Americans, and these have revealed variants, such as *CYP2C9**5, *6, *8, *11, rs7089580, rs12777823, the *GGCX*(CAA) 16/17 repeat, and *FPGS* (rs7856096) [8-12]. However, less is known about the pharmacokinetics of warfarin in African-American populations than is the case for Caucasians. Therefore, the primary goal of the present study was to characterize the pharmacokinetics of *S*-warfarin, which is mainly responsible for the anticoagulation response, and to identify predictors of *S*-warfarin clearance (CL[S]) in African-American patients by employing a population pharmacokinetics (PPK) approach.

The results of two large randomized controlled trials [13,14] to assess the clinical utility of genotype-based warfarin dosing have been reported recently. However, the outcomes of these two studies were contradictory. The EU-PACT study with a cohort predominantly comprising whites (98.6%) showed that genotype information clearly improved anticoagulation control [13]. On the other hand, the COAG study with a mixed ethnicity cohort including 27% African-Americans found no improvement in anticoagulation control [14]. In the COAG trial, the performance of the genetic algorithm was worse than that of the clinical algorithm in African-Americans who were likely to have international normalized ratios (INRs) above the therapeutic range. Therefore, the secondary goal of the present study was to search for factors potentially responsible for the discrepancy between the outcomes of the two trials by performing simulation studies with PPK parameter estimates obtained in African-Americans.

Methods

Patients

Sixty African-Americans on a fixed maintenance dose of warfarin for at least 2 weeks and had an INR within 0.1 units of the target range of 2 to 3 participated in the present study [8,15]. Race was determined based on self-report and patients taking *CYP2C9* inducers or inhibitors (e.g., phenytoin, carbamazepine, rifampin, amiodarone, metronidazole, sulfonamides), those with a documented history of hepatic disease, or those reporting nonadherence to warfarin within the previous 2 weeks were excluded.

Study protocol

Blood was collected 11.5 to 18.7 h after the last dose of warfarin during the maintenance phase. Separated plasma and blood samples for DNA extraction were stored at -80° until analysis. The study protocol was approved by the Institutional Review Boards of the

University of Illinois at Chicago, the University of Chicago and the Meiji Pharmaceutical University. Written informed consent was obtained from each patient.

Measurements of warfarin plasma concentration & response

The plasma concentration (Cp) of warfarin enantiomers was determined by chiral high-pressure liquid chromatography, as reported previously [16]. The anticoagulant effect of warfarin was assessed by the INR value, which was obtained through point-of-care testing using the ProTime® monitor (ITC, NJ, USA).

Genotyping for *CYP2C9* & *VKORC1*

Genomic DNA from warfarin-treated patients was isolated from whole blood using a Puregene kit (Qiagen, CA, USA). The *CYP2C9**2, *3, *5, *6, *11, rs7089580, rs12777823 and *VKORC1* -1639G>A alleles were determined by PCR and pyrosequencing, and the 449G>A variant (*CYP2C9**8) was determined by PCR and capillary sequencing, as described previously [17-19].

Population pharmacokinetic analysis

The Dose-Cp relationship for *S*-warfarin, Cp(S), was analyzed using NONMEM® version 7.2.0 (Icon Development Solutions, MD, USA) [20]. The first-order conditional estimation with interaction (FOCE-INTER) method was used for analysis of the Dose-Cp(S) relationship. Model selection was guided by the decrease in the objective function value (OFV). The time course of Cp(S) was analyzed using a one-compartment model with first-order absorption and elimination rate constants (Equation 1), and the population mean, interindividual error and covariates of CL(S) were estimated:

$$Cp(S)_{ij} = \frac{F \times Dose \times Ka}{Vd \times (Ka - CL(S) / Vd)} \times (e^{-CL(S)/Vd \times t} - e^{-Ka \times t}) \quad (1)$$

where Cp(S)_{ij} represents the Cp(S) in the *i*th individual at the *j*th observation, *F* is the bioavailability fixed at 1.0, *Ka* is the absorption rate constant fixed at 2 h⁻¹ and *Vd* is the volume of distribution of *S*-warfarin fixed at 13.8 l [21]. The interindividual variability in CL(S) was estimated using an exponential model and the residual intraindividual variability in Cp(S) was fixed at 1% using a relative error model.

The adequacy of the model predictions was assessed using visual diagnostic plots of the respective population (PRED) and individual predicted values (IPRED) of Cp(S) versus the corresponding observed values, and PRED versus the weighted residuals. The robustness of the final PPK model was assessed using a bootstrap procedure in which means and 95% CI were obtained for the parameter estimates using 1000 datasets resampled from the original dataset. The entire procedure was performed using Wings for NONMEM (Version 720 [22]) [23].

Multivariate analysis of covariates for CL(S)

The contribution of patients' demographic and genotypic characteristics (i.e., age, body weight, body surface area (BSA), AST, ALT and CLcr as continuous variables, and sex,

history of smoking, *CYP2C9**2, *3, *5, *6 or *11, *CYP2C9**8, rs7089580 and rs12777823 as categorical variables) to CL(S) was assessed by multivariate analyses using NONMEM. For respective continuous variables, for example, the effect of BSA on CL(S), the following two models (a power model and an exponential model) were examined:

$$CL(S) = CL(S)_{\text{mean}} \times \left(\frac{BSA_{\text{individual}}}{BSA_{\text{median}}} \right)^{\theta_{BSA}} \quad (2)$$

$$CL(S) = CL(S)_{\text{mean}} \times \exp\{\theta_{BSA} \times (BSA_{\text{individual}} - BSA_{\text{median}})\} \quad (3)$$

Multivariate analyses to select significant covariates of CL(S) involved a forward inclusion step ($p < 0.05$) and a backward deletion step ($p < 0.05$).

Impact of covariates on CL(S)

In order to assess the impact of BSA and *CYP2C9**8 on CL(S), Cp(S) values following administration of racemic warfarin at 6.5 mg/day (the median maintenance dose in this study) in 1000 African–American patients with different combinations of BSA and *CYP2C9* genotypes (1.99 vs 1.89 m² and *CYP2C9**1/*1 vs *1/*8 or *8/*8) were generated by the Monte Carlo technique using the PPK parameter estimates obtained in the final model.

Simulation of Cp(S) to compare algorithms employing loading doses or maintenance doses

We performed simulations of the time courses of Cp(S) in order to compare the respective algorithms employing loading doses (LDs) and maintenance doses. In this simulation, the maintenance doses in typical African–American patients (age 54.5 years, height 163.8 cm, body weight 90.0 kg, being the median values in the present cohort) with different combinations of *CYP2C9* (*1 vs *8) and *VKORC1* (GG vs GA vs AA) genotypes were estimated using the IWPC pharmacogenetic algorithm [4]. The 3-day loading doses were estimated using the EU-PACT algorithm [24] ($kel = 0.013 \text{ h}^{-1}$ for patients with *CYP2C9**1/*1 and $kel = 0.009 \text{ h}^{-1}$ for patients with *CYP2C9**8 and $\tau = 24 \text{ h}$), in which maintenance doses estimated by the IWPC algorithm are started from day 4.

Statistics

Associations between patients' continuous demographic data and CL(S) were examined using the Pearson correlation test. Comparisons between the median CL(S) obtained from patients with different categorical variables and those having different *CYP2C9* genotypes were performed by the Mann–Whitney U test. Probability was compared using either the χ^2 test or Fisher's exact test. Data are presented as medians with the upper and lower quartile ranges (25th and 75th percentiles) or means $\pm$ SD where appropriate. A two-tailed p-value of less than 0.05 was considered as the level of statistical significance for all analyses. All statistical analyses were performed using SPSS version 17.0.

Results

Patient characteristics

Demographic, clinical and genotypic characteristics for our 60 African–American patients are shown in Table 1. The maintenance dose in these patients (6.8 mg/day) was higher than those for Caucasian and Asian patients, as reported previously [2,25]. The patient demographics (mostly female with a larger body size) are consistent with previous reports of warfarin pharmacogenetics in larger cohorts of African–Americans [12]. There was a significant ($p < 0.01$) correlation between BSA and body weight ($r = 0.968$) and female patients had a smaller BSA (1.96 vs 2.11 m², $p < 0.05$). An association was found between *CYP2C9**8 and rs12777823 ($p < 0.05$). Minor-allele frequencies for the genetic variants (*CYP2C9**2, *3, *5, *6, *8, *11, rs7089580 and rs12777823) were consistent with those reported previously in African–American populations [8,11,12].

Relationships between dose & Cp(S)

The estimated pharmacokinetic parameters in African–Americans are summarized in Table 2. Overlapping means and 95% CIs for population parameters obtained from the original dataset and those from the bootstrap values were observed. A high predictive performance with no systematic deviations of the final PPK model was demonstrated in the diagnostic plots (Figure 1A–C). These results suggested that the parameter estimates obtained can adequately describe the time courses of Cp(S) in African–American populations.

Predictors of CL(S) & their impact

The median values (95% CI) of CL(S) for patients were 204 ml/h (92–382 ml/h, $n = 39$) for *CYP2C9**1/*1, 161 ml/h (122–251 ml/h, $n = 5$) for *CYP2C9**1/*2, 67 ml/h ($n = 1$) for *CYP2C9**1/*3, 221 ml/h ($n = 1$) for *CYP2C9**1/*5, 106 ml/h ($n = 1$) for *CYP2C9**1/*6, 125 ml/h (71–228 ml/h, $n = 12$) for *CYP2C9**1/*8 and *8/*8, 114 and 258 ml/h ($n = 2$) for *CYP2C9**1/*11, 197 ml/h (101–365 ml/h, $n = 14$) for heterozygous rs7089580 and 147 ml/h (65–303 ml/h, $n = 30$) for hetero- and homo-zygous rs12777823, respectively. During the screening step, BSA, body weight, *CYP2C9**8 and rs12777823 were extracted as significant covariates of CL(S) ($p < 0.05$). Age and *CYP2C9* SNPs (*2, *3, *5, *6 or *11) exerted no significant effect on CL(S) in this population. As a strong internal correlation was observed between BSA and body weight, a final model based on either BSA or body weight was developed, respectively. During the deletion step, rs12777823 was removed from both models. In the final model constructed on the basis of BSA, CL(S) was expressed by the following equation:

$$\text{CL(S) (ml/h)} = 179 \times 0.691^{CYP2C9*8} \times \exp\{0.518 \times (\text{BSA}_{\text{individual}} - 1.99)\} \quad (4)$$

where *CYP2C9**8 = 0 in patients with *CYP2C9**1/*1 and *CYP2C9**8 = 1 in patients with *CYP2C9**1/*8 or *8/*8 and 1.99 m² was the median BSA. As shown in Equation 4, BSA with the exponential model (Equation 3 in Methods) and the *CYP2C9**8 genotype were significant independent contributors to overall variability in CL(S), in other words, a 30% reduction in CL(S) for patients with *CYP2C9**8 relative to patients with the wild-type and a

5% reduction in CL(S) per -0.1 m^2 in BSA from 1.99 m^2 to 1.89 m^2 , which was roughly equivalent to a -10 kg reduction in body weight from 93 kg to 83 kg (height = 164 cm) (Table 2 & Figure 2). As shown in Figure 2, *CYP2C9*8* mutation had a stronger impact than BSA/body weight ($0.1 \text{ m}^2/10 \text{ kg}$) on CL(S).

Simulations of Cp(S) using algorithms with loading doses or maintenance doses

Predicted time courses of Cp(S) following administration of maintenance doses estimated using the IWPC algorithm [4] are shown in Figure 3A and those after the three loading doses estimated using the EU-PACT algorithm [24] are shown in Figure 3B. The maintenance doses estimated for typical African–American patients carrying the *CYP2C9*1/*1* and the respective *VKORC1* GG, GA or AA allele were 6.0, 4.5 and 3.5 mg/day, respectively. The predicted maintenance doses estimated using the IWPC algorithm were approximately 1.0 mg/day lower than those observed in patients with the *CYP2C9*1/*1* and the respective *VKORC1* GG or GA allele. As the IWPC algorithm does not include the *CYP2C9*8* variant as a covariate for dose prediction, patients with the *CYP2C9*8* allele would receive the same doses as patients with *CYP2C9*1/*1*, which might lead to a 1.4-times higher average Cp(S) at the steady state (Cpss) in patients with any of the three *VKORC1* genotypes. In addition, simulations showed that the determinant of the average Cpss values might be the *VKORC1* genotype (e.g., patients with the insensitive GG genotype would require a higher dose, and thus a higher Cpss, than patients carrying the sensitive AA genotype in order to maintain the therapeutic INR) as well as the *CYP2C9* genotype, while the time to reach 95% of the steady state value is determined by CL(S), i.e., the *CYP2C9* genotypes in this simulation (i.e., 10 days for patients with *CYP2C9*1/*1* and 14.5 days for patients with *CYP2C9*8*, irrespective of the *VKORC1* genotype) (Figure 3A). In the case of the loading dose algorithm (EU-PACT) being employed, the time to reach 95% of the steady state would be reduced (i.e., 2 days for patients with *CYP2C9*1/*1* and 10 days for patients with *CYP2C9*8*), whereas the same average Cpss observed after the maintenance dose would be attained (Figure 3B). When a 30% lower dose was used for patients with *CYP2C9*8* in the loading dose algorithm, the differences in the time to reach 95% of the steady state and the average Cpss between patients with the *CYP2C9* wild-type and those with *CYP2C9*8* would be eliminated (Figure 3C).

Discussion

Although many genetic variants affecting warfarin dose have been found in African–Americans [8–12], the present study employing multivariate analysis is the first to demonstrate that *CYP2C9*8* and BSA (or body weight) are the stronger contributors to CL(S) in this population. The *CYP2C9*2/*3* genotypes and age, which are major determinants of CL(S) in Caucasian and Asian patients [26,27], did not have significant effects on CL(S) in the study population. The low frequency of these variants in the African–American population may be responsible for the results we obtained. In addition, the population mean values for CL(S) estimated using PPK analysis in African–Americans with *CYP2C9*1/*1* (179 ml/h) appear to be lower than those reported in Caucasian and Asian patients (348 ml/h [26] and 240 ml/h [27], respectively). These data strongly suggest a

population difference in the relationships between dose and Cp(S) (the pharmacokinetic [PK] process) [28].

To clarify the reasons for the inconsistent outcomes of the two randomized controlled trials [13,14] which investigated the clinical utility of genotype-based warfarin dosing, we focused on differences in the study designs and populations. While the EU-PACT study involving mostly whites employed a loading dose, the COAG study in which 27% of the population was African Americans used a maintenance dose. Therefore, we compared the time courses of Cp(S) following a loading dose (Figure 3B) with those for a maintenance dose (Figure 3A) in African-Americans. Our simulations showed that the time to reach a steady state was reduced for all genotype combinations of *CYP2C9* and *VKORC1* after starting the loading dose, which may have contributed to maintenance of the therapeutic INR control from the early phase during warfarin induction observed in the EU-PACT study [13]. In addition, patients carrying *CYP2C9**8 would have a higher average Cp(S) at the steady state than patients with the wild-type, which might have partly contributed to the INRs above the therapeutic range observed for African-Americans in the COAG study [14]. Similarly, a higher Cp(S) would be observed in patients carrying *CYP2C9**5, *6, *11 and rs12777823, who have been reported to show lower CL(S) than patients with the *CYP2C9* wild-type. Moreover, if the *VKORC1* genotype is the only factor contributing to lower warfarin sensitivity in African-Americans [1], then the Cp(S) needed in order to maintain the therapeutic INR would be similar in patients with the same *VKORC1* genotype, irrespective of any difference in ethnicity. However, the average Cpss for achieving the therapeutic INR in African-Americans appears to be higher than those simulated in white and Asian patients with three different combinations of *CYP2C9**1/*1 and respective *VKORC1* GG, GA and AA genotypes [26,27], which might have contributed to the supratherapeutic INR values observed for African-American patients in the COAG study [14].

The present study had several limitations. First, the influences of African-American-specific, but rather rare SNPs, such as *CYP2C9**5, *6, *11 and rs7089580, on CL(S) could not be estimated due to the limited number of the study patients. Although the minor-allele frequency of 30% was observed for rs12777823, this SNP was excluded as a predictor of CL(S), partly because of the significant association with *CYP2C9**8. In addition to the small sample size, the majority of our patients were rather young, obese and female, and therefore they may not have been the best representatives of African-American patients receiving warfarin. With regard to the sampling point, we were unable to estimate intraindividual variability in the PPK analysis because only one sampling point at the steady state was available for patients in our clinical study. Most importantly, the present study was limited to pharmacokinetics. Pharmacodynamic studies will be necessary to clarify factors contributing to not only interindividual but also interpopulation differences in warfarin doses, and to construct population-specific dosing algorithms that will predict the warfarin requirements for all patients of any ethnic background.

Conclusion & future perspective

PK-pharmacodynamic (PD) studies of drugs in African-Americans have been very limited, and this is also the case for warfarin. As a result, the currently available genotype-based

warfarin dosing algorithms do not include African-specific variants that may influence the PK–PD of warfarin, and therefore the performance of these dosing algorithms appears to be lower in African-Americans. This study showed that African-Americans had PK predictors being independent from those of Caucasians that were mainly related to demographic and genetic factors, and that the different PK profiles could be eliminated by taking African-specific pharmacokinetic characteristics into account. Future clinical investigations identifying PK and PD determinants in minority populations will clearly be necessary. Such studies will help to improve dose prediction and anticoagulation control for patients as a whole, irrespective of their racial origin.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Financial & competing interests disclosure

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Executive summary

Predictors of *S*-warfarin clearance

- *CYP2C9**8, but not *CYP2C9**2/*3, and body surface area (BSA)/body weight were determinants of *S*-warfarin clearance (CL[S]) in African-Americans.
- The influence of *CYP2C9**8 (−30%) was larger than that of BSA/body weight (−5% per every 0.1 m² decrease in BSA or every 10 kg decrease in body weight) in reducing CL(S).

Simulations of plasma *S*-warfarin concentration to assess genotype-based warfarin dosing algorithms

- The time to reach a steady state following the loading dose will be reduced compared with the maintenance dose for all genotype combinations of *CYP2C9* and *VKORC1*.
- If currently available algorithms are used for patients with *CYP2C9**8, the plasma *S*-warfarin concentration (Cp[S]) at steady state will be 1.4-times higher than those in patients with *CYP2C9**1/*1.

Figure 1. Diagnostic plots to assess the adequacy of the estimated population parameters. Relationship between population predictions of plasma Cp(S) and the observed values (**A**), between individual predictions of Cp(S) and the observed values (**B**) and that between population predictions of Cp(S) and weighted residuals (**C**). Cp(S): *S*-warfarin concentration.

Figure 2. Impacts of predictors of clearance for *S*-warfarin extracted from population pharmacokinetics analysis.

Influences of *CYP2C9**8 mutation and BSA on *S*-warfarin clearance ($CL[S]$) in the time courses of plasma $Cp(S)$ (**A**) and the $Cp(S)_{ss,ave}$ (**B**) were predicted following administration of racemic warfarin at 6.5 mg/day in typical 1000 African–American individuals with different combinations of BSA (1.99 vs 1.89 m²)/BW (93.2 vs 83.2 kg) and *CYP2C9**8 genotypes (*1/*1 vs *1/*8 or *8/*8). These datasets were generated by the Monte Carlo technique. $Cp(S)_{ss,ave}$ (**B**) are shown as box and whisker plots. The horizontal line indicates the median and the box covers the 25–75th percentiles. Solid circles are outliers.

* $p < 0.05$ between the two groups, ** $p < 0.01$ between the two groups.

BSA: Body surface area; BW: Body weight; $Cp(S)$: *S*-warfarin concentration; $Cp(S)_{ss,ave}$: Average *S*-warfarin concentration at a steady state; NS: Not significant.

Figure 3. Influences of the different induction algorithms for warfarin (maintenance doses or loading doses) on the time courses of plasma concentration for *S*-warfarin.

The time courses of Cp(S) following administration of either maintenance doses estimated using the International Warfarin Pharmacogenetics Consortium algorithm ([A] The maintenance dose algorithm), loading doses estimated using the EU-PACT algorithm ([B] The LD algorithm*1; the LD estimated for patients with *CYP2C9**1/*1 was used for patients with *CYP2C9**8) or ([C] The LD algorithm*1,*8; a 30% lower LD estimated for patients with *CYP2C9**1/*1 was used for patients with *CYP2C9**8) were predicted in typical African–American individuals (age 54.5 years, height 163.8 cm and body weight 90 kg) with different combinations of *CYP2C9* (solid line: *1/*1 vs dashed line: *1/*8 or *8/*8) and *VKORC1* (left: GG; middle: GA; right: AA) genotypes.

Cp(S): *S*-warfarin concentration; IWPC: International Warfarin Pharmacogenetics Consortium; LD: Loading dose; MD: Maintenance dose.

Table 1.

Patient demographic, clinical and genotypic characteristics.

Characteristics [†]	n = 60
Age (years)	54.5 (32.0–81.5)
Gender (F/M)	52/8 (86.7%)
Body weight (kg)	90.0 (60.2–157.4)
Height (cm)	163.8 (152.4–184.2)
Body surface area (m ²)	1.99 (1.61–2.61)
BMI	34.2 (23.1–55.4)
Maintenance dose (mg/day)	6.79 (2.67–12.80)
INR	2.54 (1.95–3.51)
AST (IU/L)	22.0 (14.0–58.9)
ALT (IU/L)	18.5 (10.5–52.8)
Creatinine clearance (ml/min)	58.2 (22.6–107.1)
Alcohol (+/–)	2/58 (3.3%)
Smoking (+/–)	9/51 (15.0%)
Indication	
Atrial fibrillation	5 (8.3%)
Stroke	9 (15.0%)
Deep vein thrombosis	2 (3.3%)
Ventricular tachycardia	29 (48.3%)
Pulmonary embolism	27 (45.0%)
Peripheral vascular disease	4 (6.7%)
Cardiac valve replacement	2 (3.3%)
Complication	
Hypertension (+/–)	39/21 (65.0%)
Diabetes mellitus (+/–)	15/45 (25.0%)
Genotype wild/hetero/homo (minor allele frequencies)	
<i>CYP2C9</i> *2 rs1799853 (C>T)	55/5/0 (0.042)
<i>CYP2C9</i> *3 rs1057910 (A>C)	59/1/0 (0.008)
<i>CYP2C9</i> *5 rs28371686 (C>G)	59/1/0 (0.008)
<i>CYP2C9</i> *6 rs9332131 (delA)	59/1/0 (0.008)
<i>CYP2C9</i> *8 rs7900194 (G>A)	48/10/2 (0.117)
<i>CYP2C9</i> *11 rs28371685 (C>T)	58/2/0 (0.017)
<i>CYP2C9</i> rs7089580 (A>T) [‡]	42/14/0 (0.125)
rs12777823 (G>A)	30/24/6 (0.300)
<i>VKORC1</i> rs9923231 (–1639G>A)	50/10/0 (0.083)

[†]Data are median values (95% CI) or number (%).[‡]rs7089580 was determined in 56 of 60 patients.

Table 2. Population pharmacokinetic parameter estimates for the plasma *S*-warfarin concentration.

	Original dataset		Bootstrap value	
	Mean	95% CI	Mean [†]	95% CI [‡]
PK estimates: Cp(S)				
CL(S) (ml/h) [§]	179	158.8–199.2	179	159.0–203.0
Effect of BSA on CL(S)	0.518	0.187–0.849	0.516	0.178–0.859
Effect of <i>CYP2C9</i> *8 on CL(S)	0.691	0.552–0.830	0.698	0.563–0.862
Interindividual error				
$\omega_{CL(S)}$ (%)	37.5	32.7–42.4	36.5	30.2–42.9
Residual error				
σ (%)	1		1	

[†]Mean of 1000 bootstrap analyses for pharmacokinetics estimates.

[‡]The 2.5th and 97.5th values of the ranked bootstrap parameter estimates.

[§]CL(S) (ml/h) = $179 \times 0.691 \text{ CYP2C9}^{*8} \times \text{EXP}[0.518 \times (\text{BSA}_{\text{individual}} - 1.99)]$, where *CYP2C9**8 = 0 in patients with *CYP2C9**/**1* and *CYP2C9**1/**8* or **8*/*8. BSA_{median} is 1.99 m².

BSA: Body surface area; CL(S): *S*-warfarin clearance; Cp(S): *S*-warfarin concentration; PK: Pharmacokinetic.