


## ORIGINAL ARTICLE

## Population pharmacokinetics and pharmacogenomics of apixaban in Japanese adult patients with atrial fibrillation

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## AIMS

This study aimed to analyse the effects of genetic polymorphisms in drug transporters and metabolizing enzymes, and clinical laboratory data on the pharmacokinetic parameters of apixaban.

## METHODS

Data were collected from 81 Japanese patients with atrial fibrillation. Pharmacogenomic data were stratified by *ABCB1*, *ABCG2* and *CYP3A5* polymorphisms. The pharmacokinetic profile of apixaban was described by a one-compartment model with first-order absorption. Population pharmacokinetic analysis was conducted using a nonlinear mixed effect modelling (NONMEM™) program.

## RESULTS

The nonlinear relationship between oral clearance (CL/F) of apixaban and creatinine clearance (Ccr) was observed. The population mean of CL/F for a typical patient (Ccr value of 70 ml min<sup>-1</sup>) with the *CYP3A5*\*1/\*1 and *ABCG2* 421C/C or C/A genotypes was estimated to be 3.06 l h<sup>-1</sup>. When Ccr values were set to the typical value, the population mean of CL/F was 1.52 times higher in patients with the *CYP3A5*\*1/\*1 genotype compared with patients with the *CYP3A5*\*1/\*3 or \*3/\*3 genotype, while the population mean of CL/F was 1.49 times higher in patients with the *ABCG2* 421C/C or C/A genotype compared with patients with the *ABCG2* 421A/A genotype. However, no covariates affected the population mean of the apparent volume of distribution (Vd/F) of apixaban. The population mean of Vd/F was estimated to be 24.7 l.

## CONCLUSION

The present study suggests that the *ABCG2* 421A/A and *CYP3A5*\*3 genotypes and renal function are intrinsic factors affecting apixaban pharmacokinetics. These findings may provide useful information for precision medicine using apixaban, to avoid the risk of adverse reactions.

## WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The pharmacokinetic variability of apixaban is related to covariates such as renal function, sex, body weight, age and race.
- There is little information concerning the relationship between the pharmacokinetic variability of apixaban and genetic polymorphisms of drug metabolizing enzymes and transporters.

## WHAT THIS STUDY ADDS

- The population pharmacokinetic and pharmacogenomic model of apixaban in Japanese patients with atrial fibrillation is developed to estimate apixaban concentrations appropriately.
- The *ABCG2* 421A/A and *CYP3A5*\*3 genotypes as well as renal function can explain the inter-individual variability in apixaban pharmacokinetics in patients with atrial fibrillation.

## Introduction

**Apixaban** is a direct and selective inhibitor of activated coagulation factor X (FXa), which exists as a key enzyme positioned at the confluence of intrinsic and extrinsic coagulation pathways [1]. Apixaban is orally administered for the prevention of stroke or systemic embolism in patients with nonvalvular atrial fibrillation (AF) [2, 3]. The ARISTOTLE clinical trial demonstrated that in AF patients, efficacy and safety of apixaban was better than those of warfarin, which has been the most commonly used anticoagulant therapy [3–5]. Therefore, the number of prescriptions for direct FXa inhibitors such as apixaban and rivaroxaban are tending to increase while the number of prescriptions for warfarin have decreased [6–8]. However, the apixaban package insert indicates that there is no way to adjust its dosage according to monitoring anticoagulant activities such as prothrombin time (PT), international normalized ratio of PT (PT-INR), and anti-FXa activity because, unlike warfarin, the apixaban therapeutic window remains unclear [9–11]. Recent exposure–response studies showed that the area under the plasma concentration–time curve (AUC) for apixaban was related to the incidence of bleeding and venous thromboembolism [12, 13]. Additionally, underdosing anticoagulants in AF patients partly caused a high risk for stroke and thromboembolic events in the clinic [14]. Thus, monitoring the apixaban plasma concentrations in AF patients may lead to safer and more effective therapeutic dose adjustments.

Urinary unchanged apixaban represented approximately 25% of oral dose in healthy subjects, and its total metabolites represented approximately 25% of oral dose in healthy subjects [11, 15]. Apixaban is reported to be transported by the ATP-binding cassette multidrug transporters P-glycoprotein (ABCB1) and breast cancer resistance protein (**ABCG2**) in the small intestine, liver, and kidney, and is predominantly metabolized by intestinal and hepatic **cytochrome P450 (CYP) 3A4/5** [11, 16, 17]. Therefore, individual expression and/or function of these proteins can explain the pharmacokinetic variability of apixaban.

The clinical effectiveness of anticoagulants has been examined in relation to drug transporter and metabolizing enzyme single nucleotide polymorphisms (SNPs) [18–23]. Recent studies suggested that precision medicine using vitamin K epoxide reductase complex 1 (*VKORC1*) and *CYP2C9* genotypes is clinically useful for pharmacotherapy of warfarin

[18–20]. For dabigatran, a direct thrombin inhibitor, an SNP of carboxylesterase 1, which rapidly hydrolyses the prodrug (dabigatran etexilate) to dabigatran, is related to low trough concentrations of dabigatran and the risk of bleeding [21]. For both rivaroxaban and edoxaban, which are FXa inhibitors, no *ABCB1* polymorphisms affect these pharmacokinetics [22, 23]. However, our previous pharmacogenomic study indicated that trough concentrations of apixaban are associated with the *ABCG2* 421A/A and *CYP3A5*\*3 genotypes [24]. These results also suggest that *ABCG2* and *CYP3A5* genotypes can be good predictors of apixaban pharmacokinetics.

A few population pharmacokinetic studies of apixaban have suggested that the pharmacokinetic variability of apixaban was mainly explained by renal function, sex, body weight, age, race, venous thromboembolism, and patient and subject having a history of orthopaedic surgery [12, 13, 25]. However, a population pharmacokinetic analysis in Japanese AF patients has not been reported. Additionally, there is little information concerning the relationship between the pharmacokinetic variability of apixaban and genetic polymorphisms of drug metabolizing enzymes and transporters.

In this study, we conducted a population pharmacokinetic analysis of apixaban in Japanese AF patients and examined the effect of *ABCB1*, *ABCG2* and *CYP3A5* polymorphisms and clinical laboratory data on the apixaban pharmacokinetic parameters.

## Methods

### Ethics

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Review Board of Ritsumeikan University Biwako-Kusatsu Campus (Approval number BKC-IRB-2014-021) and the Ethics Boards of Shiga University of Medical Science (Approval number 26-116). Written informed consent was obtained from all patients prior to enrolment.

### Patients and study design

Japanese adult inpatients and outpatients with AF who received apixaban at the Shiga University of Medical Science Hospital from February 2015 to May 2016 were enrolled in this study. All patients took oral apixaban tablets (Eliquis<sup>®</sup>, Bristol-Myers Squibb, Princeton, NJ, USA and Pfizer Inc.,

Groton, CT, USA) twice daily at a dose of 5–20 mg day<sup>-1</sup>. For inpatients, blood samples were drawn in 3.2% citrated tubes at three time points: trough sampling and serial sampling at 0.5–2 h and 9–12 h after last apixaban dose. For outpatients, blood samples were also drawn in 3.2% citrated tubes at a single point during each hospital visit (0.3–16 h after last apixaban dose). The demographic and clinical laboratory data such as sex, age, body weight, serum creatinine (Scr), creatinine clearance (Ccr), aspartate amino transferase (AST), alanine amino transferase (ALT), and concomitant CYP3A4 and/or P-glycoprotein inhibitors or inducers were retrospectively collected from electronic medical records. Ccr was calculated using the Cockcroft–Gault equation [26]. Physicians and pharmacists asked patients about drug compliance to confirm drug exposure at each hospitalization or hospital visit. According to their assessment, we judged drug compliance of the patients. Patients who had no record of these measurements or who exhibited poor drug compliance were excluded from the analyses.

### Apixaban assay

Plasma samples from patients were separated by centrifugation at 2500 g for 15 min at 4°C and stored at –80°C until analysis. Plasma concentrations of apixaban were analysed using liquid chromatography with electrospray ionization tandem mass spectrometry (LC/MS/MS), according to our previous study [24]. The calibration curve of apixaban was linear from 2.5 to 500 ng ml<sup>-1</sup>, and the lower limit of quantification was 2.5 ng ml<sup>-1</sup>. All plasma samples were handled in this study because their concentrations of apixaban were higher than the lower limit of quantification. When plasma concentration of apixaban was more than 500 ng ml<sup>-1</sup>, the sample was diluted with blank plasma and reanalysed. The coefficient of deviation of the intra- and inter-day precision were from 1.1 to 8.0% and from 2.0 to 8.2%, respectively. The accuracy value was from 96.3 to 103.7%.

### Genotyping

After extracting genomic DNA from blood samples using a DNA Extract All Reagents Kit (Applied Biosystems, Waltham, MA, USA), the *ABCB1* 1236C>T (rs1128503), 2677G>T/A (rs2032582), 3435C>T (rs1045642), *ABCG2* 421C>A (rs2231142), and *CYP3A5* 6986A>G (rs776746; \*3) polymorphisms were analysed using a real-time PCR system (StepOnePlus™; Applied Biosystems), as described previously [24].

### Population pharmacokinetic analysis

A population pharmacokinetic analysis was conducted using a nonlinear mixed-effects modelling (NONMEM) program version 7.3.0 (Icon Development Solutions, Ellicott City, MD), using the first-order conditional estimation method with interaction. The plasma concentration–time profiles of apixaban after oral administration were analysed using a 1-compartment model with first-order absorption (ADVAN2 TRANS2). The pharmacokinetic parameters of apixaban were estimated as apparent oral clearance (CL/F) and apparent volume of distribution (Vd/F), while the absorption rate constant (*k*<sub>a</sub>) was fixed at the reported value of 0.42 h<sup>-1</sup> [13] because of a lack of data on the absorption

phase. The relationship between the observed and predicted apixaban concentrations was described by following an exponential error model, taking into account intra-individual variability ( $\epsilon$ ):

$$C_{ij} = \bar{C}_{ij} \cdot \exp(\epsilon_{ij}) \quad (1)$$

where  $C_{ij}$  and  $\bar{C}_{ij}$  designate the observed and predicted apixaban concentrations in the  $j$ th record of  $i$ th patient, respectively. The parameters CL/F, Vd/F and  $K_a$  are then described using the population mean parameters ( $\theta$ ), as in following equations:

$$(CL/F)_i = \theta_1 \cdot \exp(\eta_{1i}) \quad (2)$$

$$(Vd/F)_i = \theta_2 \cdot \exp(\eta_{2i}) \quad (3)$$

$$K_{a_i} = \theta_3 = 0.42 \quad (4)$$

where  $\eta_1$  and  $\eta_2$  designate the intra-individual variabilities for CL/F and Vd/F, respectively. Hereafter, the combination of these equations was selected as a basic model for the subsequent covariate analysis.

To develop the population pharmacokinetic model of apixaban, the influence of Ccr was first assessed in the covariate model, because renal function was known to be related to the CL/F of apixaban [12, 13, 25, 27, 28]. The CL/F of apixaban was described as the sum of apparent renal (CL<sub>R</sub>/F) and apparent nonrenal (CL<sub>NR</sub>/F) clearances as follows [13, 25]:

$$(CL/F)_i = (CL_R/F)_i + (CL_{NR}/F)_i \quad (5)$$

To assess the influence of Ccr on CL<sub>R</sub>/F, a covariate analysis was conducted using the two following equations:

$$(CL_R/F)_i = \theta_1 \cdot \left( \frac{CCR_i}{CCR_{median}} \right)^{\theta_4} \quad (6)$$

$$(CL_R/F)_i = \theta_1 \cdot \left( \frac{CCR_i}{CCR_{median}} \right) \quad (7)$$

where  $CCR_i$  and  $CCR_{median}$  denote the Ccr of the  $i$ th patients and the median Ccr, and  $\theta_4$  is the population mean estimate. The covariate models incorporating the Ccr value were selected based on the lowest objective function value (OBJ) that was calculated using the NONMEM program.

After conducting the covariate analysis for Ccr, the effects of continuous covariates such as AST, ALT, body weight, and age of patients on the parameters CL<sub>R</sub>/F, CL<sub>NR</sub>/F, and Vd/F were evaluated using the following equations:

$$P_i = \theta_5 \cdot \left( \frac{COV_i}{COV_{median}} \right)^{\theta_6} \quad (8)$$

$$P_i = \theta_5 \cdot \left( \frac{COV_i}{COV_{median}} \right) \quad (9)$$

where  $P_i$  is a pharmacokinetic parameter of the  $i$ th patients,  $COV_i$  and  $COV_{median}$  denote the covariate of the  $i$ th patients

and median of the covariate, and  $\theta_5$  and  $\theta_6$  are population mean estimates. The effects of categorical covariates such as concomitant CYP3A4 and/or P-glycoprotein inhibitors, co-morbidities and genetic polymorphisms in *ABCB1*, *ABCG2* and *CYP3A5* were also evaluated using the following equation:

$$P_i = \theta_5 \times \theta_7^A \quad (10)$$

where  $\theta_7$  is the population mean estimate, and the dichotomous parameter A is equal to 1 if the categorical covariate is present, and 0 if it was not present. For example, in the case of genetic polymorphisms, the parameter A is equal to 1 if a patient had a specific genotype, otherwise it was set to 0. Covariates were added to the basic model or the model involving Ccr using a forward stepwise inclusion method and considered statistically significant if the OBJ decreased more than 3.84 ( $P < 0.05$  with 1 degree of freedom). Subsequently, covariates were removed from the full model using a backward stepwise deletion method and considered statistically significant if the OBJ increased more than 6.63 ( $P < 0.01$  with 1 degree of freedom).

### Model evaluation

The following goodness-of-fit plots were used to investigate the models: the relationship between observed (OBS) and population predicted value (PRED) or individual predicted value (IPRED), and the relationship between conditional weighted residuals (CWRES) and time after the last dose or PRED. The final model was also assessed using a visual predictive check (VPC) and nonparametric bootstrap analysis. In VPC analysis, a total of 1000 hypothetical data sets were simulated by random sampling using the NONMEM program. The 50th percentile (median) and 90% prediction interval of the simulated concentrations were plotted using the observed concentrations. In the bootstrap analysis, a total of 1000 replication data sets were generated by random sampling using the Perl-speaks-NONMEM version 4.7.0 program [29]. The estimates of each population pharmacokinetic parameter obtained using the final model were compared with the medians and 95% prediction intervals using the bootstrap analysis.

### Model-based simulation

The AUC from 0 to 12 h at steady state ( $AUC_{0-12}$ ) as well as the CL/F of apixaban based on the final model were calculated using a Monte Carlo simulation to determine the impact of Ccr and genetic polymorphisms on apixaban exposure. Two hundred pharmacokinetic profiles were simulated for a patient with various Ccr values (30, 60, 90, and 120 ml min<sup>-1</sup>) and genetic polymorphisms, using the NONMEM program. The oral dosing schedules were set to 5 mg twice daily. The  $AUC_{0-12}$  of apixaban were calculated as its dose divided by the CL/F.

### Statistical analysis

Data are expressed as the median value unless otherwise indicated. For multiple comparisons against a control group, significant differences were evaluated using the Kruskal–

Wallis test, followed by Dunn's *post hoc* test using Prism6 software (GraphPad Software, San Diego, CA, USA). Allele frequencies of polymorphisms were evaluated according to the Hardy–Weinberg equilibrium using the  $\chi^2$  test. A probability value of less than 0.05 was considered statistically significant.

### Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [30], and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 [31, 32].

## Results

### Patient characteristics and genotype frequencies

No patients were treated with combination of apixaban and the CYP3A4 and/or P-gp inhibitors/inducers except amiodarone, verapamil and rifampicin. Because only one patient was treated with rifampicin, this patient was excluded from this analysis. As a result, a total of 276 observations from 81 patients were included in analyses and characteristics of patients are summarized in Table 1. Blood samples were drawn during hospitalization for 23 inpatients, while they were drawn during hospital visit for 32 outpatients. For 26 patients, blood samples were drawn during both hospitalization and hospital visit. Blood samples were drawn at three time points for 48 of 49 inpatients. For only one patient, blood samples were drawn at two time points (trough sampling and sampling at approximately 10 h after last apixaban dose). Twenty-three patients were treated with combinations of apixaban and the CYP3A4 and/or P-glycoprotein inhibitors amiodarone (17 patients) and verapamil (6 patients). Frequencies of the *CYP3A5*, *ABCB1* and *ABCG2* genotypes are shown in Table 2. All allele frequencies in this study were comparable to the Hardy–Weinberg equilibrium.

### Population pharmacokinetic model

To develop the population pharmacokinetic model of apixaban, the effect of Ccr on apixaban pharmacokinetics was first examined. The OBJ value with the basic model was calculated to be 2671.80. The OBJ values with covariate models including Ccr with Eqs. (6) and (7) were calculated to be 2625.89 and 2627.80, respectively, suggesting that these OBJ values were significantly smaller than those obtained using the basic model. Therefore, the covariate model including Ccr with Eq. (6) was considered to be the most suitable to characterize the relationship between CL<sub>R</sub>/F and Ccr.

The results of the covariate analyses are summarized in Table 3. A forward inclusion method revealed that Ccr significantly affected the CL<sub>R</sub>/F of apixaban, and that *CYP3A5*\*3 and *ABCG2* 421A/A genotypes had a significant impact on the CL<sub>NR</sub>/F of apixaban. A backward elimination method did not exclude these covariates. Other covariate models including AST, ALT, body weight, age of patients, three

**Table 1**Clinical characteristics and demographics of patients with atrial fibrillation<sup>a</sup>

<b>Number of patients</b>	81
<b>Number of measurements</b>	276
<b>Sex (male/female)</b>	61/20
<b>Age (years)</b>	68.1 (40.5–84.9)
<b>Body weight (kg)</b>	65.0 (41.0–92.2)
<b>Dosage of apixaban (mg day<sup>-1</sup>)</b>	10 (5–20)
<b>Plasma concentration of apixaban (ng ml<sup>-1</sup>)</b>	157.9 (15.6–673.6)
<b>Serum creatinine (mg dl<sup>-1</sup>)</b>	0.88 (0.41–1.34)
<b>Creatinine clearance (ml min<sup>-1</sup>)</b>	69.8 (30.6–145.5)
<b>Aspartate amino transferase (IU l<sup>-1</sup>)</b>	23 (13–97)
<b>Alanine amino transferase (IU l<sup>-1</sup>)</b>	19 (5–115)
<b>Number of patients treated with CYP3A4 and/or P-glycoprotein inhibitors</b>	
<b>Amiodarone</b>	17
<b>Verapamil</b>	6
<b>Number of patients with co-morbidities</b>	
<b>Hypertension</b>	36
<b>Diabetes mellitus</b>	34
<b>Heart failure</b>	20
<b>Dyslipidemia</b>	18

<sup>a</sup>Data are presented as the number or median with the range in parentheses.

*ABCB1* genotypes, co-morbidities (hypertension, diabetes mellitus, heart failure, dyslipidemia), and concomitant CYP3A4 and/or P-glycoprotein inhibitors (amiodarone and verapamil) did not affect the CL<sub>R</sub>/F or CL<sub>NR</sub>/F of apixaban. Additionally, no covariate models affected the Vd/F of apixaban. The population pharmacokinetic parameter estimates of apixaban in Japanese AF patients are shown in Table 4. The final population pharmacokinetic model for CL/F was as follows:

$$CL/F(L/h) = 1.53 \cdot \left\{ \left( \frac{CCR}{70} \right)^{0.7} + 0.312^{CYP3A5} \cdot 0.341^{ABCG2} \right\} \quad (11)$$

where the dichotomous parameter CYP3A5 is equal to 1 if patients had the *CYP3A5*\*1/\*3 or \*3/\*3 genotype, otherwise it was set to 0, and the dichotomous parameter ABCG2 is equal to 1 if patients had the *ABCG2* 421A/A genotype, otherwise it was set to 0. A nonlinear relationship between CL/F of apixaban and Ccr was observed. The population mean of CL/F for a typical patient (Ccr value of 70 ml min<sup>-1</sup>) with the *CYP3A5*\*1/\*1 and *ABCG2* 421C/C or C/A genotypes was estimated to be 3.06 l h<sup>-1</sup>. When Ccr values were set to the typical value, the population mean of CL/F was 1.52 times higher in patients with the *CYP3A5*\*1/\*1 genotype compared with patients with the *CYP3A5*\*1/\*3 or \*3/\*3 genotype, while the population mean of CL/F was 1.49

times higher in patients with the *ABCG2* 421C/C or C/A genotype compared with patients with the *ABCG2* 421A/A genotype. The CL/F value for patients with both *CYP3A5*\*1/\*3 or \*3/\*3 and *ABCG2* 421A/A genotypes was decreased further, compared with patients with both the *CYP3A5*\*1/\*1 and the *ABCG2* 421C/C or C/A genotype (Figure 1). Inter-individual variabilities for CL/F and Vd/F were 26.6% and 56.6%, respectively. The covariance between inter-individual variability for CL/F and that for Vd/F was 11.6%, and the correlation coefficient between individual CL/F and Vd/F was 0.770. The intra-individual variability was 34.0% (Table 4).

### Model evaluation

The goodness-of-fit plots for the final model are shown in Figure 2. PRED was shown to correlate reasonably with OBS, and IPRED was shown to correlate well with OBS. No systematic deviation was observed in the relationship between CWRES and time after last dose or PRED. The final model was also assessed by estimating the population pharmacokinetic parameters from 1000 bootstrap resamplings. The median values of population pharmacokinetic parameters calculated from the bootstrapping samples were generally similar to the population estimates in the final model (Table 4). The final model was further evaluated using VPC analysis. As shown in Figure 3, VPC analysis generally resulted in a reasonable predictability of the final model.

**Table 2**Allele frequencies of *ABCB1*, *ABCG2* and *CYP3A5* polymorphisms in Japanese patients

Genotype	Number of patients	Frequency	Allele frequency <sup>a</sup>
<b><i>ABCB1</i> 1236C&gt;T</b>			0.451
C/C	24	0.296	
C/T	41	0.506	
T/T	16	0.198	
<b><i>ABCB1</i> 2677G&gt;T/A</b>			0.556
G/G	20	0.247	
G/T	11	0.136	
G/A	21	0.259	
A/A	16	0.198	
T/A	11	0.136	
T/T	2	0.024	
<b><i>ABCB1</i> 3435C&gt;T</b>			0.407
C/C	31	0.383	
C/T	34	0.420	
T/T	16	0.197	
<b><i>ABCG2</i> 421C&gt;A</b>			0.315
C/C	39	0.482	
C/A	33	0.407	
A/A	9	0.111	
<b><i>CYP3A5</i> *3</b>			0.765
*1/*1	4	0.049	
*1/*3	30	0.370	
*3/*3	47	0.581	

<sup>a</sup>All allele frequencies were comparable to the Hardy–Weinberg equilibrium.

Additionally, the VPC of apixaban concentrations showed that the model fit was similar for inpatients and outpatients (Figure S1).

### Model-based simulation

To simulate the  $AUC_{0-12}$  of apixaban at steady state using the final population pharmacokinetic parameters, data sets were divided into four groups according to the combination of the *ABCG2* and *CYP3A5* genotypes in hypothetical patients, as follows: patients with the *CYP3A5*\*1/\*1 and *ABCG2* 421C/C or C/A genotype (group A); patients with the *CYP3A5*\*1/\*1 and *ABCG2* 421A/A genotypes (group B); patients with the *CYP3A5*\*1/\*3 or \*3/\*3 and the *ABCG2* 421C/C or C/A genotype (group C); and patients with *CYP3A5*\*1/\*3 or \*3/\*3 and *ABCG2* 421A/A genotypes (group D). As shown in Figure 4, the median predicted  $AUC_{0-12}$  in group A decreased from 2104 to 1329 ng h ml<sup>-1</sup> when the Ccr values were changed from 30 to 120 ml min<sup>-1</sup>. The median predicted  $AUC_{0-12}$  in group A was significantly lower compared with that in groups B, C and D independent of the Ccr values ( $P < 0.001$ ).

### Discussion

We recently reported that trough concentrations of apixaban were significantly higher in patients with the *ABCG2* 421A/A genotype than in patients with the *ABCG2* 421C/C or C/A genotype, and that trough concentrations of apixaban were significantly higher in patients with the *CYP3A5*\*1/\*3 or \*3/\*3 genotype compared with patients with the *CYP3A5*\*1/\*1 genotype. Additionally, although apixaban is known to be a substrate of *ABCB1*, no *ABCB1* polymorphism altered its trough concentration [24]. In this study, we estimated the population pharmacokinetic parameters of apixaban in Japanese AF patients and examined the impact of polymorphisms in *ABCB1*, *ABCG2*, *CYP3A5*, and clinical laboratory data on its pharmacokinetic parameters. It was reported that the  $CL_R$  of apixaban in healthy subjects administered intravenously was approximately 34% of total clearance [33]. Therefore, its  $CL/F$  was described as the sum of  $CL_R/F$  and  $CL_{NR}/F$  to examine the effect of Ccr on the  $CL_R/F$  of apixaban. Our data indicated the nonlinear relationship between  $CL/F$  and Ccr. A few population pharmacokinetic studies of apixaban showed that the

**Table 3**

Summary of the tested covariate effects on OBJs

Tested covariates	$\Delta$ OBJ (1st selection) <sup>a</sup>	$\Delta$ OBJ (2nd selection)	$\Delta$ OBJ (3rd selection)
<b>Effect on CL<sub>R</sub>/F</b>			
<i>ABCB1</i> 1236 T/T	-2.72		
<i>ABCG2</i> 421A/A	-3.93	-4.24	1.17
<i>CYP3A5</i> *3	-3.73		
Amiodarone	– <sup>b</sup>		
<b>Effect on CL<sub>NR</sub>/F</b>			
<i>ABCB1</i> 1236 T/T	-4.82	-3.31	
<i>ABCG2</i> 421A/A	-8.99	-6.80	Included
<i>CYP3A5</i> *3	-9.12	Included	Included
Amiodarone	-7.09	– <sup>b</sup>	
<b>Effect on Vd/F</b>			
BW with Eq. (8)	0.01		
BW with Eq. (9)	1.96		

<sup>a</sup>The difference from the OBJ value was calculated using the covariate model including Ccr with Eq. (6) (OBJ value, 2625.89), and it is expressed to two decimal places.

<sup>b</sup>Calculation of the OBJ value was not conducted.

**Table 4**Population pharmacokinetic parameter estimates for apixaban in AF patients<sup>a</sup>

Parameters	Original data		1000 Bootstrap sample data	
	Mean	95% CI	Median	2.5th–97.5th percentiles
$CL/F (l h^{-1}) = \theta_1 \cdot \left\{ \left( \frac{CCR}{70} \right)^{\theta_4} + \theta_5^{CYP3A5} \cdot \theta_6^{ABCG2} \right\}$				
$\theta_1 (l h^{-1})$	1.53	1.39–1.67	1.49	1.16–1.81
$\theta_4$	0.700	0.471–0.929	0.714	0.340–0.976
$\theta_5^b$	0.312	0.273–0.351	0.342	0.124–0.737
$\theta_6^c$	0.341	0.160–0.522	0.478	0.08–0.861
$Vd/F (l) = \theta_2$	24.7	15.8–33.6	25.0	19.1–43.7
$k_a (h^{-1}) = \theta_3$	0.42 fixed	–	0.42 fixed	–
<b>Inter- and intra-individual variabilities<sup>d,e</sup></b>				
$\eta_1$ (%)	26.6 (21.5)	18.7–34.5	25.9	16.4–34.4
$\eta_2$ (%)	56.6 (35.0)	8.8–104	57.3	21.3–103
$\varepsilon$ (%)	34.0 (12.0)	28.0–40.0	33.6	28.3–38.7

<sup>a</sup>The  $k_a$  was set to the literature value [13].

<sup>b</sup>If patients had the *CYP3A5*\*1/\*3 or \*3/\*3 genotype, then the dichotomous parameter *CYP3A5* was equal to 1, otherwise it was set to 0.

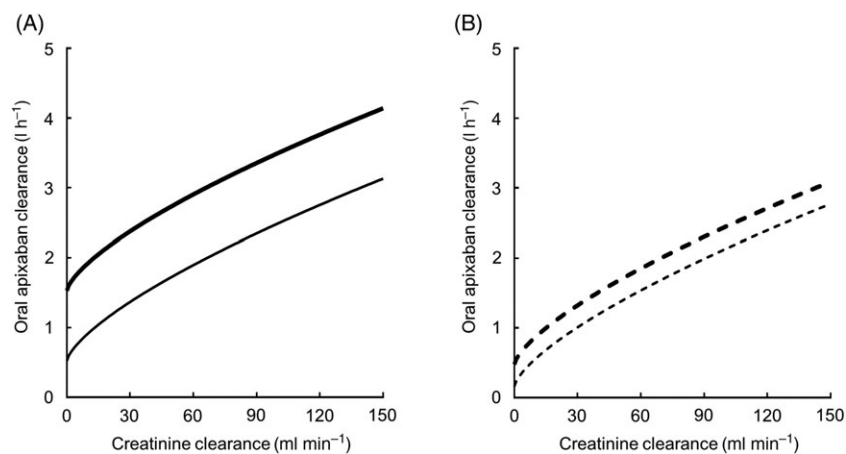
<sup>c</sup>If patients had the *ABCG2* 421A/A genotype, then the dichotomous parameter *ABCG2* was equal to 1, otherwise it was set to 0.

<sup>d</sup>The  $\eta_1$ ,  $\eta_2$  and  $\varepsilon$  values denote the inter-individual variabilities for CL/F, V/F and the intra-individual variability, respectively.

<sup>e</sup>Data are presented as the mean with the shrinkage (%) in parentheses.

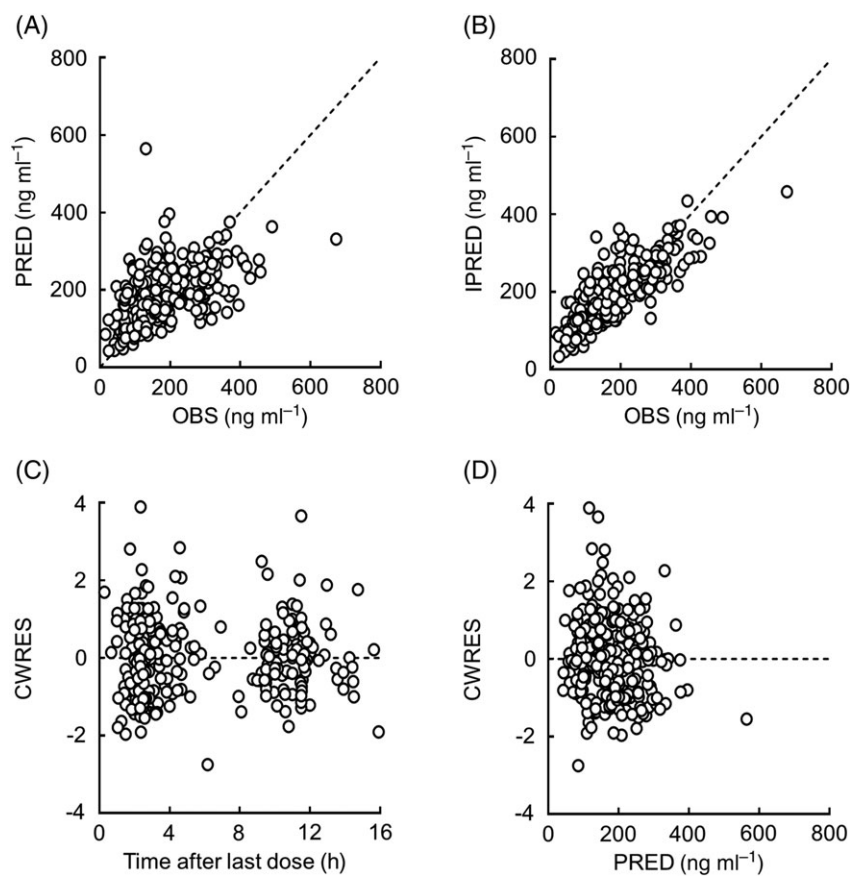
relationship between Ccr and CL<sub>R</sub>/F of apixaban was described using a linear [12],  $E_{max}$  [13], or power [25] model. Thus, our model is considered to be compatible with these models. In a subsequent covariate analysis, only the Ccr was

identified as a significant covariate for CL<sub>R</sub>/F, while the *ABCG2* 421A/A and *CYP3A5*\*3 genotypes were identified as significant covariates for CL<sub>NR</sub>/F (Figure 1, Table 3). As shown in Figures 2 and 3 and in Table 4, bootstrap and VPC analyses



**Figure 1**

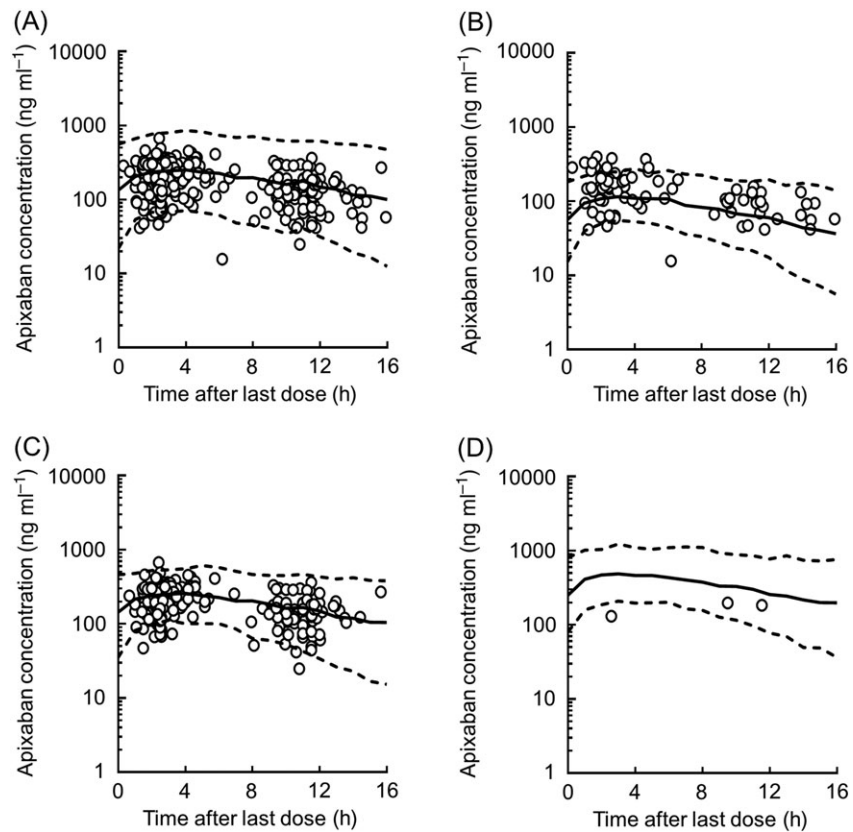
Correlation between the population mean estimates of both oral apixaban and creatinine clearance in the final model. In panel A, the thick and thin lines indicate the population mean estimates for a typical patient with the *CYP3A5*\*1/\*1 and *ABCG2* 421C/C or C/A genotype, and for a typical patient with the *CYP3A5*\*1/\*1 and *ABCG2* 421A/A genotypes, respectively. In panel B, the thick dotted and thin dotted lines indicate the population mean estimates for a typical patient with the *CYP3A5*\*1/\*3 or \*3/\*3 and *ABCG2* 421C/C or C/A genotype, and for a typical patient with the *CYP3A5*\*1/\*3 or \*3/\*3 and *ABCG2* 421A/A genotypes, respectively



**Figure 2**

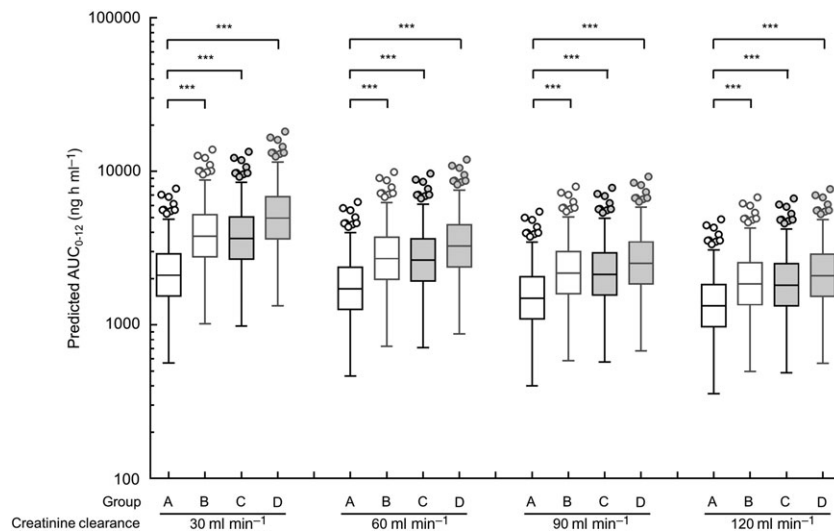
Goodness-of-fit plots for the final model of apixaban. The relationship between the observed concentrations (OBS) and population predictions (PRED), and individual predictions (IPRED), are shown in panels A and B, respectively. The relationship between conditional weighted residuals (CWRES) and the time after last dose, and PRED are shown in panels C and D, respectively. Open circles indicate the observed values. Each dotted line shows a line of identity





**Figure 3**

Visual predictive checks of apixaban concentrations with data obtained from the final model. Open circles show the observed concentrations in all patients (A), patients administered twice daily a dose of 5 mg day<sup>-1</sup> (B), 10 mg day<sup>-1</sup> (C) and 20 mg day<sup>-1</sup> (D). The top dotted, middle solid, and bottom dotted lines are shown as the 95th, 50th, and 5th percentiles, respectively, as calculated from 1000 simulated data sets



**Figure 4**

Simulations of AUC from time 0 to 12 h (AUC<sub>0-12</sub>) for apixaban in the 200 replication data sets in a patient administered a typical dose of 5 mg twice daily. These simulations are conducted using the final model. The box-and-whisker plots are presented according to the Tukey style. Open circles show the outliers. Four groups consist of patients with the *CYP3A5*\*1/\*1 and *ABCG2* 421C/C or C/A genotype (group A), patients with the *CYP3A5*\*1/\*1 and *ABCG2* 421A/A genotypes (group B), patients with the *CYP3A5*\*1/\*3 or \*3/\*3 and *ABCG2* 421C/C or C/A genotype (group C), and patients with the *CYP3A5*\*1/\*3 or \*3/\*3 and *ABCG2* 421A/A genotypes (group D). \*\*\*  $P < 0.001$  by the Kruskal-Wallis test, followed by Dunn's multiple comparison test

indicated that the final model provided a robust and unbiased fit to the data. Our results indicated for the first time the impact of the genetic polymorphisms of *ABCG2* and *CYP3A5* on pharmacokinetic parameters of apixaban.

The CL/F value of apixaban in this study was smaller than values for a typical patient or healthy subject in previous population pharmacokinetic analyses ( $4.29\text{--}4.53\text{ l h}^{-1}$ ), while the Vd/F value of apixaban ( $24.7\text{ l}$ ) in this study was comparable to that of previous population pharmacokinetic analyses ( $22.9\text{--}43.9\text{ l}$ ) [12, 13, 25]. Recently, it was reported that the CL/F value in the Asian race was lower than that in non-Asian races [24]. This racial difference in the CL/F value of apixaban may be explained by the higher allele frequency of *ABCG2* 421C>A in East Asians (30–60%) compared with Caucasians and African-Americans (5–10%) [34]. The CL/F value in Japanese AF patients in our study is slightly lower than that in healthy male Japanese subjects [35]. Therefore, the racial and pathological differences among AF patients or subjects were considered to affect the pharmacokinetics of apixaban.

It was reported that renal clearance of apixaban in healthy Japanese subjects after intravenous administration ranged from  $15.2$  to  $17.8\text{ ml min}^{-1}$ , which corresponds to 22.2–33.2% of its [35]. Apixaban exhibits 87% protein binding in plasma [36], and renal clearance corrected by the unbound apixaban concentration is estimated to range from  $116.9$  to  $136.9\text{ ml min}^{-1}$ , which is close to normal glomerular filtration rate of nearly  $100\text{ ml min}^{-1}$ . These results suggest that *ABCG2* protein may contribute to the hepatic and/or intestinal secretion of apixaban rather than its renal excretion. Further studies are required to clarify the relative contribution of *ABCG2* and *CYP3A5* to hepatic, intestinal and renal clearances of apixaban.

There is little information concerning the relationship between the pharmacokinetics of non-vitamin K anticoagulants and co-morbidities in AF patients. It was reported that heart failure significantly affected the CL/F of dabigatran in AF patients, although patients with heart failure showed a 6.7% decreased CL/F [37]. In the present study, co-morbidities did not affect the apixaban pharmacokinetics. Thus, the influence of co-morbidities on pharmacokinetics of non-vitamin K anticoagulants in AF patients may be considered to be negligible.

The concomitant *CYP3A4* and/or P-glycoprotein inhibitors (amiodarone and verapamil) had no effects on the CL/F of apixaban in this study (Table 3). Drug–drug interactions between apixaban and inhibitors (ketoconazole and diltiazem) or inducers (rifampicin) of both *CYP3A4* and P-glycoprotein were observed in healthy subjects [33, 38], and a previous population pharmacokinetic analysis indicated that strong/moderate *CYP3A4* and P-glycoprotein inhibitors decreased the CL/F of apixaban by 20.3% [25]. In the present study, all patients treated with apixaban and amiodarone had the *CYP3A5*\*1/\*3 or \*3/\*3 genotype, and five of six patients treated with apixaban and verapamil also had these genotypes. Thus, we expected that it would be difficult to separately evaluate the effect of amiodarone, verapamil and the *CYP3A5*\*3 genotype on the pharmacokinetic parameters of apixaban.

There is no information concerning the exposure–response study for AF patients. Recent exposure–response studies for patients undergoing orthopaedic surgery have

shown that an increase in the daily AUC at steady state of  $1000\text{ ng h ml}^{-1}$  would increase the odds ratio for bleeding by 0.118 and decrease the odds ratio for venous thromboembolism (VTE) by 0.0499 [12]. The present simulation study showed that the median of the predicted steady-state apixaban  $\text{AUC}_{0-12}$  had large variability using the final model (Figure 4). For example, the predicted  $\text{AUC}_{0-12}$  of apixaban at steady state was calculated to range from 463 to  $3254\text{ ng h ml}^{-1}$  when a patient with Ccr of  $60\text{ ml min}^{-1}$  took apixaban at a typical dose of 5 mg twice daily. The predicted  $\text{AUC}_{0-12}$  of apixaban in a patient with a Ccr of 30, 90 or  $120\text{ ml min}^{-1}$  was similarly variable compared with a patient with a Ccr of  $60\text{ ml min}^{-1}$ . Therefore, the *ABCG2* and *CYP3A5* genotypes as well as apixaban concentrations can predict the rate of bleeding and VTE events because apixaban exhibits considerable inter-individual variabilities for these events. Further exposure–response studies in AF patients are needed to clarify the effect of the *ABCG2* and *CYP3A5* genotypes as well as apixaban concentrations on the rate of bleeding and VTE events.

A limitation of this study is the small number of patients with *CYP3A5*\*1/\*1 or *ABCG2* 421A/A genotype. It was reported that the false positive rate (type 1 error inflation) of covariate effects tended to be high when the frequency of covariate was less than 20% [39]. In the present study, the frequency of the *ABCG2* 421A/A and *CYP3A5*\*1/\*1 genotypes were only 11.1% and 4.9%, respectively (Table 1). The covariate effects of these genotypes on apixaban pharmacokinetics may be poorly estimated, although our population pharmacokinetic and pharmacogenomics model was validated (Table 4). The population pharmacokinetics and pharmacogenomics of apixaban in a larger number of Japanese population will need to be examined in the future.

In conclusion, our results suggest that the *ABCG2* 421A/A and *CYP3A5*\*3 genotypes and renal function are significant predictors of apixaban pharmacokinetics. These findings may provide useful information for individualized apixaban pharmacotherapy to prevent the risk of adverse reactions.

## Competing Interests

There are no competing interests to declare.

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## Contributors

S.U., D.H., M.H., T.T. and T.K. were involved in the conception and design of the study. S.U., D.H., Y.K., R.F., C.T., T.Y., M.H., T.T. and T.K. were involved in the analysis and/or interpretation of data. D.H., Y.T., T.O., H.I. and S.O. were involved in the acquisition of data. S.U. drafted the manuscript. D.H., M.H., T.T. and T.K. revised the manuscript critically for

important intellectual content. All authors gave approval of the final version of the manuscript to be published.

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## Supporting Information

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<http://onlinelibrary.wiley.com/doi/10.1111/bcp.13561/supinfo>

**Figure S1** Visual predictive checks of apixaban concentrations with data obtained from the final model. Open circles show the observed concentrations in inpatients (A) and outpatients (B). The top dotted, middle solid, and bottom dotted lines are shown as the 95th, 50th, and 5th percentiles, respectively, as calculated from 1000 simulated data sets