

# Impacts of pregnane X receptor and cytochrome P450 oxidoreductase gene polymorphisms on trough concentrations of apixaban in patients with non-valvular atrial fibrillation.

**Junichi Nakagawa**

Hirosaki University Hospital

**Takahiko Kinjo**

Hirosaki University Graduate School of Medicine

**Naoya Aiuchi**

Hirosaki University Hospital

**Kayo Ueno**

Hirosaki University Hospital

**Hirofumi Tomita**

Hirosaki University Graduate School of Medicine

**Takenori Niioka** (✉ [t-niioka@hirosaki-u.ac.jp](mailto:t-niioka@hirosaki-u.ac.jp))

Hirosaki University Graduate School of Medicine

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## Research Article

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# Abstract

**Purpose:** We examined the impact of polymorphisms in genes encoding cytochrome P450 (CYP) 3A5 (gene code *CYP3A5*), P-glycoprotein (*ABCB1*), breast cancer resistance protein (*ABCG2*), cytochrome P450 oxidoreductase (*POR*) and pregnane X receptor (PXR; *NR1I2*) on the steady-state trough concentrations ( $C_{0h}$ ) of apixaban.

**Methods:** The analyses included 86 patients with non-valvular atrial fibrillation (NVAf) undergoing AF catheter ablation. The *CYP3A5*\*3; *ABCG2* 421C>A; *ABCB1* 1236C>T, 2677G>A/T, 3435C>T, and 2482-2236G>A; *NR1I2* 11156A>C, 11193T>C, and 8055C>T; and *POR*\*28 genotypes were determined. The combination of the noted *NR1I2* genotypes determined the *PXR*\*1B haplotype.

**Results:** Stepwise selection multiple linear regression analyses demonstrated that decreased creatinine clearance and the *PXR*\*1B/ \*1B genotype correlated with increased apixaban  $C_{0h}$ , while the presence of the *POR*\*28 allele correlated with decreased  $C_{0h}$  (partial  $R^2 = 0.196, 0.057, \text{ and } 0.046$ , all  $P < 0.05$ ). The  $C_{0h}$  of apixaban showed a moderate correlation with prothrombin time ( $r = 0.594 P < 0.001$ ).

**Conclusion:** The *PXR*\*1B haplotype and *POR*\*28 genotype statuses, which involve genes that impact the expression of multiple drug-metabolizing enzymes and drug-transporters, may affect the  $C_{0h}$  of apixaban.

## 1. Introduction

Apixaban, a direct oral anticoagulant and reversible inhibitor of factor Xa (FXa), is widely used for the prevention of venous thrombosis or thrombus formation in non-valvular atrial fibrillation (NVAf) and in the treatment of venous thromboembolism [1]. In the ARISTOTLE trial, apixaban was shown to more effectively reduce the risk of stroke, systemic thromboembolism, and major bleeding in patients with NVAf, as compared to warfarin [2]. Therefore, apixaban is increasingly prescribed to patients newly diagnosed with NVAf [3].

The dose of apixaban is typically determined based on the patient's age, weight, and serum creatinine level without routine anticoagulant testing or plasma drug concentration monitoring [1]. However, achieving accurate dosing of apixaban is important, as it has been reported that increased plasma concentrations and areas under the plasma concentration-time curves (AUC) are associated with bleeding risk and thromboembolic events [4–6]. In addition, large interindividual variabilities in the pharmacokinetics (PK) of apixaban have been observed [7, 8]. For example, Gulilat et al. reported that the coefficient of variations of peak concentration ( $C_{max}$ ) and trough concentration ( $C_{0h}$ ) at the steady-state were 55.0% and 47.3%, respectively, in NVAf patients receiving 5 mg of apixaban twice daily [9].

These variations may be due to variables impacting the clearance pathways of apixaban. Orally administered apixaban is absorbed mainly in the small intestine, where the bioavailability is approximately 50%. Apixaban is then metabolized by cytochrome P450 (CYP) in the liver and is excreted through the kidneys. Renal excretion as the unchanged form accounts for 27% of the total clearance, and

excretion as metabolites in the urine and feces accounts for 25% of the administered dose. The elimination half-life of apixaban is reported to be approximately 12 h [10].

Apixaban is mainly metabolized by CYP3A4/5, with minor contributions from CYP1A2, CYP2C8/9/19, and CYP2J2 [11]. Furthermore, apixaban is also a substrate of the efflux transporter P-glycoprotein (P-gp; gene code *ABCB1*) and breast cancer resistance protein (BCRP; gene code *ABCG2*) [12]. These transporters are involved in absorption from the small intestine, in excretion from hepatocytes into bile, and in renal tubular secretion of substrate drugs [13].

Although several studies have investigated the effects of polymorphisms of genes encoding drug metabolic enzymes and drug transporters on the PK of apixaban, the results have been inconsistent. Ueshima et al. reported that the oral clearance of apixaban was lower in Japanese AF patients with the *CYP3A5\*3* allele than those with the *CYP3A5\*1/\*1* genotype and was lower in those with *ABCG2* 421A/A genotype than those with *ABCG2* 421G allele [14, 15]. Dimatteo et al. reported that the  $C_{max}$ , not the  $C_{0h}$ , was higher in Caucasian patients taking apixaban with *ABCB1* 2482 – 2236 A/A genotype than those with the G allele [16]. On the other hand, Roşian et al. reported that there were no significant differences in the trough or peak plasma concentrations of apixaban between the genotype groups of *ABCB1* 3435C > T and *ABCB1* 2482-2236G > A [17]. In addition, Lenoir et al. reported that there were no significant differences in the  $AUC_{0-6h}$  of apixaban between the genotype groups of *ABCB1* 1236C > T, 2677G > A/T, 3435C > T, and *CYP3A*, including *CYP3A5\*3* [18].

Several systems are known to be involved in the induction of drug-metabolizing enzymes or transporters, and variations in these genes may help to further explain differences in the PK of apixaban PK. For example, the nuclear receptor, pregnane X receptor (PXR), regulates the transcription of genes encoding several drug-metabolizing enzymes, such as CYP2 and CYP3A, and drug transporters, such as P-gp, and it thus facilitates the elimination of xenobiotics from the body [19, 20]. Accordingly, polymorphisms in *PXR* (also known as *NR1I2*) have been found to affect the induction of its target genes [21]. The *PXR\*1B* haplotype cluster, which is common in Asian populations [22], has been characterized by the combination of *NR1I2* 8055C > T, 11156A > C, and 11193T > C genotypes [22, 23] (Supplementary Table 1).

Another relevant system is P450 oxidoreductase (*POR*), which transfers electrons from NADPH oxidase to CYP enzymes, increasing CYP activity and affecting the metabolism of drug substrates [24, 25]. Among several single nucleotide polymorphisms (SNPs) of *POR*, the most common variant is *POR\*28* (c.1508 C > T, rs1057868), and its allele frequency in the Japanese population is approximately 40% [26]. According to an *in vitro* study, this SNP is associated with increased activity of multiple CYPs, including CYP1A2, CYP2C19, and CYP3A4/5 [27, 28]. Therefore, the statuses of the *PXR\*1B* haplotype and the *POR\*28* genotype may affect blood levels of drugs that are substrates for the above-mentioned metabolic enzymes and transporters, but the impacts of these polymorphisms on the PK of apixaban have not been investigated.

The first purpose of this study was to investigate the impact of polymorphisms of genes encoding the major metabolic enzymes and transporters, *CYP3A5*, *ABCG2*, and *ABCB1*, that directly affect the PK of apixaban, on steady-state  $C_{0h}$  of apixaban in Japanese patients with NVAf. The second goal was to investigate the effects of polymorphisms of *PXR* and *POR*, which affect the expression or activity of CYPs and/or ATP-binding cassette (ABC) transporters, on this  $C_{0h}$ .

## 2. Methods

### 2.1 Patients

This single-center study enrolled 86 patients undergoing AF catheter ablation at the Hirosaki University Hospital from June, 2018, through October, 2020. These patients had been taking 5 mg apixaban twice daily. Patients who were taking apixaban twice daily at 2.5 mg were excluded from the study due to a small sample size ( $n = 18$ ). Patients who received verapamil or rifampicin during the study period were excluded from the analyses. A pharmacist or nurse confirmed the time of day of each apixaban dose. Apixaban was skipped only in the morning of the day of AF ablation. Blood collection for measurement of  $C_{0h}$  was performed at 10 to 12 h after the last apixaban administration on the morning of the day of AF ablation. The study protocol was approved by the Ethics Committee of Hirosaki University Graduate School of Medicine (project identification code: 2018-011-02), and all patients provided written informed consent before the study. With the exception of amiodarone (AMD), patients who were taking drugs that affect the PK of apixaban were excluded from the analysis due to small sample sizes (verapamil,  $n = 1$ , and rifampin,  $n = 1$ ) [1, 29].

### 2.2 Analysis of plasma concentrations of apixaban

Blood samples were centrifuged at 3,500 rpm for 10 min at 4 °C, and the separated plasma was stored at -30 °C until analysis. Plasma concentrations of apixaban were measured by ultra-performance liquid chromatography (UPLC) tandem mass spectrometry using an ACQUITY UPLC System (Waters, MA, USA). Plasma (100  $\mu$ L) was mixed with 150  $\mu$ L of acetonitrile and 10  $\mu$ L of an internal standard (IS), which consisted of 1,000 ng/mL rivaroxaban. The mixture was vortexed for 30 s and centrifuged at 13,500 rpm for 5 min at room temperature. The supernatant (100  $\mu$ L) was diluted with 100  $\mu$ L MilliQ water. The sample was transferred to an autosampler vial, and 5  $\mu$ L was then injected into an ACQUITY UPLC phenyl column (1.7  $\mu$ m, 75 mm  $\times$  2.1 mm) at 40 °C. The mobile phase consisted of (A) MilliQ water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. The flow rate was 0.4 mL/min. Gradient conditions were as follows: 0–1.0 min, held in 5% B; 1.0–6.0 min, linear from 5–95% B; 6.0–7.0 min, held in 95% B; 7.0–7.1 min, linear from 95–5% B; and 7.1–10.0 min, held in 5% B.

The analyte and IS were ionized and detected using a Xevo TQD mass spectrometer (Waters). Positive electrospray ionization was performed in the multiple reaction monitoring mode. Ion transitions of apixaban and IS were  $m/z$  460.2→443.0 and 436.1→144.9. Cone voltages and collision energies were 40 V and 20 eV for apixaban and 40 V and 30 eV for IS. The calibration curve was linear in the range of 5 to

400 ng/mL. If the plasma concentration of the analyte exceeded the upper limit of the calibration curve, the plasma sample was diluted twice with an equal volume of blank human plasma and remeasured. Calibration curves showed good linearity, with  $R^2 > 0.99$ . The intra- and inter-day accuracy values (CV%) were all within  $\pm 15\%$ , and precision values (CV%) were all less than 15% within the range of each calibration curve.

## 2.3 Genotyping

DNA was extracted from peripheral blood samples with a QIAamp Blood Kit (Qiagen, Hilden, Germany) and was stored at  $-30\text{ }^\circ\text{C}$  until analysis. The following SNPs were determined by real-time PCR using TaqMan SNP Genotyping Assays from Thermo Fisher Scientific (Waltham, MA, USA): *CYP3A5\*3* (c.6986A > G, rs776746); *ABCG2* 421C > A (rs2231142); *ABCB1* 1236C > T (rs1128503), 2677G > A/T (rs2032582), 3435C > T (rs1045642), and 2482-2236G > A (rs4148738); *POR\*28* (A503V, rs1057868); and *NR1I2* 11156A > C (rs3814057), 11193T > C (rs3814058), and 8055C > T (rs2276707). Cycle conditions were as follows:  $95\text{ }^\circ\text{C}$  for 3 min, followed by 40 cycles of  $95\text{ }^\circ\text{C}$  for 15 s and  $60\text{ }^\circ\text{C}$  for 15 s. Genotypes were detected using a CFX-Connect Real-Time PCR system (Bio-Rad Laboratories Inc., Hercules, CA, USA).

## 2.4 Prothrombin time test

Blood collection for testing of prothrombin time (PT) was performed at the same time as blood collection for measuring of the plasma concentration of apixaban. PT was determined using Thromborel S on a CS-5100 coagulation system (Sysmex, Kobe, Japan) according to the manufacturer's instructions.

## 2.5 Statistical procedures

The Shapiro-Wilk test was used to assess distribution of data. Allele frequencies of gene polymorphisms were evaluated according to the Hardy-Weinberg equilibrium using  $\chi^2$  tests. The Kruskal-Wallis test or Mann-Whitney U test was used to determine differences in continuous values between groups. Spearman's rank correlation coefficient test was used to assess correlations in continuous values between groups, and all results were expressed as Spearman's  $\rho$  values. Stepwise selection multiple linear regression analysis was performed to determine the effects of all factors in univariate analyses. Each PK-related gene polymorphism was categorized into "wild-type homozygotes and other genotypes" and "mutant-type homozygotes and other genotypes." That is, they were replaced by dummy variables and entered into the stepwise selection multiple linear regression analysis. The percent variation that could be explained by the stepwise selection multiple regression equation was expressed as a coefficient of determination ( $R^2$ ). An interaction between categorical variables (*PXR\*1B* haplotype and *POR\*28* genotype) identified as independent variables affecting the dependent variable (apixaban  $C_{0h}$ ) in multiple linear regression analysis was evaluated using the analysis of covariance (ANCOVA). Here, continuous variables (creatinine clearance, Ccr), which were also identified as independent variables, were entered into ANCOVA as covariates, and the interaction was evaluated based on estimated marginal means. Differences with  $P$  values of less than 0.05 were considered statistically significant. Statistical analysis was performed with SPSS 26.0 for Windows (SPSS IBM Japan Inc., Tokyo, Japan).

## 3. Results

### 3.1 Patient characteristics

The demographic and clinical characteristics of the patients with NVAF taking apixaban are listed in Table 1. The means  $\pm$  standard deviations (SDs) of age, body weight, and Ccr as estimated by the Cockcroft and Gault equation [30] were  $67.3 \pm 9.0$  years,  $65.2 \pm 12.0$  kg, and  $76.8 \pm 25.4$  mL/min, respectively. There were no patients with serious hepatic dysfunction, as noted by a Child-Pugh score of greater than 2. During the study period, 13 patients were taking AMD.

The distributions of *CYP3A5*\*3, *ABCG2* 421C > A; *ABCB1* 1236C > T, 2677G > A/T, 3435C > T, and 2482-2236G > A; *POR*\*28; and *NR1I2* 11156A > C, 11193T > C, and 8055C > T genotypes were in agreement with the Hardy-Weinberg equilibrium ( $P = 0.284, 0.555, 0.175, 0.423, 0.360, 0.497, 0.577, 0.667, 0.667,$  and  $0.688$ , respectively). *NR1I2* 11156A > C and 11193T > C were completely linked. The *PXR*\*1B haplotype status was non-*PXR*\*1B:  $n = 25$ , *PXR*\*1B:  $n = 41$  and *PXR*\*1B/\*1B:  $n = 20$ . The *ABCB1* haplotype status was 1236TT-2677TT-3435TT:  $n = 11$  and others:  $n = 75$ .

### 3.2 Relationships of apixaban $C_{0h}$ to patient characteristics

The apixaban  $C_{0h}$  did not follow a normal distribution (Fig. 1). Comparisons and correlations between apixaban  $C_{0h}$  and patient demographic and clinical characteristics are listed in Table 2. In univariate analyses, apixaban  $C_{0h}$  was found to be significantly correlated with age and Ccr ( $\rho = 0.501$  and  $-0.462$ , respectively; both  $P < 0.01$ ). On the other hand, there were no significant differences or correlations in other patient factors. In addition, there were no significant differences found among all genotypes (all  $P > 0.05$ ) (Table 3). In stepwise selection multiple linear regression analyses, Ccr, *PXR*\*1B/\*1B carrier, and *POR*\*28 carrier were found to be independent factors influencing apixaban  $C_{0h}$  (partial  $R^2 = 0.196, 0.057$  and  $0.046$ , respectively; all  $P < 0.05$ ) (Table 4). There was no interaction between the effects of the *PXR*\*1B haplotype status and the *POR*\*28 genotype status on the  $C_{0h}$  of apixaban ( $P = 0.849$ ) (Fig. 2).

### 3.3 Effect of plasma concentration of apixaban on PT

A significant correlation was observed between the  $C_{0h}$  of apixaban and PT, as measured with Thromborel S ( $\rho = 0.594$ ,  $P < 0.001$ ) (Fig. 3).

## 4. Discussion

This is the first study to show that *PXR* and *POR* polymorphisms affect the steady-state  $C_{0h}$  of apixaban. A decrease in Ccr and the *PXR*\*1B/\*1B genotype correlated with an increased  $C_{0h}$  of apixaban, while the presence of the *POR*\*28 allele correlated with a decreased  $C_{0h}$  in Japanese patients with NVAF.

Unlike previous reports [14], no significant differences in the  $C_{0h}$  of apixaban among patients with *CYP3A5* or *ABCG2* polymorphisms were found in this study. One of the reasons for this discrepancy may

be that the numbers of patients with the *CYP3A5*\*1/\*1 or *ABCG2* A/A genotypes were small and the differences in the  $C_{0h}$  between these gene polymorphisms were not large enough to be detectable. On the other hand, significant differences were found in the  $C_{0h}$  of apixaban among patients with *PXR* or *POR* polymorphisms. It has been suggested that *PXR* polymorphisms may affect the expression level of the genes encoding CYP1A2, CYP2C8/9/19, CYP3A4, and P-gp, as well as CYP3A5 and BCRP [31]. In addition, *POR* polymorphisms have also been suggested to potentially affect the activities of CYP1A2, CYP2C19 and CYP3A4/5 [27, 28]. Because it is known that multiple CYPs and ABC transporters are involved in the PK of apixaban [10], it is possible that *PXR* and *POR* polymorphisms, which affect the activities of these multiple clearance pathways, may have affected the  $C_{0h}$  of apixaban.

The patient factor that was found to most strongly influence the  $C_{0h}$  of apixaban in this study was Ccr. The significance of Ccr to the PK of apixaban is consistent with the Cockcroft-Gault equation, which is used to determine the appropriate dosage of apixaban for patients with NVAf in the United States and Japan and which takes into account age, weight, and serum creatinine [1]. Although the contribution of renal excretion to overall apixaban elimination is limited to approximately 27% [10], renal function remains the most important factor for predicting apixaban  $C_{0h}$ . However, it has been reported that in cases of renal dysfunction, the presence of mutant alleles of *ABCB1*, *ABCG2*, and *CYP3A5* can lead to impairment of multiple apixaban elimination pathways, in turn substantially increasing its exposure [32]. It thus will be necessary to investigate the effect of polymorphisms of PK-related genes on the  $C_{0h}$  of apixaban in patients with renal deficiencies in the future. In addition, there was no significant effect of the concomitant use of AMD on the  $C_{0h}$  of apixaban. This result was in agreement with a report by Ueshima et al. [14], but Gulilat et al. reported a dose-dependent effect of AMD on plasma concentrations of apixaban [9]. However, Gulilat et al. also noted that these effects were minor in patients taking less than 400 mg of AMD per day. Because the maximum dose of AMD in the present study was 100 mg/day, our finding of no significant effect supports these previous results.

The  $C_{0h}$  of apixaban showed a moderate correlation with PT as measured with Thromborel S in this study ( $\rho = 0.594$ ). Although it is well known that the sensitivity of PT for each direct FXa inhibitor depends on the reagent used for the PT measurement [33], over the range of apixaban  $C_{0h}$  and PT levels observed in this study, the correlation was similar to those reported by Shin et al. [34]. However, the results of coagulation tests performed on multiple populations have shown that neither PT nor activated partial thromboplastin time (aPTT) is sensitive enough to predict the apixaban plasma concentration [35–37]. Although FXa inhibitors, including apixaban, are known to exhibit a strong correlation between plasma drug concentration and anti-Xa activity [38], knowing the associations between routine coagulation tests and apixaban plasma concentration levels may be also important. Although PT at the trough was not sensitive enough for the prediction of apixaban  $C_{0h}$  in this study, our results did show that increased PT was associated with increased plasma concentrations of apixaban.

There were several limitations in this study. First, although O-desmethyl apixaban (M2) is the predominant metabolite produced by CYP3A4/5 [11], we did not measure the plasma concentrations of

M2. Therefore, the effect of *CYP3A5* polymorphisms on apixaban  $C_{0h}$  could not be fully evaluated. By examining the effects of *PXR\*1B* and *POR\*28* polymorphisms on the ratio of M2 to apixaban, it would have been possible to partially assess the effect of CYP3A4/5 activity on the clearance of apixaban. Second, as the number of patients enrolled in the study was small, we were unable to detect the effects of the interaction of the *POR\*28* and *CYP3A5\*3* genotypes on the  $C_{0h}$  of apixaban (data not shown). It has been reported that the dose- and weight-adjusted  $C_{0h}$  of tacrolimus, a substrate of CYP3A5, was different between *POR\*28* genotypes in renal transplant recipients with the *CYP3A5\*1* allele but not in those with the *CYP3A5\*3/\*3* genotype [39]. Therefore, the effect of this interaction on the PK of apixaban requires further investigation. Third, changes in plasma concentration levels of apixaban over time were not monitored in this study, and the effects of *PXR* and *POR* polymorphisms on steady-state  $C_{max}$  and AUC of apixaban could not be determined. Considering that blood concentration of apixaban and anti-FXa activity are expected to fluctuate within a dosing interval, in contrast to the blood concentration of warfarin and the international normalized ratio [40], the influence of polymorphisms of PK-related genes examined in this study on  $C_{max}$  should also be examined. Despite these limitations, however, this study identified new polymorphisms of PK-related genes that affect the  $C_{0h}$  of apixaban and that are easily monitored in patients with NVAf. If the impact of *PXR* and *POR* polymorphisms on the  $C_{0h}$  of apixaban in patients who meet the criteria for dose reduction to twice daily at 2.5 mg is clarified, even more detailed individualization of dosing may be possible for these patients.

In conclusion, the haplotype status of *PXR\*1B*, which impacts the expression of multiple drug-metabolizing enzymes and transporters, correlates with the  $C_{0h}$  of apixaban in NVAf patients. The genotype status of the *POR\*28* gene, which impacts the activity of the main metabolic enzyme of apixaban, CYP3A4/5, also correlates with the  $C_{0h}$  of apixaban. Further studies are needed to determine whether these genetic polymorphisms can be useful indicators for individualizing the dosage of apixaban.

## Declarations

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### Conflicts of interest

Dr. Tomita received research funding from Boehringer Ingelheim, Bayer, and Daiichi-Sankyo and speakers' bureau/honoraria from Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Daiichi-Sankyo and Pfizer. Dr



Niioka received speakers' honoraria from Daiichi-Sankyo. The remaining authors have nothing to disclose.

### **Availability of data and material**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### **Authors' contributions**

Conceptualization, T.N.; investigation, T.K. and N.A.; plasma concentration measurements, J.N. and K.U.; formal analysis, J.N.; writing—original draft preparation, J.N.; writing—review and editing, H.T. and T.N. All authors read and approved the final manuscript.

### **Ethics approval**

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of Hirosaki University Graduate School of Medicine (project identification code: 2018-011-02) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

### **Consent to participate**

Informed consent was obtained from all individual participants included in this study.

### **Consent for publication**

Not applicable.

## **References**

1. Bristol-Myers Squibb Company PI. Eliquis (apixaban) prescribing information. 2021. [http://packageinserts.bms.com/pi/pi\\_eliquis.pdf](http://packageinserts.bms.com/pi/pi_eliquis.pdf). Accessed 28 Apr 2022.
2. Granger CB, Alexander JH, McMurray JJ, Lopes RD, Hylek EM, Hanna M et al (2011) Apixaban versus warfarin in patients with atrial fibrillation. *N Engl J Med* 365:981–992. <https://doi.org/10.1056/NEJMoa1107039>
3. Zhu J, Alexander GC, Nazarian S, Segal JB, Wu AW (2018) Trends and variation in oral anticoagulant choice in patients with atrial fibrillation, 2010–2017. *Pharmacotherapy* 38:907–920. <https://doi.org/10.1002/phar.2158>
4. Leil TA, Frost C, Wang X, Pfister M, LaCreta F (2014) Model-based exposure-response analysis of apixaban to quantify bleeding risk in special populations of subjects undergoing orthopedic surgery. *CPT Pharmacometrics Syst Pharmacol* 3:e136. <https://doi.org/10.1038/psp.2014.34>

5. Bhagirath VC, Eikelboom JW, Hirsh J, Coppens M, Ginsberg J, Vanassche T et al (2017) Apixaban-calibrated anti-FXa activity in relation to outcome events and clinical characteristics in patients with atrial fibrillation: Results from the AVERROES Trial. *TH Open* 1:e139-e145. <https://doi.org/10.1055/s-0037-1613679>
6. Nosál V, Petrovičová A, Škorňová I, Bolek T, Dluhá J, Stančiaková L et al (2022) Plasma levels of direct oral anticoagulants in atrial fibrillation patients at the time of embolic stroke: a pilot prospective multicenter study. *Eur J Clin Pharmacol* 78:557–564. <https://doi.org/10.1007/s00228-022-03280-8>
7. Upreti VV, Wang J, Barrett YC, Byon W, Boyd RA, Pursley J et al (2013) Effect of extremes of body weight on the pharmacokinetics, pharmacodynamics, safety and tolerability of apixaban in healthy subjects. *Br J Clin Pharmacol* 76:908–916. <https://doi.org/10.1111/bcp.12114>
8. Frost C, Shenker A, Gandhi MD, Pursley J, Barrett YC, Wang J et al (2014) Evaluation of the effect of naproxen on the pharmacokinetics and pharmacodynamics of apixaban. *Br J Clin Pharmacol* 78:877–885. <https://doi.org/10.1111/bcp.12393>
9. Gulilat M, Keller D, Linton B, Pananos AD, Lizotte D, Dresser GK et al (2020) Drug interactions and pharmacogenetic factors contribute to variation in apixaban concentration in atrial fibrillation patients in routine care. *J Thromb Thrombolysis* 49:294–303. <https://doi.org/10.1007/s11239-019-01962-2>
10. Byon W, Garonzik S, Boyd RA, Frost CE (2019) Apixaban: A clinical pharmacokinetic and pharmacodynamic review. *Clin Pharmacokinet* 58:1265–1279. <https://doi.org/10.1007/s40262-019-00775-z>
11. Wang L, Zhang D, Raghavan N, Yao M, Ma L, Frost CE et al (2010) In vitro assessment of metabolic drug-drug interaction potential of apixaban through cytochrome P450 phenotyping, inhibition, and induction studies. *Drug Metab Dispos* 38:448–458. <https://doi.org/10.1124/dmd.109.029694>
12. Zhang D, He K, Herbst JJ, Kolb J, Shou W, Wang L et al (2013) Characterization of efflux transporters involved in distribution and disposition of apixaban. *Drug Metab Dispos* 41:827–835. <https://doi.org/10.1124/dmd.112.050260>
13. Ieiri I (2012) Functional significance of genetic polymorphisms in P-glycoprotein (MDR1, ABCB1) and breast cancer resistance protein (BCRP, ABCG2). *Drug Metab Pharmacokinet* 27:85–105. <https://doi.org/10.2133/dmpk.dmpk-11-rv-098>
14. Ueshima S, Hira D, Fujii R, Kimura Y, Tomitsuka C, Yamane T et al (2017) Impact of ABCB1, ABCG2, and CYP3A5 polymorphisms on plasma trough concentrations of apixaban in Japanese patients with atrial fibrillation. *Pharmacogenet Genomics* 27:329–336. <https://doi.org/10.1097/FPC.0000000000000294>
15. Ueshima S, Hira D, Kimura Y, Fujii R, Tomitsuka C, Yamane T et al (2018) Population pharmacokinetics and pharmacogenomics of apixaban in Japanese adult patients with atrial fibrillation. *Br J Clin Pharmacol* 84:1301–1312. <https://doi.org/10.1111/bcp.13561>

16. Dimatteo C, D'Andrea G, Vecchione G, Paoletti O, Tiscia GL, Santacroce R et al (2016) ABCB1 SNP rs4148738 modulation of apixaban interindividual variability. *Thromb Res* 145:24–26. <https://doi.org/10.1016/j.thromres.2016.07.005>
17. Roşian AN, Roşian ŞH, Kiss B, Ştefan MG, Trifa AP, Ober CD et al (2020) Interindividual variability of apixaban plasma concentrations: Influence of clinical and genetic factors in a real-life cohort of atrial fibrillation patients. *Genes (Basel)* 11:438. <https://doi.org/10.3390/genes11040438>
18. Lenoir C, Terrier J, Gloor Y, Gosselin P, Daali Y, Combescure C et al (2022) Impact of the genotype and phenotype of CYP3A and P-gp on the apixaban and rivaroxaban exposure in a real-world setting. *J Pers Med* 12:526. <https://doi.org/10.3390/jpm12040526>
19. Torres-Vergara P, YS, Espinoza F, Nualart F, Escudero C, Penny J (2020) The constitutive androstane receptor and pregnane X receptor in the brain. *Br J Pharmacol* 177:2666–2682. <https://doi.org/10.1111/bph.15055>
20. Mackowiak B, Hodge J, Stern S, Wang H (2018) The roles of xenobiotic receptors: Beyond chemical disposition. *Drug Metab Dispos* 46:1361–1371. <https://doi.org/10.1124/dmd.118.081042>
21. Mbatchi LC, Brouillet JP, Evrard A (2018) Genetic variations of the xenoreceptors NR1I2 and NR1I3 and their effect on drug disposition and response variability. *Pharmacogenomics* 19:61–77. <https://doi.org/10.2217/pgs-2017-0121>
22. Sandanaraj E, Lal S, Selvarajan V, Ooi LL, Wong ZW, Wong NS et al (2008) PXR pharmacogenetics: association of haplotypes with hepatic CYP3A4 and ABCB1 messenger RNA expression and doxorubicin clearance in Asian breast cancer patients. *Clin Cancer Res* 14:7116–7126. <https://doi.org/10.1158/1078-0432.CCR-08-0411>
23. Yuan JJ, Ma XJ, Li ZS, Chang YZ, Zhang W, Kan QC et al (2016) Effect of Pregnane X receptor\*1B genetic polymorphisms on postoperative analgesia with fentanyl in Chinese patients undergoing gynecological surgery. *BMC Med Genet* 17:87.
24. Hubbard PA, Shen AL, Paschke R, Kasper CB, Kim JJ (2001) NADPH-cytochrome P450 oxidoreductase. Structural basis for hydride and electron transfer. *J Biol Chem* 276:29163–29170. <https://doi.org/10.1074/jbc.M101731200>
25. Masters BS (2005) The journey from NADPH-cytochrome P450 oxidoreductase to nitric oxide synthases. *Biochem Biophys Res Commun* 338:507–519. <https://doi.org/10.1016/j.bbrc.2005.09.165>
26. Tadaka S, Katsuoka F, Ueki M, Kojima K, Makino S, Saito S et al (2019) 3.5KJPNv2: an allele frequency panel of 3552 Japanese individuals including the X chromosome. *Hum Genome Var* 6:28. <https://doi.org/10.1038/s41439-019-0059-5>
27. Agrawal V, Huang N, Miller WL (2008) Pharmacogenetics of P450 oxidoreductase: effect of sequence variants on activities of CYP1A2 and CYP2C19. *Pharmacogenet Genomics* 18:569–576. <https://doi.org/10.1097/FPC.0b013e32830054ac>
28. Oneda B, Crettol S, Jaquenoud Sirot E, Bochud M, Ansermot N, Eap CB (2009) The P450 oxidoreductase genotype is associated with CYP3A activity in vivo as measured by the midazolam

- phenotyping test. *Pharmacogenet Genomics* 19:877–883.  
<https://doi.org/10.1097/FPC.0b013e32833225e7>
29. Steffel J, Collins R, Antz M, Cornu P, Desteghe L, Haeusler KG et al (2021) 2021 European Heart Rhythm Association practical guide on the use of Non-Vitamin K antagonist oral anticoagulants in patients with atrial fibrillation. *Europace* 23:1612–1676. <https://doi.org/10.1093/europace/euab065>
  30. Cockcroft DW, Gault MH (1976) Prediction of creatinine clearance from serum creatinine. *Nephron* 16:31–41. <https://doi.org/10.1159/000180580>
  31. Mbatchi LC, Brouillet JP, Evrard A (2018) Genetic variations of the xenoreceptors NR1I2 and NR1I3 and their effect on drug disposition and response variability. *Pharmacogenomics* 19:61–77.  
<https://doi.org/10.2217/pgs-2017-0121>
  32. Huppertz A, Grond-Ginsbach C, Dumschat C, Foerster KI, Burhenne J, Weiss J et al (2019) Unexpected excessive apixaban exposure: case report of a patient with polymorphisms of multiple apixaban elimination pathways. *BMC Pharmacol Toxicol* 20:53. <https://doi.org/10.1186/s40360-019-0331-9>
  33. Dunois C (2021) Laboratory Monitoring of Direct Oral Anticoagulants (DOACs). *Biomedicines* 9:445.  
<https://doi.org/10.3390/biomedicines9050445>
  34. Shin H, Cho MC, Kim RB, Kim CH, Choi NC, Kim SK et al (2018) Laboratory measurement of apixaban using anti-factor Xa assays in acute ischemic stroke patients with non-valvular atrial fibrillation. *J Thromb Thrombolysis* 5:250–256.
  35. Schmitz EM, Boonen K, van den Heuvel DJ, van Dongen JL, Schellings MW, Emmen JM et al (2014) Determination of dabigatran, rivaroxaban and apixaban by ultra-performance liquid chromatography - tandem mass spectrometry (UPLC-MS/MS) and coagulation assays for therapy monitoring of novel direct oral anticoagulants. *J Thromb Haemost* 12:1636–1646. <https://doi.org/10.1111/jth.12702>
  36. Hillarp A, Gustafsson KM, Faxälv L, Strandberg K, Baghaei F, Fagerberg Blixter I et al (2014) Effects of the oral, direct factor Xa inhibitor apixaban on routine coagulation assays and anti-FXa assays. *J Thromb Haemost* 12:1545–1553. <https://doi.org/10.1111/jth.12649>
  37. Gosselin R, Grant RP, Adcock DM (2016) Comparison of the effect of the anti-Xa direct oral anticoagulants apixaban, edoxaban, and rivaroxaban on coagulation assays. *Int J Lab Hematol* 38:505–513. <https://doi.org/10.1111/ijlh.12528>
  38. Goto E, Horinaka S, Ishimitsu T, Kato T (2020) Factor Xa inhibitors in clinical practice: Comparison of pharmacokinetic profiles. *Drug Metab Pharmacokinet* 35:151–159.  
<https://doi.org/10.1016/j.dmpk.2019.10.005>
  39. Lee DH, Lee H, Yoon HY, Yee J, Gwak HS (2022) Association of P450 oxidoreductase gene polymorphism with tacrolimus pharmacokinetics in renal transplant recipients: A systematic review and meta-analysis. *Pharmaceutics* 14:261. <https://doi.org/10.3390/pharmaceutics14020261>
  40. Gulilat M, Tang A, Gryn SE, Leong-Sit P, Skanes AC, Alfonsi JE et al (2017) Interpatient variation in rivaroxaban and apixaban plasma concentrations in routine care. *Can J Cardiol* 33:1036–1043.  
<https://doi.org/10.1016/j.cjca.2017.04.008>

## Tables

Tables 1 to 4 are available in the Supplementary Files section.

## Figures

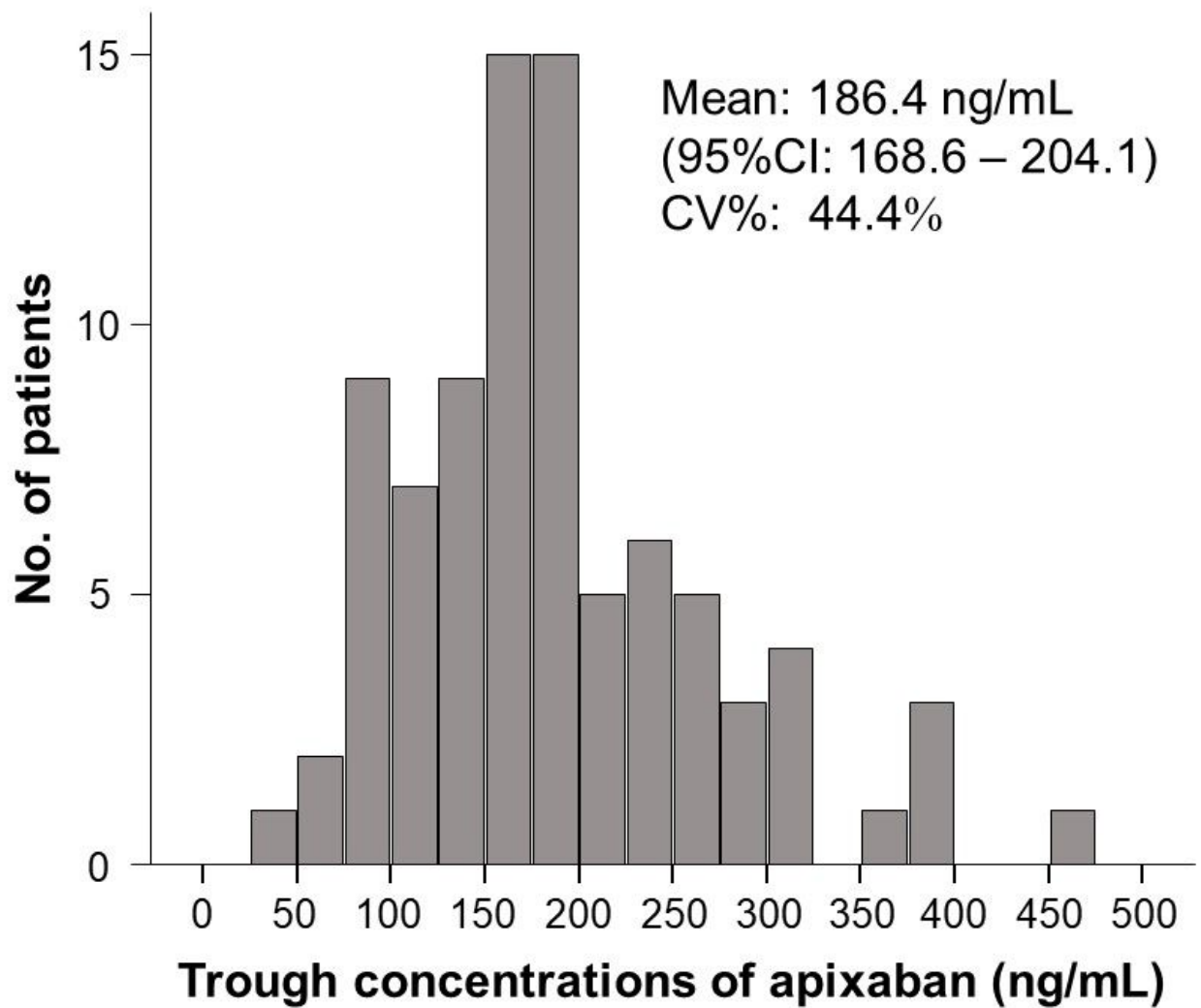


Figure 1

Histograms of trough concentrations of apixaban

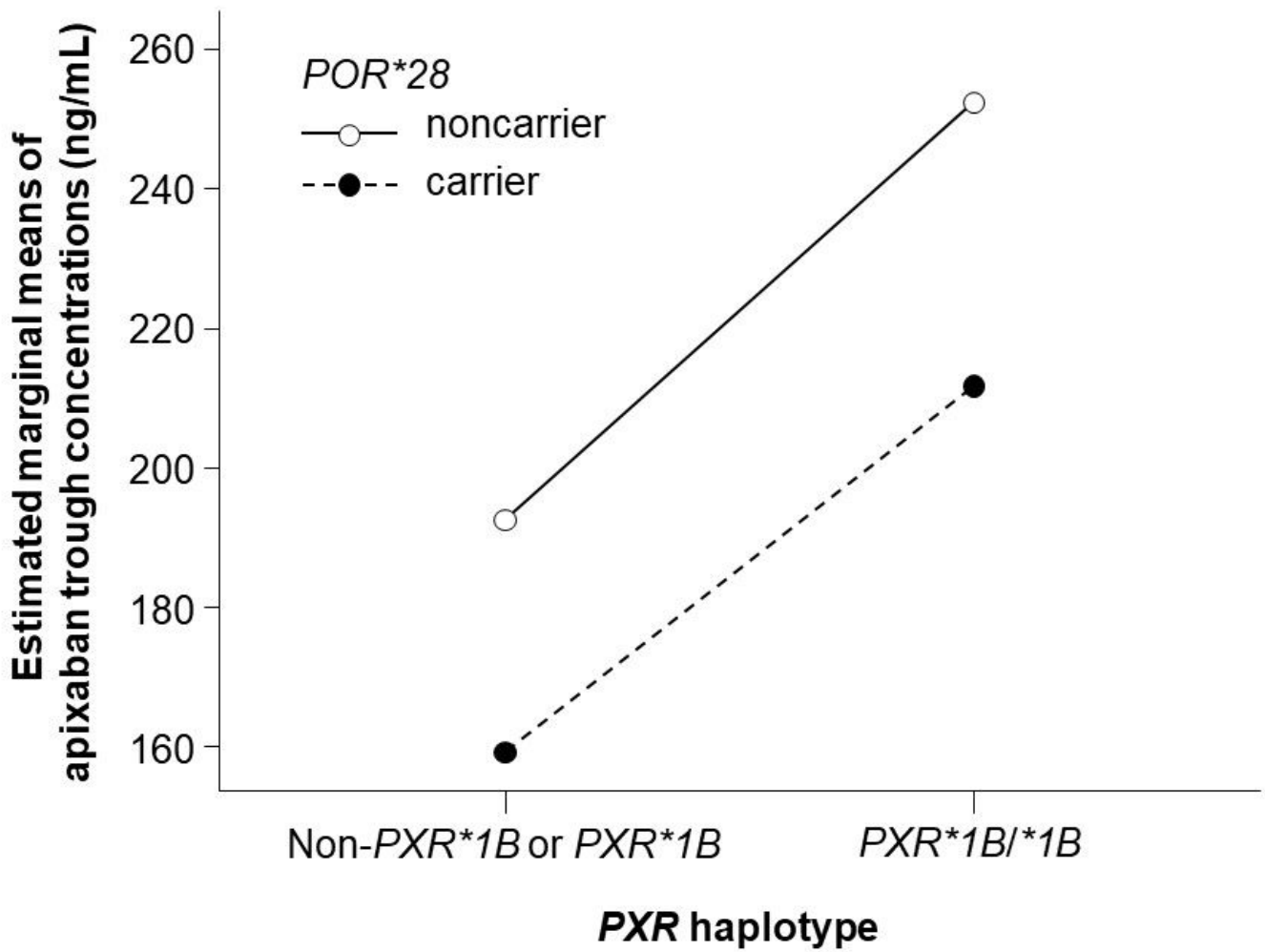
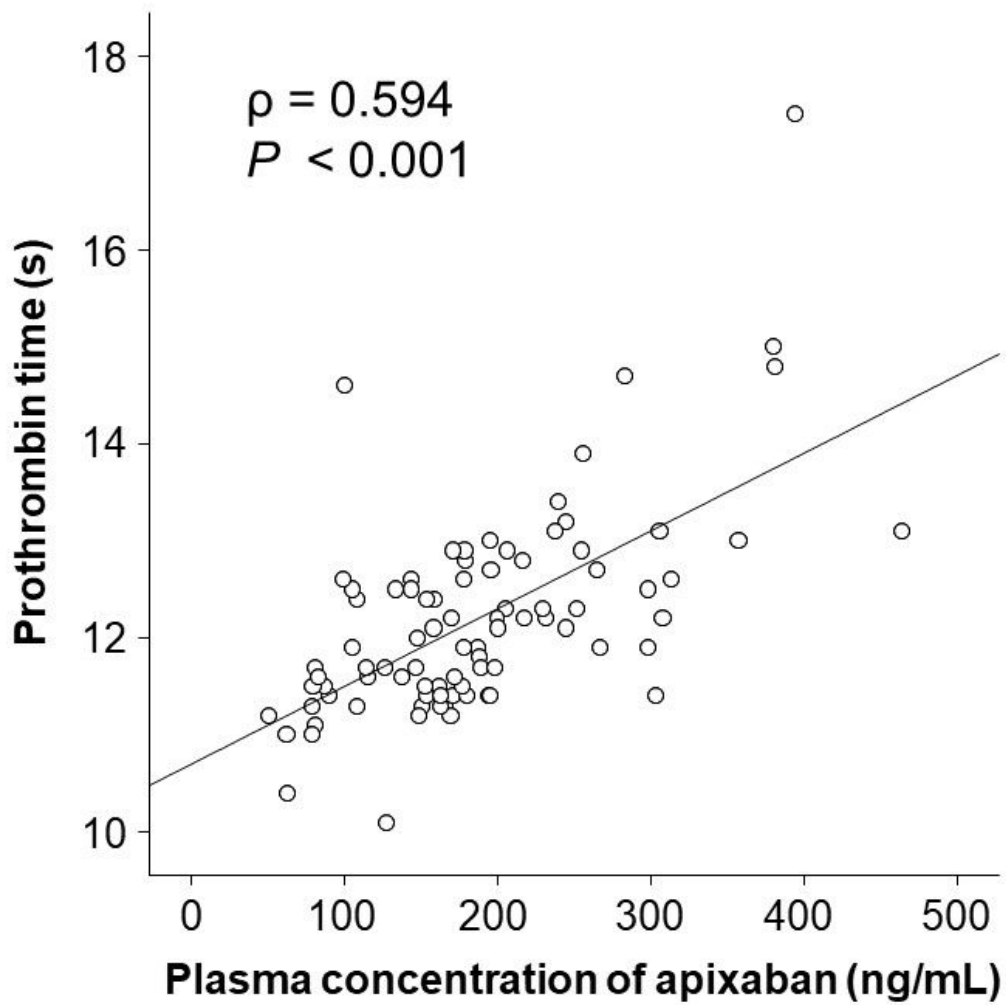


Figure 2

Effects of interactions between the *PXR\*1B* haplotype and the *POR\*28* genotype on the trough concentration of apixaban



**Figure 3**

Relationship between prothrombin time and plasma concentration of apixaban at the trough

## Supplementary Files

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