



# NAT2 phenotype alters pharmacokinetics of rivaroxaban in healthy volunteers

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## ABSTRACT

Rivaroxaban is a direct inhibitor of factor Xa, a member of direct oral anticoagulant group of drugs (DOACs). Despite being a widely extended alternative to vitamin K antagonists (i.e., acenocoumarol, warfarin) the inter-individual variability of DOACs is significant, and may be related to adverse drug reaction occurrence or drug inefficacy, namely hemorrhagic or thromboembolic events. Since there is not a consistent analytic practice to monitor the anticoagulant activity of DOACs, previously reported polymorphisms in genes coding for proteins responsible for the activation, transport, or metabolism of DOACs were studied. The study population comprised 60 healthy volunteers, who completed two randomized, crossover bioequivalence clinical trials between two different rivaroxaban formulations. The effect of food, sex, biogeographical origin and 55 variants (8 phenotypes and 47 single nucleotide polymorphisms) in drug metabolizing enzyme genes (such as *CYP2D6*, *CYP2C9*, *NAT2*) and transporters (namely, *ABCB1*, *ABCG2*) on rivaroxaban pharmacokinetics was tested. Individuals dosed under fasting conditions presented lower  $t_{max}$  (2.21 h vs 2.88 h,  $\beta = 1.19$ ,  $R^2 = 0.342$ ,  $p = 0.012$ ) compared to fed volunteers. NAT2 slow acetylators presented higher  $AUC_{\infty}$  corrected by dose/weight ( $AUC_{\infty}/DW$ ; 8243.90 vs 7698.20 and 7161.25 h\*ng\*mg /ml\*kg,  $\beta = 0.154$ ,  $R^2 = 0.250$ ,  $p = 0.044$ ), higher  $C_{max}/DW$  (1070.99 vs 834.81 and 803.36 ng\*mg /ml\*kg,  $\beta = 0.245$ ,  $R^2 = 0.320$ ,  $p = 0.002$ ), and lower  $t_{max}$  (2.63 vs 3.19 and 4.15 h,  $\beta = -0.346$ ,  $R^2 = 0.282$ ,  $p = 0.047$ ) than NAT2 rapid and intermediate acetylators. No other association was statistically significant. Thus, slow NAT2 appear to have altered rivaroxaban pharmacokinetics, increasing  $AUC_{\infty}$  and  $C_{max}$ . Nonetheless, further research should be conducted to verify NAT2 involvement on rivaroxaban pharmacokinetics and to determine its clinical significance.

## 1. Introduction

Rivaroxaban is a direct inhibitor of coagulation factor Xa, a member of direct oral anticoagulant group of drugs (DOACs). DOACs are mainly prescribed for prevention of deep vein thrombosis (DVT) and pulmonary embolism (PE), prevention of venous thromboembolism in adults who underwent surgery to replace a hip or knee and prevention of stroke and systemic embolism in adults with non-valvular atrial fibrillation [1,2]. Rivaroxaban absolute oral bioavailability varies according to the dose.

For the 2.5–10 mg range, bioavailability varies between 80% and 100%, and it is not affected by food intake. For the 20 mg dose, bioavailability is 66% under fasting conditions, although it increases with food up to 99% [1,2]. The time to reach maximum plasma concentration ( $t_{max}$ ) after oral administration ranges between 2 and 4 h after intake. It is highly bound to plasmatic proteins (92–95%), predominantly albumin. Approximately 33% of the administered dose is excreted via urine as unchanged active substance, and the remaining 67% of the administered dose is metabolized and eliminated via urine and feces. The metabolism

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of rivaroxaban is mediated by CYP3A4, CYP2J2 and CYP-independent mechanisms, i.e. MAO-A and B pathway [1–3]. Moreover, rivaroxaban is a substrate of the ATP-binding cassette transporters ABCB1 and ABCG2. The elimination half-life ( $t_{1/2}$ ) of rivaroxaban ranges between 5 and 9 h in healthy subjects aged 20–45 years. Neither rivaroxaban pharmacokinetics nor pharmacodynamics are influenced by sex or race [1,2].

Despite being a widely extended alternative to vitamin K antagonists (i.e., acenocoumarol, warfarin), DOACs may still cause a number of adverse drug reactions (ADRs), including bleeding events, which can occur in up to 5% of the patients [1,2]. As a consequence, several authors and medical professionals rose the concern of therapeutic monitoring of DOACs [4,5]. Since there is not a consensual analytic practice to monitor the anticoagulant activity of DOACs, the activated partial thromboplastin time, dilute thrombin time, ecarin chromogenic assay, ecarin clotting time, prothrombin time or international normalized ratio, fibrinogen and thrombin time may be used [4,6–8]. Other drugs such as warfarin, an oral anticoagulant vitamin K antagonist, benefit from dose adjustment algorithms that include pharmacogenetic variants of genes such as CYP2C9 and VKORC1 [9]. As a consequence, this missed opportunity for monitoring renders the idea to research for pharmacogenetic markers that would bring personalized treatment for such a number of potential patients.

However, scarce work is available nowadays in this regard and no clinically relevant biomarkers have been validated to date for this drug. Henceforth, the primary goal of this study was to determine the effect of 55 genetic variants in 32 candidate genes coding for proteins responsible for transport or metabolism of rivaroxaban, including *CES1*, *ABCB1*, *ABCG2* and *CYP3A4* [7,10–12], and genes potentially related to rivaroxaban pharmacokinetic variability, such as *CYP3A5* or *CYP2C19* on rivaroxaban pharmacokinetics and adverse drug reactions. The genetic variants comprised 8 phenotypes (inferred from 64 polymorphisms) and 47 additional polymorphisms analyzed individually. The secondary goals of the study were to determine the effect of food, sex and biogeographical origin on rivaroxaban pharmacokinetics and adverse drug reactions.

## 2. Material and methods

### 2.1. Population and study design

The study population comprised 60 healthy volunteers, males and females, that completed two randomized, crossover bioequivalence clinical trials between two different rivaroxaban formulations, performed at the Clinical Trial Unit of Hospital Universitario de La Princesa (UECHUP), and that provided informed consent to participate in this pharmacogenetic study. Prior to inclusion into the study, complete physical examination, heart rate and systolic and diastolic blood pressure recording, electrocardiogram, breath alcohol intake, haematology, coagulation, biochemistry, serology, urinalysis determinations, cotinine and drug abuse analysis were performed. The inclusion criteria for both clinical trials were: male or female subjects aged from 18 to 55; with no clinically significant organic or psychic conditions; without clinically significant abnormalities in medical records, hematology, coagulation, biochemistry, serology (Ag HBs, HC antibodies, HIV antibodies) and urinalysis and with no clinically significant alterations in vital signs and electrocardiogram.

Both clinical trials were reviewed and approved by the Independent Ethics Board (IEB) of Hospital Universitario de La Princesa, and by the Spanish Drug Agency (AEMPS) (EUDRA-CT: 2020–005613–41 and 2020–005614–18). In addition, they were performed in accordance with the guidelines of the International Conference on Harmonization for Good Clinical Practice (ICH-GCP), current Spanish legislation and the Revised Declaration of Helsinki [13]. The pharmacogenetic study (SFC-FG-2020–1) was likewise approved by the IEB of Hospital Universitario de La Princesa (registry number 4176, July 9th 2020).

The first clinical trial (Fasting-CT) was designed as a phase I, oral single-dose, open-label, crossover and randomized bioequivalence clinical trial, with two sequences, two periods with a washout period of eight days, in which subjects were hospitalized from 10 h before to 12 h after dosing 10 mg of rivaroxaban under fasting conditions. The clinical trial was blinded only for the plasma concentrations determination of rivaroxaban. From 36 subjects, 33 provided informed consent to participate in the pharmacogenetic analysis.

The second clinical trial (Fed-CT) was designed as a phase I, oral single-dose, open-label, crossover and randomized bioequivalence clinical trial, with two sequences, two periods with a washout period of eight days, in which subjects were hospitalized from 10 h before to 12 h after dosing 20 mg of rivaroxaban under fed conditions. The breakfast consisted of a high-fat, high-calorie meal served 30 min before dosing, according to EMA guidelines [14]. The study was blinded only for the plasma concentrations determination of rivaroxaban. From 36 subjects that were dosed, 35 subjects completed the study. Finally, 27 volunteers provided informed consent to participate in the pharmacogenetic analysis.

### 2.2. Pharmacokinetic analysis

During the clinical trials performance, volunteers self-reported standardized biogeographical group [15] and weight and age were recorded. Baseline blood sample was obtained at 8:00. Breakfast was administered only on Fed CT at 8:30. Drug was administered at 9:00 on both clinical trials. Each volunteer provided 23 blood samples (EDTA K2 tubes, 3 ml each) in each period between baseline and 72 h after the administration of each formulation. Samples were centrifugated and plasma was extracted for rivaroxaban plasma level determination at each time. Drug level determination in plasma was performed by an external laboratory, with a validated method according to European Medicines Agency (EMA) guidelines for bioanalytical determinations [14]. The method involved liquid-liquid extraction procedure with tert-butyl methyl ether, followed by high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), with a lower limit of quantification (LLOQ) of 0.5 ng/ml.

Pharmacokinetic parameters were calculated using WinNonlin Professional Edition, current version (Scientific Consulting, Inc, Cary USA) for model-independent approach. The pharmacokinetic data used in the analysis were obtained as the mean value of each pharmacokinetic parameter for both rivaroxaban formulations. These comprised: the area under the time versus drug concentration curve (AUC), from time 0 to infinity ( $AUC_{\infty}$ ); the maximum plasma concentration observed ( $C_{max}$ );  $t_{max}$  and  $t_{1/2}$ .  $AUC_{\infty}$  was calculated according to the linear trapezoidal rule, as the AUC from time 0 to the last observation ( $AUC_{0-t}$ ) plus the extrapolation to infinity ( $AUC_{t-\infty}$ ).  $AUC_{t-\infty}$  was calculated as the last detectable concentration (C) divided by the slope of the best adjusted straight line obtained by means of lineal regression, starting from the points corresponding to the phase of elimination of the drug (k), and  $t_{1/2}$  was calculated as  $-\ln 2/k$ .

### 2.3. Safety

Adverse events (AE) were recorded during both bioequivalence clinical trials. Volunteers were asked for AEs at the beginning of each visit and at the moment of blood extractions. Furthermore, spontaneously reported AEs were recorded. AEs were classified according to the algorithm of Spanish Pharmacovigilance System based on their causal relationship with drug intake [16]. For the present study, definite, possible and probable AEs were considered ADRs.

### 2.4. Genotyping

The pharmacogenetic study was designed as a candidate gene study. DNA was extracted from peripheral blood samples with a Maxwell RSC

automated DNA extractor (Promega, United States) following the manufacturer's instructions. The genotyping was conducted in a QuantStudio 12k Flex real-time PCR system along with a custom TaqMan® OpenArray® qPCR array (Thermo Fisher Scientific, United States). This customized array included the polymorphisms included in [Supplementary Table 1](#). The most important pharmacogenes were included, along with other genes of interest potentially related to rivaroxaban and other direct oral anticoagulants metabolism and transport (i.e. *NAT2*, *CES1*) [1,2,7]. A *CYP2D6* copy number variation assay (CNV) was performed in the same thermal cycler with a 96-well thermal block, performed with TaqMan® technology as previously described [17].

## 2.5. Haplotyping and phenotyping

Genotyping results obtained with real-time PCR do not inform the phase of variants (i.e., whether variants occur within the same or in different chromosomes). However, it is widely accepted in pharmacogenetics to default alleles following PharmVar core allele rules or the Clinical Pharmacogenetics Implementation Consortium (CPIC) allele definition tables. The following CPIC guidelines and allele definition tables were used: *CYP2C19* and voriconazole [18]; *CYP2D6* and opioids [19]; *CYP3A5* and tacrolimus [20]; *SLCO1B1*, *ABCG2* and *CYP2C9* and statins [21]; and *UGT1A1* and atazanavir [22]. Following these guidelines' criteria, the possible phenotypes were ultra-rapid (UM), rapid (RM), normal (NM), intermediate (IM) and poor metabolizer (PM) for drug metabolizing enzymes, and normal (NF) and decreased function (DF) for transporters. *CYP2C8* alleles were defined according to PharmVar database [23]. *NAT2* alleles were defined according to the Arylamine N-acetyltransferase Gene Nomenclature Committee [24,25]. *NAT2* phenotypes were assigned as rapid acetylators (RA), intermediate acetylators (IA) and slow acetylators (SA) [26]. *SLC22A1* allele inference was performed complying with previous research [27] for \* 2, \* 3 and \* 5. Accordingly, *SLC22A1* rs628031 G>A intronic variant was analyzed separately. The remaining polymorphisms were analyzed individually.

## 2.6. Statistical analysis

The Shapiro-Wilk test was applied to verify dependent variables normality. Results are showed as mean (coefficient of variation) for normally distributed variables or median (interquartile range) for non-normally distributed variables.  $AUC_{\infty}$  and  $C_{max}$  were adjusted by the dose/weight ratio, becoming  $AUC_{\infty}/DW$  and  $C_{max}/DW$ .  $AUC_{\infty}/DW$  and  $C_{max}/DW$  were logarithmically transformed and analyzed by means of t-test (two categories within a variable) or one-way ANOVA (three or more categories within a variable). When parametric tests were not applicable, a Mann-Whitney U test (two categories within a variable) or a Kruskal-Wallis one-way analysis of variance test (three or more categories within a variable) were used. For statistically significant variables with three or more groups, a pairwise comparison Bonferroni post hoc analysis was performed. A multivariate analysis was performed by means of linear regression. The significant variants from the univariate analysis and the study design were considered the independent variables for the multivariate analysis of pharmacokinetic parameters, which were established as dependent variables. Variables with three or more groups were clustered following Bonferroni post hoc results. The Benjamini and Hochberg correction for multiple comparisons was performed, i.e., false discovery rate (FDR) after univariate analysis. After FDR correction,  $p$  values lower than 0.05 were considered statistically significant;  $p$  values lower than 0.05 before FDR correction were considered nominally significant. For the ANOVA or t-test, the  $p$  value is shown for nominally significant relationships ( $p_{ANOVA}$  or  $p_{t-test}$ , respectively), and for the Mann-Whitney U test or Kruskal-Wallis one-way analysis the  $p$  value is shown for nominally significant relationships ( $p_{KW}$  or  $p_{U-test}$ , respectively). For the multivariate analysis, significance was

indicated with the unstandardized  $\beta$ -coefficient,  $R^2$  value,  $p$  of multivariate analysis ( $p_{MV}$ ), and  $p$  after FDR ( $p_{FDR}$ ). All calculations were computed in R version 4.0.2 software (R Core Team, 2020).

Concerning treatment safety, the incidence of ADRs depending on phenotypes, genotypes, biogeographical group of origin, and clinical trial design was analyzed by means of  $\chi^2$  or Fisher exact test, when suitable. The resulting statistically significant variables were used to calculate the risk of developing those ADRs by means of logistic regression, including genetic variables, sex, clinical trial design and biogeographical group.

## 3. Results

### 3.1. Demographic results

A total of 60 volunteers participated in the study, 27 female and 33 males, aged from 20 to 55 (mean age of 30), with mean body mass index (BMI) of  $24.73 \pm 3.05$  kg/m<sup>2</sup>. The biogeographical groups were represented by 48 Latino and 12 European individuals. Study population demographics are shown in [Table 1](#). Males presented higher height and weight than females ( $p < 0.005$ ). No differences in age, BMI, frequency of sex and biogeographical groups were observed according to sex, biogeographical group and CT design.

### 3.2. Pharmacokinetic results

Volunteers in Fasting-CT presented lower median  $AUC_{\infty}$  and  $C_{max}$  (1247.62 (457.97) h\*ng/ml and 123.45 (41.42) ng/ml, respectively) than volunteers in Fed-CT (2295.77 (751.13) h\*ng/ml  $p < 0.001$ , and 309.82 (86.45) ng/ml,  $p < 0.001$ , respectively). After DW correction, study population showed a mean  $AUC_{\infty}/DW$  of 8261.29 h\*ng\*mg/ml\*kg,  $C_{max}/DW$  of 993.73 ng\*mg/ml\*kg, median  $t_{max}$  of 2.36 h and  $t_{1/2}$  of 8.66 h. Volunteers who participated in the Fasting-CT presented lower  $C_{max}/DW$  ( $p_{t-test} = 0.011$ ,  $p_{FDR} = 0.277$ ), lower  $t_{max}$  ( $\beta = 1.19$ ,  $R^2 = 0.342$ ,  $p_{MW} = 0.012$ ) and higher  $t_{1/2}$  ( $p_{U-test} < 0.001$ ,  $p_{FDR} = 0.004$ ) compared to volunteers in Fed-CT, but not higher  $AUC_{\infty}/DW$  ([Table 2](#)). No other pharmacokinetic association was significant regarding demographic characteristics of study population.

All genotypes were analyzed. All results are displayed in [Table S2](#). *NAT2* SAs presented higher  $AUC_{\infty}/DW$  ( $\beta = 0.154$ ,  $R^2 = 0.250$ ,  $p_{MV} = 0.044$ ), higher  $C_{max}/DW$  ( $\beta = 0.245$ ,  $R^2 = 0.320$ ,  $p_{MV} = 0.002$ ), and lower  $t_{max}$  ( $\beta = -0.346$ ,  $R^2 = 0.282$ ,  $p_{MV} = 0.047$ ) than RAs and IAs ([Fig. 1](#)). *ABCB1* rs1045642 G/A volunteers presented higher  $AUC_{\infty}/DW$  ( $p_{ANOVA} = 0.026$ ,  $p_{FDR} = 0.493$ ) compared to A/A and G/G individuals. *ABCC2* rs2273697 G/G volunteers presented lower  $C_{max}/DW$  ( $p_{ANOVA} = 0.032$ ,  $p_{FDR} = 0.631$ ) than G/A and A/A individuals. Volunteers

**Table 1**  
Demographic characteristics of study population.

Variable		Age (years)	Weight (kg)	Height (m)	BMI (kg/m <sup>2</sup> )
Sex	Female (n = 27)	34.15 (28.69%)	62.91 (16.08%)	1.59 (4.25%)	24.7 (13.48%)
	Male (n = 33)	29.48 (28.14%)	73.98 (13.41%)*	1.73 (3.06)*	24.75 (11.52%)
Biogeographical group	Latino (n = 48)	31.54 (27.37%)	69.24 (16.25%)	1.66 (5.12%)	25.08 (11.69%)
	European (n = 12)	31.75 (36.98%)	68.04 (18.08%)	1.71 (5.94%)	23.32 (13.86%)
	Fasting-CT (n = 33)	29.64 (23.41%)	69.93 (17.88%)	1.68 (5.29%)	24.76 (12.86%)
Clinical Trial design	Fed-CT (n = 27)	33.96 (32.68%)	67.85 (14.64%)	1.66 (5.50%)	24.69 (11.89%)

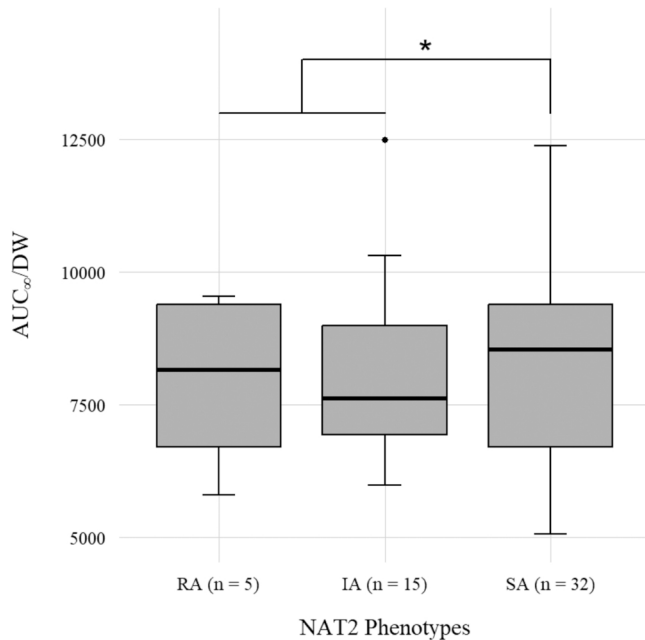
Results are presented as mean (coefficient of variation). CT: Clinical Trial. \*: Statistically significant after Mann-Whitney U-test.

**Table 2**

Pharmacokinetic characteristics of study population by Sex, Study design and Biogeographical group of origin.

Variable		AUC <sub>∞</sub> /DW (h*ng*mg/ ml*kg)	C <sub>max</sub> /DW (ng*mg/ ml*kg)	t <sub>max</sub> (h)	t <sub>1/2</sub> (h)
Sex	Female (n = 27)	8007.06 (24.02%)	989.36 (26.48%)	2.21 (2.16)	9.99 (4.92)
	Male (n = 33)	8469.30 (26.24%)	997.31 (27.14%)	2.50 (1.42)	8.46 (3.12)
Clinical Trial design	Fasting-CT (n = 33)	8666.04 (28.13%)	<b>919.06</b> <b>(28.00%)</b>	<b>2.21</b> <b>(1.00)</b>	<b>10.38</b> <b>(2.77)</b>
	Fed-CT (n = 27)	7766.60 (18.78%)	1085.00 (22.87%)	2.88 (3.02)	6.74 (2.39)
Biogeographical group of origin	Latino (n = 48)	8405.49 (24.31%)	1010.50 (26.29%)	2.34 (1.65)	8.99 (4.00)
	European (n = 12)	7684.50 (29.40%)	926.67 (28.07%)	3.00 (1.97)	6.92 (5.39)
	Total (n = 60)	8261.29 (25.29%)	993.73 (26.62%)	2.36 (5.44)	8.66 (9.61)

AUC<sub>∞</sub>/DW: area under the time-concentration curve from time 0 to infinity, corrected by dose/weight ratio; C<sub>max</sub>/DW: maximum drug concentration in plasma corrected by dose/weight ratio; t<sub>max</sub>: time to reach C<sub>max</sub>; t<sub>1/2</sub>: elimination half-life. AUC<sub>∞</sub>/DW and C<sub>max</sub>/DW data are presented as mean (coefficient of variation), t<sub>max</sub> and t<sub>1/2</sub> data are presented as mean (Inter Quartile Range). Nominally significant results are highlighted in bold. \*: Nominally significant results after t-test or Mann-Whitney U-test, †: Statistically significant results after multiple linear regression



**Fig. 1.** AUC<sub>∞</sub>/DW of the different NAT2 phenotypes. AUC<sub>∞</sub>/DW is displayed in h\*ng\*mg/ml\*kg. \*: *p*-value < 0.05 after multivariate analysis; RA: rapid acetylators; IA: intermediate acetylators; SA: slow acetylators.

with CYP3A4 rs61469810 A/A diplotype showed higher C<sub>max</sub>/DW (*p*<sub>ANOVA</sub>=0.011, *p*<sub>FDR</sub>=0.277) than A/- and -/- diplotype carriers. SLC22A1 rs628031 A/A homozygotes presented lower C<sub>max</sub>/DW (*p*<sub>ANOVA</sub>=0.003, *p*<sub>FDR</sub>=0.191) than A/G and G/G individuals. UGT1A3 rs2008584 G/G volunteers showed higher AUC<sub>∞</sub>/DW (*β* = 0.201, *R*<sup>2</sup> = 0.250, *p*<sub>MV</sub>=0.029) and higher t<sub>max</sub> (*p*<sub>KW</sub>=0.028, *p*<sub>FDR</sub>=0.493) than A/G and A/A volunteers. UGT2B7 rs7668258 T/T individuals showed lower t<sub>1/2</sub> (*p*<sub>KW</sub>=0.005, *p*<sub>FDR</sub> = 0.212) than T/C and C/C volunteers (Table 3). No other pharmacokinetic association remained significant after multivariate analysis.

### 3.3. Safety

No serious ADRs were reported. Six volunteers presented at least one ADR: five volunteers in Fasting-CT and one volunteer in Fed-CT. The most frequent ADR was headache (n = 3), followed by nausea (n = 2), dizziness (n = 1), epistaxis (n = 1) and menorrhagia (n = 1). No other relationship between polymorphisms, sex or biogeographical group of origin with ADR occurrence was found.

### 4. Discussion

Pharmacogenetics is a valuable tool for the adjustment and personalization of pharmacological treatments, improving their efficacy and tolerability [28–31]. This diagnostic tool is routinely used at the Clinical Pharmacology Department of Hospital Universitario de La Princesa (Madrid, Spain) as a consequence of Prime-PGx initiative [32], and could be potentially useful for the prescription of rivaroxaban. In addition, multiple studies analyzed the relationship between different pharmacogenetic variants and pharmacokinetics, safety, and efficacy of rivaroxaban [7]. Nonetheless, the evidence is inconclusive and contradictory results were found. Consequently, this study aimed to explore pharmacogenes involved in transport and metabolism of rivaroxaban.

Study population showed median AUC<sub>∞</sub>, C<sub>max</sub>, t<sub>max</sub> and t<sub>1/2</sub> similar to previously reported [1,2]. Median AUC<sub>∞</sub> and C<sub>max</sub> in Fasting-CT volunteers constituted almost half of median AUC<sub>∞</sub> and C<sub>max</sub> in Fed-CT volunteers, which might be anticipated since the dose was 10 mg for Fasting-CT and 20 mg for Fed-CT. No significant differences regarding sex and biogeographical origin were detected, as expected [1, 2]. No significant differences in AUC<sub>∞</sub>/DW were observed between fed and fasting conditions of administration. This was expected since the different administration conditions achieve equalized bioavailability despite the different dosages [1,2]. On the contrary, volunteers that participated in Fasting-CT presented lower C<sub>max</sub>/DW, lower t<sub>max</sub> and higher t<sub>1/2</sub> compared to volunteers in Fed-CT. Only t<sub>max</sub> remained significant in the multivariate analysis. The difference in t<sub>max</sub> might originate in the delayed gastric emptying and time of absorption produced by the food. The higher occurrence of ADRs under fasting conditions is consistent with these observations and with the tendency towards a higher AUC<sub>∞</sub>/DW compared to fed conditions (15% vs 0%). These outcomes highlight the importance of following drug label instructions for drug administration, particularly, the fed conditions depending on the dose prescribed.

As the main result of this study, NAT2 SA presented higher AUC<sub>∞</sub>/DW, higher C<sub>max</sub>/DW, and lower t<sub>max</sub> than RA and IA, which were confirmed in the multivariate analysis. The impact of NAT2 variants has a high level of evidence for this type of drugs, and is related to alterations in pharmacokinetics and incidence of ADRs [33,34]. Besides, NAT2 variants have been associated with alterations in the pharmacokinetics of other drugs such as diazepam and rasagiline [35,36]. The associations found in the present study correlate with the potential effect of the alleles and the pharmacokinetics variables, namely, a decreased or no function allele would encode a protein with lower activity, augmenting the levels of rivaroxaban in plasma. About 18% of rivaroxaban has been reported to be hydrolyzed through the CYP3A4 pathway, 14% through CYP2J2, and another 14% is hydrolyzed by CYP-independent pathways [37]. In addition, 11% fraction of rivaroxaban is metabolized via unidentified mechanisms [38–40]. Therefore, it is plausible that NAT2 or other enzymes were involved in its metabolism [37]. To the best of our knowledge, this is the first study to propose the involvement of NAT2 in the metabolism of rivaroxaban. This result might be important for rivaroxaban management. Despite this association, no increased incidence of ADRs was observed in NAT2 SAs. This may be a consequence of the reduced number of ADRs observed due to the short duration of rivaroxaban treatment. As it is a single dose study, a short number of ADRs are expected. Nevertheless, further research should be performed to better describe NAT2 involvement on



**Table 3**

Pharmacokinetic characteristics of study population by genotype or phenotype.

Gene	Genotype/Phenotype		AUC <sub>∞</sub> /DW (h*ng*mg/ml*kg)	C <sub>max</sub> /DW (ng*mg/ml*kg)	t <sub>max</sub> (h)	t <sub>1/2</sub> (h)
ABCB1	rs1045642	C/C (n = 24)	7746.43 (22.55%)	1006.35 (26.13%)	2.44 (1.98)	8.66 (3.39)
		T/C (n = 24)	<b>9193.41 (23.37%)*<sup>1</sup></b>	1024.06 (29.46%)	2.34 (1.60)	8.03 (4.32)
		T/T (n = 12)	7426.77 (27.38%)	907.85 (19.08%)	2.50 (2.00)	9.55 (5.22)
ABCC2	rs2273697	G/G (n = 41)	7978.59 (24.17%)	<b>939.35 (26.94%)*<sup>2</sup></b>	2.38 (1.50)	9.19 (3.99)
		G/A (n = 18)	8717.76 (26.43%)	1095.52 (23.23%)	2.60 (1.91)	6.72 (3.72)
CYP3A43	rs61469810	A/A (n = 48)	8245.75 (24.15%)	1015.77 (25.34%)	2.44 (1.58)	8.71 (4.40)
		A/delA + delA/delA (n = 10)	8091.16 (31.5%)	<b>826.35 (28.71%)*<sup>3</sup></b>	2.67 (2.17)	8.46 (2.15)
NAT2	Phenotype	RA (n = 5)	7161.25 (32.37%)	803.36 (34.96%)	3.66 (1.58)	7.62 (3.49)
		IA (n = 15)	7698.20 (26.79%)	834.81 (21.85%)	2.66 (2.00)	8.69 (4.43)
		SA (n = 32)	<b>8243.90 (23.17%)*<sup>1</sup>†<sup>1</sup></b>	<b>1070.99 (24.38%)*<sup>4</sup>†<sup>1</sup></b>	<b>2.15 (1.54)*<sup>4</sup>†<sup>1</sup></b>	8.54 (3.80)
SLC22A1	rs628031	G/G (n = 32)	8462.65 (25.1%)	981.66 (26.1%)	2.44 (1.63)	8.54 (3.81)
		G/A (n = 21)	8395.92 (21.88%)	1069.73 (20.65%)	2.67 (3.16)	8.54 (3.65)
		A/A (n = 4)	6366.17 (19.51%)	<b>680.22 (21.88%)*<sup>5</sup></b>	1.85 (1.53)	11.55 (9.44)
UGT1A3	rs2008584	A/A (n = 14)	8849.47 (27.4%)	972.08 (26.24%)	2.19 (0.79)	7.60 (4.07)
		A/G (n = 27)	7747.45 (20.87%)	1007.17 (21.88%)	2.34 (1.67)	8.54 (3.66)
		G/G (n = 8)	<b>8593.79 (29.94%)*<sup>2</sup>†<sup>2</sup></b>	1067.87 (38.51%)	<b>3.66 (2.08)*<sup>6</sup></b>	8.12 (3.80)
UGT2B7	rs7668258	T/T (n = 8)	7368.28 (29.96%)	954.62 (23.17%)	2.67 (2.95)	<b>6.42 (0.63)*<sup>7</sup></b>
		T/C (n = 19)	8168.46 (23.63%)	1049.24 (26.9%)	2.66 (1.81)	8.54 (2.23)
		C/C (n = 19)	7779.93 (25.21%)	938.26 (29.77%)	1.83 (1.35)	10.43 (4.24)
Total (n = 60)			8261.29 (25.29%)	993.73 (26.62%)	2.36 (5.47)	8.66 (6.88)

AUC<sub>∞</sub>/DW: area under the time-concentration curve from time 0 to infinity, corrected by dose/weight ratio; C<sub>max</sub>/DW: maximum drug concentration in plasma corrected by dose/weight ratio; t<sub>max</sub>: time to reach C<sub>max</sub>; t<sub>1/2</sub>: elimination half-life; RA: rapid acetylators; IA: intermediate acetylators and PA: poor acetylators. AUC<sub>∞</sub>/DW and C<sub>max</sub>/DW data are presented as mean (coefficient of variation), t<sub>max</sub> and t<sub>1/2</sub> data are presented as mean (Inter Quartile Range). \*<sup>1</sup>: Statistically significant after Bonferroni post hoc A/G vs G/G + A/A; \*<sup>2</sup>: Statistically significant after Bonferroni post hoc G/G vs G/A; \*<sup>3</sup>: Statistically significant after Bonferroni post hoc A/A vs A/delA + delA/delA; \*<sup>4</sup>: Statistically significant after Bonferroni post hoc SA vs RA + IA; \*<sup>5</sup>: Statistically significant after Bonferroni post hoc A/A vs G/G + G/A; \*<sup>6</sup>: Statistically significant after Bonferroni post hoc G/G vs A/A + A/G; \*<sup>7</sup>: Statistically significant after Bonferroni post hoc T/T vs T/C + C/C; †<sup>1</sup>: Statistically significant results after multiple linear regression G/G vs A/G + A/A; †<sup>2</sup>: Statistically significant results after multiple linear regression SA vs RA + IA.

rivaroxaban pharmacokinetics.

The association between *ABCB1* and *UGT1A3* genotypes and AUC<sub>∞</sub>/DW and C<sub>max</sub>/DW suggest a heterozygote advantage, which is not possible in a codominance model. Consequently, they may be explained by type 1 error and should be rejected. In addition, *ABCC2*, *CYP3A43*, *SLC22A1* and *UGT2B7* variants did not remain significant after multivariate analysis therefore an impact of them on rivaroxaban pharmacokinetics is unlikely.

Despite the strengths of this study, several limitations should be acknowledged. First, the number of individuals is limited, especially in terms of studying the effect of less frequent polymorphisms. Volunteers were genotyped on a mass genotyping platform with 95% efficiency. This means that certain polymorphisms failed genotyping, and therefore, that samples were excluded from the sub-analysis of that polymorphism, which explains why the total number of individuals in each gene is lower in certain cases than the total number of participants. This entails a relevant absence of results related to the main metabolizing enzymes: *CYP3A4* and *CYP2J2*. Eight polymorphisms of *CYP3A4*, including \* 17 and \* 20 (two known reduced function alleles), were included in this study. Nonetheless, the low prevalence of these polymorphisms and the limited sample size of our study did not yield sufficient variability to perform statistical analysis. Additionally, and despite the interest of *CYP2J2*, the screening of its polymorphisms was not possible in our study. It is partially justified as *CYP2J2* is not yet well characterized and its alleles are not definitively established. Moreover, as this is a study of healthy volunteers, we cannot study the effect of genetic variability on the efficacy of rivaroxaban. The fact that this is a single-dose study is also limiting, as it makes it difficult to detect adverse effects due to the short time of exposure to the drug. Finally, the clinical relevance of the significant difference is yet to be established.

## 5. Conclusion

As a conclusion, slow NAT2 appear to have altered kinetics which increases the AUC<sub>∞</sub> and C<sub>max</sub>. Despite the exposed limitations, this study is the first to propose an effect of NAT2 phenotype on rivaroxaban pharmacokinetics. Nonetheless, the statistical significance does not

imply clinical significance, and it would be necessary to perform studies with larger number of patients with longer exposure to rivaroxaban in order to assess clinical relevance. Additionally, the study contributes to further characterize previously reported rivaroxaban pharmacogenetic biomarkers associated with rivaroxaban pharmacotherapy, such as *ABCB1* and *ABCC2*.

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## CRedit authorship contribution statement

**Gonzalo Villapalos-García:** Conceptualization, Formal analysis, Writing – original draft. **Pablo Zubiaur:** Conceptualization, Formal analysis, Writing – original draft. **Dolores Ochoa:** Investigation, Resources. **Paula Soria-Chacartegui:** Investigation. **Marcos Navares-Gómez:** Investigation. **Miriam Matas:** Investigation. **Gina Mejía-Abril:** Investigation. **Ana Casajús-Rey:** Investigation. **Diana Campodónico:** Investigation. **Manuel Román:** Investigation. **Samuel Martín-Vilchez:** Investigation. **Carmen Candau-Ramos:** Investigation. **Marina Aldama-Martín:** Investigation. **Francisco Abad-Santos:** Conceptualization, Resources, Project administration, Funding acquisition. All authors: Writing – review & editing.

## Declaration of Competing Interest

Francisco Abad-Santos and Dolores Ochoa have been consultants or

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Data availability

Data will be made available on request.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.biopha.2023.115058](https://doi.org/10.1016/j.biopha.2023.115058).

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