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Galaxy Publication

Influence of Food Effect and ABCB1 Genetic Polymorphism (rs4148738) on Dabigatran Pharmacokinetics: A Population Modeling Study in Chinese Adults

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ABSTRACT

The influence of ABCB1 and CES1 genetic variants on dabigatran plasma exposure is still uncertain. This investigation aimed to quantitatively evaluate how these polymorphisms affect dabigatran ester concentrations in healthy Chinese participants using a population pharmacokinetic (PopPK) framework. A total of 1,926 PK samples obtained from 123 healthy volunteers who received 150 mg of oral dabigatran under either fasting or fed conditions were examined through a PopPK analysis. The data were best represented by a two-compartment model featuring first-order absorption.

Food intake and the ABCB1 rs4148738 variant emerged as significant covariates. Under post-meal dosing, lag time (ALAG) and clearance (CL) increased by 2.65% and 0.51%, respectively, while the absorption rate constant (KA) decreased by 0.24%. Furthermore, carriers of the CT genotype for ABCB1 (rs4148738) exhibited a 0.38% rise in central distribution volume (V2). Overall, the PopPK model reliably described dabigatran pharmacokinetics in healthy Chinese adults and confirmed that both dietary state and ABCB1 polymorphism meaningfully alter dabigatran absorption and circulating levels.

Keywords: Population pharmacokinetics, Food effects, Dabigatran, Genetic polymorphism, ABCB1, CES1

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Introduction

Atrial fibrillation (AF), affecting many millions globally, represents the most prevalent type of arrhythmia. Individuals with AF face elevated risks of systemic embolism and stroke [1]. Dabigatran, a direct thrombin inhibitor, is recommended to prevent thromboembolic stroke in those with non-valvular AF (NVAF) [2, 3]. When compared with warfarin, dabigatran offers comparable protective efficacy with a reduced likelihood of bleeding events [4].

Its consistent pharmacokinetic behavior allows the use of fixed dosing without routine coagulation monitoring [5, 6]. Nonetheless, both PK and PD responses to dabigatran display considerable person-to-person variability [7, 8]. For example, the inter-individual coefficient of variation in dabigatran levels among AF patients ranges from 51% to 64%, while intra-individual variation lies between 32% and 40% [9]. Even in healthy subjects, PK variability between individuals approaches 30% [10].

Emerging evidence suggests that genetic variants contribute to differences in dabigatran plasma exposure [7, 11, 12]. Dabigatran etexilate serves as a substrate for P-glycoprotein (P-gp), encoded by ABCB1, which mediates intestinal efflux [13]. Its conversion to the active drug is primarily catalyzed by carboxylesterase 1 (CES1) encoded by the CES1 gene [13, 14]. Consequently, ABCB1 and CES1 polymorphisms may modulate dabigatran etexilate's absorption and metabolic activation.

Moreover, ABCB1 and CES1 show tissue-specific expression and sex-related differences, potentially influencing pharmacokinetic behavior. ABCB1 is found mainly in the adrenal glands, renal tissue, and gastrointestinal tract

[15, 16]. CES1, on the other hand, is predominantly expressed in the liver, gallbladder, and digestive system [17, 18].

In the RE-LY study, the single-nucleotide polymorphisms (SNPs) rs2244613 and rs8192935 in CES1, as well as the ABCB1 SNP rs4148738, were found to be linked to the concentrations of dabigatran, either at its peak or trough levels [7]. Various studies have indicated that the CES1 variants rs8192935 and rs2244613 influence dabigatran plasma levels [13, 19, 20]. Earlier research also proposed that ABCB1 SNPs, including rs4148738 and rs1045642, may impact dabigatran's equilibrium peak concentration [21]. Furthermore, the connection between ABCB1 polymorphisms (rs4148738 and rs1045642) and an increased likelihood of major hemorrhagic events was first highlighted in a Chinese population study [22]. However, Ji *et al*. reported that no significant differences in dabigatran pharmacokinetics or pharmacodynamics were observed in participants with the ABCB1 variant SNPs rs4148738 and rs1045642 [13]. Recent findings also support that polymorphisms in ABCB1 (rs4148738) and CES1 (rs2244613) have no meaningful effect on dabigatran levels [19]. Consequently, the influence of genetic variations in the ABCB1 and CES1 genes on dabigatran concentrations remains uncertain.

Population pharmacokinetic (PopPK) modeling is frequently used to estimate pharmacokinetic parameters within specific populations, as well as to identify covariates contributing to the variability observed in pharmacokinetics [23]. The role of genetic polymorphisms in the ABCB1 and CES1 genes in altering dabigatran plasma levels is still debated, but PopPK modeling serves as a useful tool to investigate this. Furthermore, the current dabigatran dosing schedules (75 mg, 110 mg, or 150 mg, twice daily) may still lead to adverse events like hemorrhages or embolisms in certain individuals [24, 25]. The available studies on the impact of genetic polymorphisms on dabigatran plasma levels in Chinese populations are limited. Using healthy participants for these analyses eliminates many confounding factors, leading to a more accurate understanding of the role genetic variations play. Thus, the goal of this study was to quantitatively examine the impact of genetic polymorphisms on dabigatran etexilate in Chinese participants using PopPK analysis, leveraging comprehensive pharmacokinetic data.

Materials and Methods

Study population

The data for this analysis were sourced from a bioequivalence study involving 29 female and 94 male healthy Chinese participants, with a total of 1926 pharmacokinetic (PK) samples. Only data from the reference formulation were included in the PopPK analysis (Boehringer Ingelheim International GmbH, Germany). The study followed standard inclusion criteria for bioequivalence trials, ensuring all participants were healthy based on medical history and physical assessments. Participants' ages ranged from 18 to 43 years, weights varied from 45.2 to 82.0 kg, heights were between 145.5 and 182.5 cm, and their body mass index (BMI) ranged from 19.2 to 25.9 kg/m². All subjects provided written informed consent before participation. The trial adhered to the Helsinki Declaration, Good Clinical Practices, and national regulations, with ethical approval from the Guizhou Medical University ethics committee (Ethical batch number: 2024037K). The study was registered on ClinicalTrials.gov (NCT06387407).

Study design and sampling

Each participant received a single dose of 150 mg dabigatran etexilate (Pradaxa) dissolved in 240 mL of warm boiled water, either on an empty stomach or within 30 minutes of a standard high-fat meal. Blood samples were taken from both fasting and postprandial groups at various time intervals: predose (0 h), 0.25 h, 0.5 h, 0.75 h, 1 h, 1.33 h (80 min), 1.67 h (100 min), 2 h, 2.5 h, 3 h, 3.5 h, 4 h, 5 h, 6 h, 8 h, 12 h, 24 h, 36 h, and 48 h, for a total of 19 points. Each sample was approximately 4 mL, collected in pre-cooled EDTA-K2 tubes, and processed by centrifugation at 4°C and 1700 g for 10 minutes within one hour. After centrifugation, the samples were stored at −60°C for subsequent analysis.

Plasma dabigatran concentration analysis

The total dabigatran concentration in plasma was measured using HPLC-MS/MS [26, 27], with a range from 1.000 ng/mL to 300.0 ng/mL and a lower limit of quantification of 1.000 ng/mL. The pharmacokinetic parameters were determined using WinNonlin 8.2 or higher software, employing a non-compartmental model. The parameters calculated for each subject included Cmax, AUC0-t, AUC0-∞, Tmax, and t1/2. Statistical measures such as mean, standard deviation, coefficient of variation, median, maximum, minimum, and geometric mean were computed

for each parameter. Blood concentration-time (C-t) data were analyzed by plotting individual and mean concentration-time curves, including semilogarithmic plots, and statistical summaries of the concentrations at each time point were provided.

Genotype analysis

For this study, approximately 50 μL of blood was used to extract genomic DNA using the Blood Genomic DNA Extraction Kit (TIANGEN BIOTECH, Beijing, China). The DNA concentration and purity were assessed before proceeding. Genotyping was conducted using the PrimeSTAR® Max DNA Polymerase (TaKaRa) in a Rapid PCR process.

The primers used for the genotyping were as follows: for ABCB1 rs4148738, the forward primer was 5′CTGCAAGGAGATTTAACCCC 3′, and the reverse primer was 5′AAGACACCTCAAACTTGGCC 3′. For ABCB1 rs1045642, the forward primer was 5′ACAAGGAGGGTCAGGTGATC 3′, and the reverse primer was 5′GAACTCTTGTTTTCAGCTGC 3′. For CES1 rs2244613, the forward primer was 5′GCCCTGTATTCTTGGTGTTT 3′, and the reverse primer was 5′AGGACTTGCCCAAATCATAG 3′. For CES1 rs8192935, the forward primer was 5′TTATGGTTCAATACCCAATG 3′, and the reverse primer was 5′AGAAGCAGTTAAGCAGGTGA 3′.

The PCR conditions for genotyping CES1 rs8192935 involved an initial denaturation at 95°C for 10 seconds, followed by a denaturation at 98°C for 10 seconds, then annealing at 53°C for 15 seconds, and an extension at 72°C for 30 seconds, repeated for 35 cycles. For the other polymorphisms (CES1 rs2244613, ABCB1 rs1045642, and ABCB1 rs4148738), the reaction started with denaturation at 95°C for 10 seconds, followed by 98°C for 10 seconds, annealing at 55°C for 10 seconds, and a 30-second extension at 72°C for 35 cycles.

Model development

Population pharmacokinetic (PopPK) modeling of dabigatran etexilate was performed using NONMEM software (version 7.5.1, Icon Development Solutions, Ellicott, Maryland, USA), applying nonlinear mixed-effects modeling. Graphical representations and simulations from NONMEM were produced with the help of R software (version 4.3.2, R Foundation for Statistical Computing). The modeling method utilized was First-Order Conditional Estimation with Interaction (FOCEI). A two-compartment model with first-order absorption was employed to characterize dabigatran etexilate's pharmacokinetics. Covariates such as age, weight, height, BMI, and genetic variations were incorporated in the model, with variable selection done using both forward selection and backward elimination techniques.

The robustness of the final model was assessed through bootstrap simulations, and its predictive accuracy was compared to observed data. The goodness of fit was evaluated using standard diagnostic plots, likelihood ratio tests, and visual predictive checks (VPC). Additional model evaluation was conducted with R and PsN (version 5.0.0) software. Further visualization and support were provided using tools like Pirana (version 23.1.1) and Xpose (version 4.7.1).

In NONMEM modeling, individual variability in parameters (IIV) is often modeled using an exponential function. This model reflects the relationship between the individual parameters (θi) and the typical population parameters (θtv), as shown in Eq. 1.

$$\theta_i = \theta_{tv} * \exp^{\eta i} \quad (1)$$

In the model, ηi represents the random effect for each individual, which is generally assumed to follow a normal distribution with a mean of 0 and a variance of ω^2, i.e., $\eta i \sim N(0, \omega^2)$.

Residual variability is commonly modeled using a combined error structure. This allows for more flexibility in capturing complex patterns in the residual errors, leading to a better representation of the variability observed in the dataset. The mathematical representation of this residual error is typically given by the following equations (Eq. 2):

$$Y_{ij} = F_{ij} * (1 + \varepsilon_{ij,2}) + \varepsilon_{ij,1} \quad (2)$$

In this equation, Yij represents the measured value for the ith individual at the jth time point, while Fij is the model's predicted value for that same individual and time. The terms εij,1 and εij,2 represent the additive and

proportional sources of variation within the individual, both assuming a mean of 0 and variances σ_1^2 and σ_2^2, respectively.

Covariate selection and model evaluation

Following the establishment of the base model, individual parameter values were examined for their relationship with various covariates. Covariates with potential influence on the base model's parameters were selected for further analysis based on their statistical relevance and biological feasibility. Stepwise covariate modeling (SCM) was used to determine the effect of demographic and genetic factors on dabigatran's pharmacokinetics (PK). In this method, the critical value for including covariates during forward selection was 3.84 ($P < 0.05$), while for backward elimination, it was 10.83 ($P < 0.001$). Significant covariates were added or removed through these steps to finalize the covariates included in the model. The covariates considered included age, height, weight, BMI, sex, diet, and the ABCB1 and CES1 gene variants of the participants. The way a covariate is incorporated into the model depends on its type: continuous covariates are represented with exponential modeling (Eq. 3), while categorical covariates are included using piecewise functions (Eq. 4).

$$\theta_i = \theta_{tv} * \exp^{\eta i} * \theta_{COV}^{\frac{cov_i}{COV_{median}}} \quad (3)$$

$$\theta_i = \theta_{tv} * (1 + COV * \theta_{COV}) \quad (4)$$

Where θi denotes the parameter for the ith individual, θtv represents the typical value of the parameter, θcov refers to the typical values for covariate parameters, and ηi is the random error term, assumed to have a mean of 0 and variance ω^2. COVi stands for a continuous covariate, while COVmedian represents its median. If there is a categorical covariate, COV is set to 1, otherwise, it is 0. θcov acts as the adjustment factor for covariates within the model's parameters.

Results and Discussion

Participant demographics

The study enrolled 123 healthy individuals, yielding a total of 1,926 blood concentration measurements. The demographic characteristics of these participants are provided in **Table 1**. Participants were split into two groups: 61 in the fasting group and 62 in the fed group, each receiving a single 150 mg dose of dabigatran etexilate. In the fasting group, the median values for age, height, weight, and BMI were 25 years, 165 cm, 58.8 kg, and 21.5 kg/m², respectively. In the postprandial group, the corresponding median values were 25 years, 164.8 cm, 59.7 kg, and 21.9 kg/m².

Table 1. Participant Demographics.

Characteristic	Fasting Group (n = 123)	Postprandial Group (n = 123)
Sex, n (%)		
Male	47 (38.21)	47 (38.21)
Female	14 (11.38)	15 (12.20)
Age (years), mean ± SD	25.20 ± 4.17	24.94 ± 5.08
Body weight (kg), mean ± SD	58.82 ± 7.18	59.74 ± 7.71
Height (cm), mean ± SD	165.2 ± 8.13	164.77 ± 8.02
Body mass index (kg/m²), mean ± SD	21.50 ± 1.57	21.95 ± 1.74
Trough concentration (ng/mL), mean ± SD	33.15 ± 12.06	33.91 ± 17.46
Peak concentration (ng/mL), mean ± SD	159.50 ± 57.28	114.89 ± 36.96

Genotype distribution

Genotyping of four SNPs was performed on 99 healthy individuals who took a single 150 mg dose of dabigatran etexilate. The results of the genotyping analysis are summarized in **Table 2**.

Table 2. Frequency of Alleles by Loci for CES1 and ABCB1 in the Study Participants.

Gene	SNP	Genotype	Number of Subjects (N)	Minor Allele	Minor Allele Frequency (MAF, %)
CES1					
	rs2244613	GG	40	T	36.87
		GT	45		
		TT	14		
	rs8192935	AA	49	G	26.77
		AG	47		
		GG	3		
ABCB1					
	rs4148738	CC	20	C	44.44
		CT	48		
		TT	31		
	rs1045642	AA	15	A	39.29
		AG	47		
		GG	36		

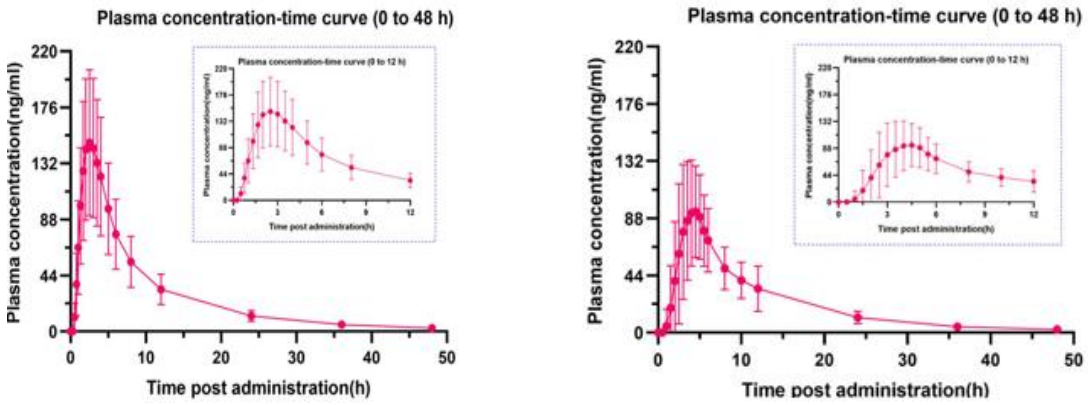
SNP: single nucleotide polymorphism; MAF: minor allele frequency.

For the CES1 SNP rs2244613, genotyping showed that 40 subjects had the GG genotype, 14 had the TT genotype, and 45 were heterozygous (GT), with a minor allele (T) frequency of 36.87%. For the CES1 SNP rs8192935, 49 subjects had the AA genotype, 3 had the GG genotype, and 47 were heterozygous (AG), with a minor allele (G) frequency of 26.77%.

For ABCB1 SNP rs4148738, 20 subjects had the CC genotype, 31 had the TT genotype, and 48 were heterozygous (CT), with a minor allele (C) frequency of 44.44%. Lastly, for ABCB1 SNP rs1045642, 15 participants carried the AA genotype, 36 had the GG genotype, and 47 were heterozygous (AG), with a minor allele (A) frequency of 39.29%.

Pharmacokinetics

Figure 1 presents the mean plasma concentration-time curves for dabigatran in both fasting and post-meal conditions. The data clearly show that peak plasma concentrations were significantly lower when the drug was taken after a high-fat meal compared to when taken on an empty stomach.



a) b)

Figure 1. Plasma concentration-time profiles for total dabigatran under fasting (a) and postprandial (b) conditions. The figure compares the average plasma concentrations of dabigatran over time in healthy Chinese participants under both fasting (a) and post-meal (b) conditions. The error bars represent the standard deviation (SD).

The pharmacokinetic parameters, including the effects of food intake, sex, and genetic polymorphisms, as analyzed using non-compartmental methods, are presented in **Table 3**. For a single oral dose of dabigatran etexilate, the peak time (Tmax) in healthy Chinese adults was approximately 2.4 hours when taken on an empty stomach and 4 hours after a meal. Both conditions followed a pattern indicative of linear kinetics. The mean AUC values were considerably greater in the fasting group compared to the post-meal group. Additionally, females showed slightly elevated Cmax and AUC values when compared to males. Among carriers of CES1 SNP rs2244613, those with the TT genotype had higher Cmax and AUC values than both GG and GT carriers. For the CES1 SNP rs8192935, GG genotype carriers exhibited notably higher Cmax and AUC than AA and AG carriers. In contrast, the ABCB1 SNPs (rs4148738 and rs1045642) did not demonstrate a significant effect on Cmax or AUC.

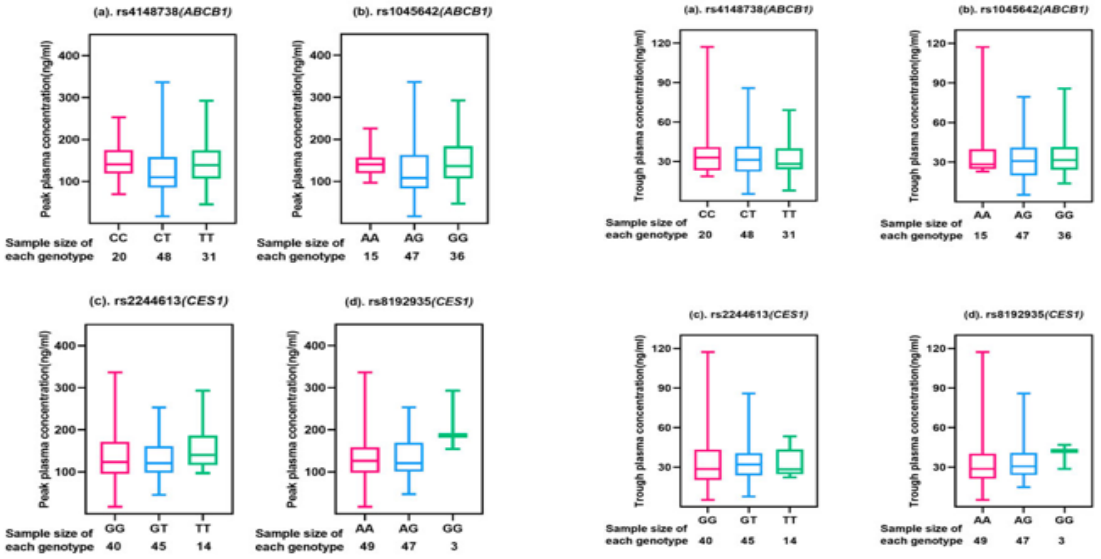
Table 3. Results from the non-compartmental analysis of dabigatran plasma concentrations.

Covariate / Group	N	Cmax (ng/mL)	Tmax (h)	AUC0–t (ng·h/mL)	AUC0–∞ (ng·h/mL)	t½ (h)
Food effect						
Fasting	61	159.56 ± 57.25	2.39 ± 0.60	1352.14 ± 454.75	1395.65 ± 466.86	10.07 ± 2.33
Postprandial	62	114.89 ± 36.96	4.44 ± 2.08	1049.14 ± 313.73	1071.37 ± 313.71	9.24 ± 1.32
Sex						
Male	94	136.33 ± 52.73	3.11 ± 1.09	1177.60 ± 435.79	1218.66 ± 446.37	9.79 ± 2.04
Female	29	139.36 ± 54.32	4.45 ± 3.07	1270.09 ± 347.61	1299.67 ± 361.98	9.19 ± 1.48
CES1 rs2244613	99					
GG	40	135.32 ± 62.60	3.63 ± 2.35	1187.89 ± 508.40	1216.80 ± 524.69	9.336 ± 1.90
GT	45	131.71 ± 49.90	3.55 ± 1.82	1176.47 ± 390.59	1216.03 ± 403.55	9.68 ± 1.79
TT	14	156.23 ± 52.73	2.76 ± 1.04	1286.87 ± 377.30	1322.27 ± 382.42	9.54 ± 1.20
CES1 rs8192935	99					
AA	49	134.43 ± 55.14	3.34 ± 2.06	1187.01 ± 464.69	1218.24 ± 478.55	9.59 ± 1.90
AG	47	134.20 ± 53.48	3.68 ± 1.93	1177.71 ± 403.16	1215.71 ± 414.47	9.50 ± 1.66
GG	3	211.03 ± 72.34	2.22 ± 0.69	1652.17 ± 382.85	1691.93 ± 389.58	9.17 ± 0.77
ABCB1 rs4148738	99					
CC	20	149.52 ± 45.69	3.40 ± 2.26	1312.07 ± 446.42	1321.88 ± 452.86	9.10 ± 1.46
CT	48	126.45 ± 58.26	3.73 ± 1.89	1145.77 ± 448.85	1192.18 ± 467.69	9.87 ± 2.15
TT	31	144.10 ± 56.54	3.11 ± 1.92	1201.09 ± 413.61	1236.92 ± 425.15	9.27 ± 1.01
ABCB1 rs1045642	98					
AA	15	145.85 ± 31.47	3.47 ± 2.50	1315.01 ± 374.43	1312.08 ± 372.97	9.22 ± 1.24
AG	47	126.51 ± 60.71	3.51 ± 1.54	1120.27 ± 466.94	1163.98 ± 480.75	9.63 ± 2.24
GG	36	145.82 ± 56.70	3.45 ± 2.30	1246.37 ± 421.31	1292.01 ± 438.79	9.54 ± 1.10

Cmax: maximum plasma concentration; Tmax: time to reach the maximum concentration; AUC0-t: area under the plasma concentration-time curve from 0 to the last measurable concentration; AUC0-∞: area under the concentration-time curve extrapolated to infinity; t1/2: half-life. All values are presented as means with standard deviation (SD).

Impact of genetic variants on peak and trough concentrations

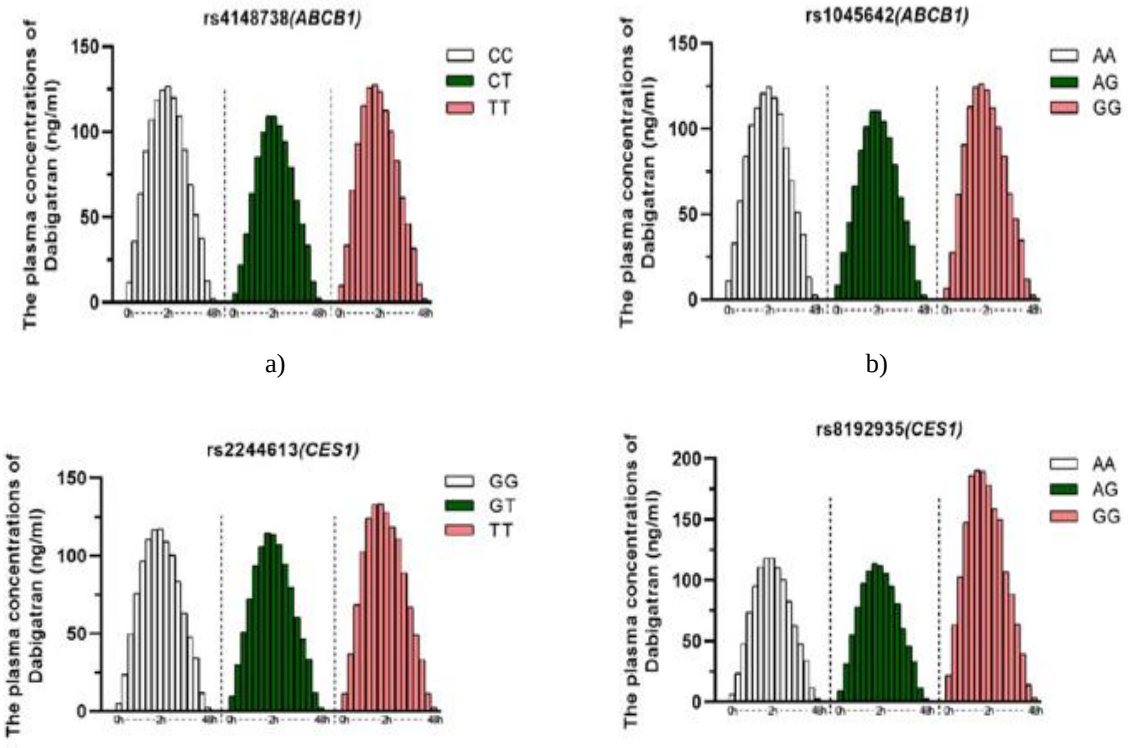
The study included 123 participants, with 99 individuals genotyped for ABCB1 (rs4148738, rs1045642) and CES1 (rs2244613, rs8192935) SNPs. Trough concentrations of dabigatran in plasma were defined as the concentration measured 12 hours after drug administration [22]. The genetic influences on both peak and trough plasma levels of dabigatran are depicted in **Figure 2**. Statistical analysis revealed no significant differences in peak plasma concentrations between males and females for any of the SNPs, but notable differences were observed in trough concentrations for the genotypes rs4148738 (ABCB1) TT, rs2244613 (CES1) GT, and rs8192935 (CES1) AT. Additionally, **Figure 3** provides histograms illustrating dabigatran plasma levels across different genetic groups.



a) b)

Figure 2. Distribution of peak and trough (a, b) plasma concentrations of dabigatran across different genotypes for four SNPs. The box-and-whisker plots show median values, interquartile ranges (IQR), and outliers.

(a) For ABCB1 SNP rs4148738: Genotypes CC (red), CT (blue), TT (green); Sample sizes: CC (20), CT (48), TT (31). (b) For ABCB1 SNP rs1045642: Genotypes AA (red), AG (blue), GG (green); Sample sizes: AA (15), AG (47), GG (36). (c) For CES1 SNP rs2244613: Genotypes GG (red), GT (blue), TT (green); Sample sizes: GG (40), GT (45), TT (14). (d) For CES1 SNP rs8192935: Genotypes AA (red), AG (blue), GG (green); Sample sizes: AA (49), AG (47), GG (3).



a) b)

c) d)

Figure 3. Plasma concentrations of dabigatran for different genetic subpopulations. The x-axis represents time after administration (0–48 hours), and the y-axis shows plasma concentration.

(a) For ABCB1 SNP rs4148738: Genotypes CC (white, 20), CT (green, 48), TT (red, 31); (b) For ABCB1

SNP rs1045642: Genotypes AA (white, 15), AG (green, 47), GG (red, 36); (c) For CES1 SNP rs2244613: Genotypes GG (white, 40), GT (green, 45), TT (red, 14); (d) For CES1 SNP rs8192935: Genotypes AA (white, 49), AG (green, 47), GG (red, 3).

In **Figure 2a**, there were no significant differences in the distribution of peak plasma concentrations among different genotypes, indicating little to no effect of these SNPs on the peak drug concentration. However, **Figure 2b** shows significant genotype-dependent variation in the median and range of dabigatran's trough concentrations. For rs4148738 (ABCB1), the CT genotype displayed a lower median trough concentration (around 10 ng/mL) with a narrow variability range. Similarly, in rs1045642 (ABCB1), the GG genotype had a low median trough concentration (~10 ng/mL) with limited variability. The rs2244613 (CES1) TT genotype also showed a low median concentration (~10 ng/mL), with most values clustered around the lower end, although the sample size was small. For rs8192935 (CES1), the AA genotype had a higher median concentration (~20 ng/mL) with a wide range, while the AG genotype had a slightly lower median and a smaller range of variability. The GG genotype (with only 3 samples) exhibited lower concentrations, concentrated around 10 ng/mL. Overall, SNPs in the ABCB1 gene had a more pronounced effect on the trough concentration of dabigatran compared to those in the CES1 gene.

Pharmacokinetic (PK) modeling

To determine the best model for dabigatran's pharmacokinetic profile, we compared one-compartment, two-compartment, and three-compartment models. The two-compartment model demonstrated a significantly lower OFV than the one-compartment model, indicating a better fit and more stable parameter estimates. However, the three-compartment model did not show a substantial improvement over the two-compartment model and was found to have unnecessary complexity. Consequently, a two-compartment model with a lag time for first-order absorption was chosen as the most appropriate. This model provided a superior fit for the observed data and aligned with findings from prior studies on dabigatran ester concentrations [10, 28]. A combined residual error model was applied to account for intra-individual variability.

Key pharmacokinetic parameters estimated by the final model are summarized in **Table 4**. The values for the primary parameters were as follows:

- Central compartment clearance (CL): 122 L/h
- Central volume of distribution (V2): 245 L
- Intercompartmental clearance (Q): 70.80 L/h
- Peripheral volume of distribution (V3): 756 L
- Absorption rate constant (Ka): 0.38 h^{-1}
- Lag time (ALAG): 0.48 h

Inter-individual variability (IIV) for CL, V2, and ALAG was 12%, 8%, and 10%, respectively. The model's residual variance was 6.31 ng/mL.

Table 4. Final model estimates and bootstrap results for pharmacokinetic parameters.

Parameter	Final Model Estimate (RSE %) [Shrinkage %]	95% CI of Estimate	Bootstrap Mean	Bootstrap 95% CI
Fixed effects				
Absorption rate constant, KA (h^{-1})	0.38 (16.2%)	0.26 – 0.50	0.34	0.28 – 0.40
Absorption lag time, ALAG (h)	0.48 (5%)	0.44 – 0.53	0.55	0.48 – 0.63
Apparent clearance, CL/F (L/h)	122 (5.6%)	108.61 – 135.39	125	115.23 – 144.98
Central volume of distribution, V_2 (L)	245 (14.1%)	177.18 – 312.82	240	195.44 – 276.26
Inter-compartmental clearance, Q (L/h)	70.80 (7.9%)	59.80 – 81.80	65	55.31 – 73.82
Peripheral volume of distribution, V_3 (L)	756 (10.6%)	599.20 – 912.80	789	704.23 – 954.93
Effect of postprandial state on KA (fold change)	−0.24 (30%)	−0.39 – −0.10	−0.18	−0.27 – −0.03

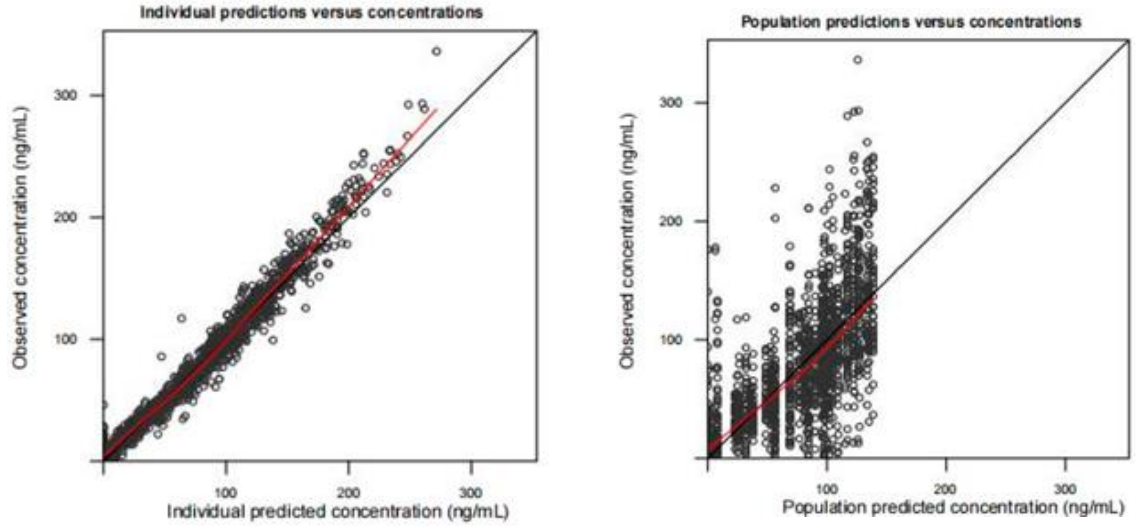
Effect of postprandial state on ALAG (fold change)	2.65 (8.7%)	2.20 – 3.10	2.57	2.06 – 2.79
Effect of postprandial state on CL/F (fold change)	0.51 (25.5%)	0.26 – 0.77	0.39	0.09 – 0.51
Effect of ABCB1 rs4148738 CT vs CC on V_2 (fold change)	0.38 (45.5%)	0.04 – 0.71	0.33	0.04 – 0.55
Inter-individual variability (ω^2, CV%)				
IIV on CL/F	0.31 (12%) [7.2%]	−0.18 – 0.79	0.27	0.15 – 0.39
IIV on V_2	0.27 (8%) [0.1%]	−0.04 – 0.58	0.34	0.26 – 0.55
IIV on ALAG	0.19 (10%) [14.6%]	−0.19 – 0.57	0.15	0.08 – 0.19
Residual variability				
Proportional error (SD)	0.08 (24.9%)	0.04 – 0.11	0.08	0.06 – 0.10
Additive error (SD, ng/mL)	6.31 (14.7%)	4.50 – 8.12	6.63	5.40 – 8.08
Additive error for low concentrations	1 (fixed) [2.4%]	—	1 (fixed)	—

RSE: Relative Standard Error; Shr: Shrinkage (%); Ka: Absorption rate constant; ALAG: Lag time; CL: Central compartment clearance; V2: Central compartment volume; Q: Intercompartmental clearance; V3: Peripheral volume.

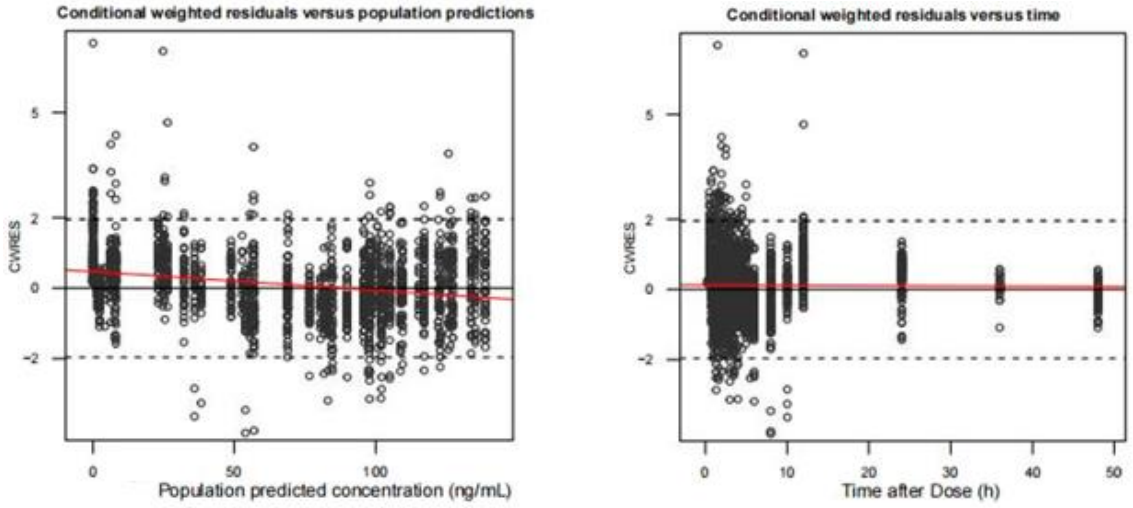
Covariate analysis and model validation

Upon examining covariates, food intake was found to significantly affect ALAG, CL, and Ka. The CT genotype of ABCB1 SNP rs4148738 also influenced the central compartment's apparent volume of distribution (V2). The shrinkage of random effects (η) between individuals was found to be under 15%, while the bootstrap analysis showed a 99.9% minimization success rate. The median and 95% confidence intervals (CIs) from the bootstrap closely mirrored the parameter estimates of the final model, further validating its robustness.

Figure 4 shows the model's fit, where the goodness-of-fit plot reveals a strong relationship between population predictions (PRED) and individual predictions (IPRED) compared to observed data. The points are dispersed around the diagonal line, with the PRED trend line aligning almost perfectly with it, indicating excellent model fit. The visual predictive check (VPC) in **Figure 5** further supports the model's predictive performance, as the 95% CI for predicted values encompasses the majority of observed data.



a) b)



c) d)

Figure 4. Goodness-of-fit analysis for the final population PK model.
(a) Individual predicted values vs. observed values. (b) Population predicted values vs. observed values. (c) Conditioned weighted residuals (CWRES) vs. population predictions. (d) Time vs. conditioned weighted residuals.

The black solid line represents the reference line, and the red solid line shows the trend line. Residuals (CWRES) are ideally distributed within ±2 to indicate good accuracy.

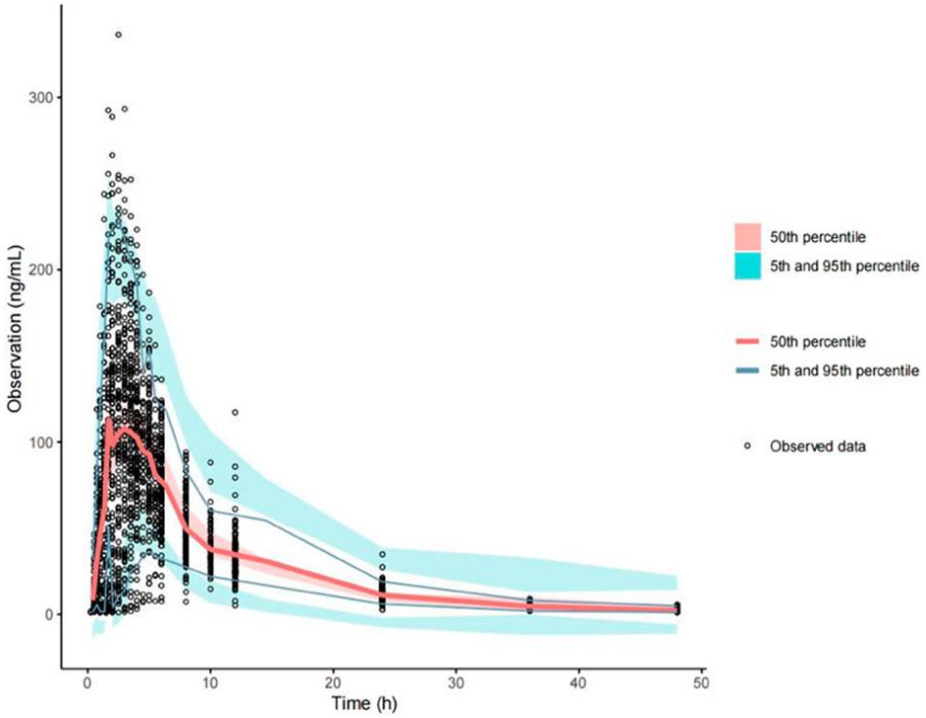


Figure 5. Visual Predictive Check (VPC) of the final model.

Hollow circles represent observed concentrations, and solid lines represent the 5th, 50th, and 95th percentiles based on model simulations. Red shading shows the 95% confidence interval for the median predicted by the model, while the light blue area indicates the 95% confidence intervals for the 5th and 95th percentiles.
NONMEM is a parametric population pharmacokinetic software that improves parameter estimation and modeling across diverse subgroups, aiding in the accurate understanding of their pharmacokinetic behaviors. This tool also provides insights into how drugs behave in the body and the role genotypes play in modifying pharmacokinetic parameters. The current research is the first to create a PopPK model using an extensive dataset

of dabigatran pharmacokinetics to investigate the relationship between specific genetic variations and the metabolism of dabigatran esters in healthy subjects. Our study utilizes demographic information, pharmacokinetic data, and genotype findings from healthy individuals for PopPK analysis.

Previous studies have identified various factors that influence dabigatran plasma levels, such as renal function, gender, BMI, age, comorbidities, and genetic variants. Notably, certain SNPs in the ABCB1 (rs1045642, rs4148738) and CES1 (rs2244613, rs8192935) genes have a significant effect on dabigatran's pharmacokinetics [29, 30]. In this investigation, the effects of renal function and concomitant medications were excluded by focusing on healthy subjects. The age range (18–43 years) and BMI (19.2–25.9 kg/m²) were well-suited for the study design. However, the limited number of female participants restricted a full assessment of gender-based pharmacokinetic differences. As a result, the final model incorporated variables such as food intake and one ABCB1 genotype. The pharmacokinetic predictions from the model aligned closely with observed data, confirming its reliability in representing dabigatran's PopPK in vivo.

In line with previous studies, analysis of blood concentration-time curves and non-compartmental modeling revealed that the mean AUC in females was about 8% higher than in males [31, 32]. Additionally, the time to peak concentration after taking the drug post-meal was delayed by around 2 hours compared to fasting, which mirrors results from other research [33]. While food intake did not significantly alter the extent of dabigatran absorption [33], our data showed that fasting AUC was approximately 28.86% higher than postprandial AUC, with high-fat meals potentially altering the AUC as well. Moreover, the final model indicated that postprandial administration led to a 2.65% and 0.51% increase in ALAG and CL, respectively, and a 0.24% decrease in KA, suggesting that food, especially high-fat meals, can slow down or diminish absorption, while the drug's elimination is less influenced. These findings are consistent with animal studies [34]. It's also been proposed that taking dabigatran on an empty stomach might heighten digestive side effects, potentially compromising its effectiveness [35]. For patients on dabigatran, it’s advised to follow a consistent eating pattern in line with the Cardiovascular Disease Prevention Guidelines [35]. Given the potential for interactions between dabigatran and food, special attention should be given to older adults, those with bleeding risks, individuals with renal impairment, and those on complex medications [36].

This study genotyped 99 healthy Chinese participants, with genotype frequencies similar to those found in previous research [19]. Our non-compartmental analysis revealed that individuals with the GG genotype of the CES1 SNP rs8192935 exhibited significantly higher mean Cmax and AUC values compared to those with AA or AG genotypes. This suggests that the GG allele may influence dabigatran plasma concentrations. This observation aligns with studies conducted on 202 Chinese individuals [13] and 92 Caucasians [8]. In the RE-LY study, the CES1 SNP rs2244613 was linked to increased bleeding risk and trough drug concentrations [7]. However, a study involving 60 Caucasian patients with venous thromboembolism found no impact of the ABCB1 SNPs (rs1045642 and rs4148738) or the CES1 SNP rs2244613 on dabigatran concentrations [37]. In contrast, our research showed that carriers of the TT genotype for CES1 SNP rs2244613 had higher Cmax and AUC values than those with GG and GT genotypes. No significant effect was observed from the ABCB1 SNPs on Cmax and AUC.

When considering the genetic polymorphisms on plasma dabigatran concentrations, we found no significant differences in peak concentrations across genotypes. However, variations within the ABCB1 gene appeared to affect trough concentrations more significantly than the CES1 gene. The final model did not show a significant effect of the CES1 polymorphism on dabigatran concentrations, which supports previous findings [14]. Despite this, CES1 polymorphisms may have a different impact on plasma dabigatran levels in the Chinese population, but due to the limited sample size, we could not draw definitive conclusions. The small sample size, especially with rare genotypes (e.g., only three subjects with the GG genotype of rs8192935), may affect the reliability of the data.

Our model also indicated a 0.38% increase in the central volume of distribution (V2) in individuals with the CT genotype of ABCB1 SNP rs4148738. It is known that genetic variations in ABCB1 affect the systemic concentration of dabigatran [11, 38]. Studies have shown that the SNP rs4148738 in the ABCB1 gene is associated with elevated peak plasma levels of dabigatran [7, 11, 37].

The limited number of studies on CES1 polymorphisms in the Chinese population and the relatively small sample sizes highlight the need for further research on how these genetic variations impact dabigatran plasma concentrations. Larger studies are required to validate these findings and explore their implications for dabigatran exposure. In clinical practice, patients on dabigatran who experience adverse reactions or ineffective therapeutic outcomes despite standard factors (age, gender, renal and liver function, concurrent medications) may benefit

from investigating genetic factors. This research could serve as a foundation for future investigations into the relationship between genetic polymorphisms and dabigatran pharmacokinetics.

Conclusion

This study assessed the population pharmacokinetics of dabigatran in healthy subjects, considering different genotypes and dietary conditions. The final pharmacokinetic model successfully captured the drug's behavior in vivo. Our findings showed that dietary factors influenced the absorption and elimination of dabigatran, while the ABCB1 SNP rs4148738 impacted its distribution. Therefore, identifying genotypes and adjusting dietary habits may improve the safety and therapeutic outcomes of dabigatran, especially in patients at higher bleeding risk. This study provides a basis for further exploration of the relationship between genetic variations, dietary factors, and dabigatran exposure in vivo.

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