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Pharmacogenomic Profiling of ABCB1 and CES1 in Atrial Fibrillation Patients on Dabigatran from a Multiethnic Malaysian Cohort

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Abstract

Direct oral anticoagulants (DOACs) are preferred for atrial fibrillation (AF) due to their efficacy and safety, though response variabilities raises concerns about fixed-dose regimens. This study investigates the association between *ABCB1* and *CES1* genetic polymorphisms with Dabigatran trough drug levels (DL), clotting time (CT), and clinical outcomes in a multiethnic Malaysian cohort. A total of 180 AF patients in Dabigatran were sequenced across the entire length of *ABCB1* and *CES1* genes using the Illumina MiSeq platform. Trough Dabigatran levels and clotting time were measured by LC-MS/MS and viscoelastic assay (Clotpro®), respectively. Patients were followed up for one year to assess major adverse cardiovascular and cerebrovascular events (MACCE). The mean dabigatran level was 34.7 ± 45.4 ng/ml (CV: 130%), and clotting time was 374.6 ± 207.9 s (CV: 55.5%). Trough levels were significantly correlated with clotting time ($r = 0.663$, $p < 0.001$).). Multiple non-coding variants in ABCB1 and CES1 showed nominal associations (unadjusted $p < 0.05$) with drug level (35 SNPs) and/or clotting time (32 SNPs), although none remained statistically significant after false discovery rate correction. A total of 17 SNPs overlapped, and were associated with both. Overall, these findings suggest that non-coding regulatory variation may contribute to inter-individual variability in dabigatran pharmacokinetics and pharmacodynamics, but the results are exploratory and require replication in larger cohorts. This study highlights the importance of population-specific pharmacogenomic research for future investigations on personalized anticoagulation strategies.

Keywords: *ABCB1*, *CES1*, genetic polymorphisms, direct oral anticoagulants, dabigatran

Introduction

Atrial fibrillation (AF) is highly prevalent globally, with an estimated lifetime risk of 1 in 3 to 5 individuals after the age of 45 (1). In Malaysia, the prevalence of AF among hospitalized patients is 2.8% (2). Oral anticoagulation (OAC) therapy, either with vitamin K antagonist or direct oral anticoagulants (DOACs), has been shown to significantly reduce stroke risk by approximately two-thirds. With the introduction of generic DOACs, these medications are expected to become more widely prescribed, replacing warfarin, which was previously the cornerstone therapy in Malaysia due to cost consideration. Additionally, the East-Asian Paradox suggested that patients from Asian countries experienced lower rates of all-cause mortality and bleeding compared to those from other regions, alongside differences in treatment approaches. Furthermore, DOACs have been more effective in reducing mortality and stroke rates among Asian patients (3).

Despite the use of fixed-dose regimens, considerable inter-individual variability in DOAC pharmacokinetics (PK), pharmacodynamics (PD), and clinical outcomes has been reported
(4). According to the 2021 European Heart Rhythm guideline, no studies have yet determined whether adjusting DOAC dosages based on drug levels and coagulation tests can improve long-term outcomes. Therefore, routine monitoring and dosage adjustments based on plasma levels are not generally recommended. However, laboratory assessment may be useful in selected clinical scenarios, such as bleeding, urgent procedures, extreme body weight, or severe renal impairment (5). The reasons of inter-individual variability remain incompletely understood and may, in part, be attributable to genetic factors(6).

In Malaysia, Dabigatran, Apixaban, Rivaroxaban, and Edoxaban are included in the Ministry of Health Formulary and are available for prescription to eligible patients. At the Sarawak Heart Centre, all direct oral anticoagulants (DOACs) are subjected to quota-based distribution. Although DOAC utilization has increased in recent years, Warfarin remained the primary anticoagulant at the time this study was initiated, largely due to its lower cost. Among the available DOACs, Dabigatran was more frequently prescribed at our center due to relatively greater availability. After being absorbed, Dabigatran etexilate is rapidly converted into the active form of dabigatran (the main metabolite of the drug) via liver enzymes such as carboxylesterases (CEs), encoded by *CES1* gene. Genetic variants of *CES1* gene were found to regulate the pharmacokinetics of dabigatran, therefore, its plasma concentrations. All DOACs are substrates of the *ABCB1* transporter, also known as P-glycoprotein, which functions as an active efflux pump. One key interaction mechanism for DOACs is their gastrointestinal resecretion over a P-glycoprotein (P-gp) after Dabigatran etexilate is absorbed in the gut. Strong inducers of P-gp could reduce the bioavailability of DOACs, thus lowering the plasma concentrations, potentially decreasing and increasing the bleeding and thrombotic risks. Conversely, strong inhibitors of P-gp can increase the bioavailability of Dabigatran(7).

Genetic polymorphisms in the drug transporter *ABCB1* gene, and drug metabolism *CES1* gene had been shown to affect Dabigatran concentrations (6, 8-10). Studies by Sychev et al. (11) and Pare et al. (8) had demonstrated that genetic polymorphisms in *ABCB1* were associated with both increased and decreased Dabigatran plasma concentrations (8, 11). The effect of both genes on Dabigatran has been demonstrated in landmark trials, primarily conducted in populations of European and North American origin(8), with some research also involving Chinese populations(9, 10). Although past pharmacogenomics research on DOACs primarily focused on the *ABCB1* and *CES1* genes, recent studies have started to investigate other genomic loci via whole exome sequencing (9). While emerging research has begun to explore additional genomic loci using broader sequencing approaches, pharmacogenomic data on DOACs in Malaysia remain scarce. Moreover, evidence linking genetic variants to pharmacodynamic measures or clinical outcomes, such as bleeding or thromboembolic events, remains limited(6).

Given the increasing use of DOACs in Malaysia, partly driven by the availability of generic formulations following the expiration of original patents, there is a growing need to better understand the factors contributing to inter-individual variability in DOACs response. Although fixed-dose regimens are currently the standard of care, considerable variability in Dabigatran plasma concentrations and anticoagulant effects has been observed, which may affect both therapeutic efficacy and bleeding risk. Genetic polymorphisms of transport and drug metabolism genes, particularly *ABCB1 and CES1*, have been implicated in influencing Dabigatran pharmacokinetics and pharmacodynamics. However, the relevance of these variants in the Malaysian population remains unclear. Given Malaysia's multiethnic population, the frequency and functional implications of genetic variants may differ substantially from those reported in other regions. Therefore, investigating the pharmacogenetic determinants of Dabigatran response in this context is essential to moving toward more personalized anticoagulation strategies.

This study aims to explore the relationship between genetic polymorphisms in *ABCB1* and *CES1*, Dabigatran trough drug level (DL), clotting time (CT), and 1-year major adverse cardiovascular events (MACCE) in a multiethnic Malaysian population. Additionally, this study also aims to determine the genetic diversity of *ABCB1* and *CES1* in the target population; examine the association between the genetic polymorphisms with trough drug level and clotting time; and investigate the association between clotting time and trough drug levels.

Materials and Methods:

This is an investigator initiated, observational, pragmatic study conducted jointly by multidisciplinary team of doctors, pharmacists, nurses, and research assistants. All patients with non-valvular atrial fibrillation (NVAF) at the Sarawak Heart Centre prescribed with Dabigatran were screened for inclusion in the study. Informed consent was obtained from all subjects. Patients who had at least 3 days of Dabigatran therapy, 150mg twice daily or 110mg twice daily were included in the study. The inclusion criteria were as follows: a) adults aged ≥18 years old ; b) patients with NVAF on Dabigatran therapy; c) patients who could provide informed consent. The exclusion criteria included: a) patients with valvular AF on DOACs therapy; b) those with moderate to severe mitral stenosis; c) patients who are unable to provide informed consent. Clinical data were recorded using structured case report forms, which included demographic information, relevant clinical characteristics, and thrombotic and bleeding risk scores, including CHA_2DS_2-VASc and HAS-BLED.

Approximately 12mls of venous blood was obtained via venipuncture at least ten hours after the preceding dose and before the next due dose of Dabigatran (for trough sampling). A total of 6mls (3ml × 2) of blood were taken in two citrate tubes. Whole blood in the first citrate tube was used for clotting time analysis with Clotpro®, while the second citrate tube was centrifuged at 3000 rpm for 10 minutes at 4°C. The plasma was then aliquoted into two labelled cryovials (one for storage, one for drug concentration analyses via LCMS-MS/MS). Another 6mls (3ml × 2) of blood was collected in two EDTA tubes. Following centrifugation using the same parameters as above, plasma and buffy coats were extracted. This study was conducted in accordance to the Declaration of Helsinki and it was approved by the Malaysian Ministry of Health Medical Research and Ethics Committee (NMRR-21-31-58059).

Follow up and Study End-points

Subjects were followed up for one month, sixth months, and one year through either telephone interviews, or outpatient clinic assessments. The telephone interview took approximately 5-10 minutes. Patients' compliance, major adverse cardiovascular and cerebrovascular events (MACCE) and bleeding outcomes were assessed. MACCE was defined as composite of all-cause death, myocardial infarction, stroke, repeat revascularisation, readmissions of arrythmias, heart failure. All deaths were considered cardiovascular unless a clear non-cardiovascular cause was demonstrated. The cause of death was determined by the treating clinicians based on available clinical documentation. In cases of sudden death occurring outside the hospital (e.g., death at home) without an identifiable non-cardiovascular cause, the event was classified as cardiovascular death(12).

The primary safety primary safety endpoint was major or minor bleeding, defined according to the Thrombolysis in Myocardial Infarction (TIMI) criteria (TIMI major and TIMI minor bleeding).(13)

Anti thrombin-Activity (clotting time) (pharmacodynamic assessment)

Clotting time was assessed through point-of-care instrument, Clotpro®, by Dynabyte Germany. Clotpro® is a viscoelastometry analyzer which analyses the clotting time (CT), ie. the duration of the clotting of the blood. Prolonged clotting time was associated with reduced thrombin activity. The reagent was Ecarin test (ECA test). Ecarin, a thrombin activator, derived from venom of the saw-scaled viper, activates prothrombin into thrombin from the blood sample. The Dabigatran in the blood sample inhibited the thrombin, therefore causing the blood to clot in longer duration, thus increasing clotting time. Whole blood collected in citrate tube was used for this assessment. The time from blood insertion to test completion was approximately one hour.

Trough Drug Level (pharmacokinetic assessment)

Plasma concentration of Dabigatran of 180 subjects were assessed through the gold standard, triple quadrupole Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) method. The instrument used was Agilent 1290 Infinity Liquid Chromatography system coupled with Agilent 6490 Triple Quadrupole LC/MS system (Agilent Technologies, USA). Dabigatran was extracted from plasma using protein precipitation method. The analytical column used for separation was Poroshell 120EC-C18, 2.7µm (2.1 x 50 mm) column, with an attached UHPLC Guard 3PK column, 2 µm (Agilent Technologies, USA). The internal standard and dabigatran were eluted under gradient conditions using a flow rate of 0.35 ml/min. Standard curve samples were prepared by spiking the appropriate known working solution with internal standard into the plasma. At certain concentrations, known concentration of internal standard was spiked together with Dabigatran into the plasma to create internal standard curves for quality control purposes. After validation, this method was used to quantitate Dabigatran plasma concentrations.

Next Generation Sequencing

The DNA was extracted from the buffy coat collected in EDTA tube by using Qiacube® as per QIAamp DNA Blood Mini DNA Extraction Protocol. Purity and concentration were checked with nanophotometer Spectrophotometer (IMPLEN, CA, United States), and concentration checked with Fluorometer, DeNovix dsDNA High Sensitivity Kit 1000 Assays. Libraries were prepared using a custom panel targeting whole *ABCB1* (7q21.12) and *CES1* (16q12.2) genes, using the AmpliSeq Library PLUS kit (Illumina, 96 reactions) following Agilent's standard protocol and subsequently sequenced using the Illumina MiSeq® platform with the MiSeq Reagent Kit v2 (300 cycles), generating paired-end reads of 2 × 150 bp, with Illumina PhiX Control v3 included as an internal sequencing control. The raw fastq files underwent quality filtering and adapter trimming using fastp (14), followed by mapping to the human reference genome hg19 using bwa (15). The average sequencing depth over the two genes was 15.5x. The resulting bam files were used for variant calling using GATK's best practices for germline short variant discovery (16), resulting in 3,393 initial variants. Variants were further filtered to remove indels, multiallelic variants, and those with poor quality (QUAL <30, Read depth DP <10, Mapping Quality MQ <40). The final vcf file containing 2,564 SNPs was converted to plink format (17) and further filtered to remove variants with <95% genotyping rate and <1% Minor Allele Frequency (MAF). The final dataset for downstream analysis consists of 436 and 83 variants for *ABCB1* and *CES1*, respectively. Variants were annotated using ANNOVAR with RefGene, ClinVar, and SIFT databases for predicting the gene structure, clinical significance, and functional impacts, respectively(18).

Statistical analysis

Statistical analyses were performed with SPSS Version 16 and R software version 4.4.2 (R Core Team, 2024) via RStudio 2024.04.2+764. Descriptive statistics includes percentage, mean with standard deviation, or median with interquartile range was reported, whichever appropriate. Continuous variables will be analysed using independent t-test (two variables only), or Mann-Whitney test (two variables only) depending on the nature of underlying distributions. Multiple linear regression of Dabigatran trough levels and clotting time was performed in PLINK 1.09, assuming an additive contribution of each minor allele.

Q–Q plots (Supplementary Figure 2) were constructed for Dabigatran plasma concentrations and clotting times. Both variables were log-transformed to approximate a normal distribution due to initial non-normality. Log-transformed Dabigatran concentrations and clotting times were analyzed using linear regression, adjusted for age, creatinine level, Dabigatran dose, gender, and the first two principal components (PC1 and PC2). These variables were chosen because age and gender were found to associated with Dabigatran drug level and clotting time in univariate analyses (except creatinine and dose). Considering the sample size of 180 patients, and this was not a whole genome sequencing, but targeted gene association analysis, we adopted a nominal significance threshold of $p < 0.05$ to identify exploratory suggestive single nucleotide polymorphisms (SNPs). To account for multiple testing across all analysed single nucleotide polymorphisms (SNPs), p-values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) method. Associations that remained significant after FDR correction were considered statistically significant. In addition, nominal associations (unadjusted $p < 0.05$) are reported as exploratory and hypothesis-generating findings.

SNPs demonstrating nominal associations ($p<0.05$) with pharmacokinetic or pharmacodynamic outcomes were subsequently evaluated in cross tabulation analyses to explore potential associations with clinical outcomes, including major adverse cardiovascular and cerebrovascular events (MACCE) and bleeding events. Given the limited number of clinical events, these analyses were considered exploratory. All statistical tests were two-sided.

Independent samples t-tests were used to compare drug levels and clotting times between genotype groups of each SNP. Equality of variances was assessed using Levene's test. When variances were equal (Levene's $p \geq 0.05$), Student's t-test was applied. When variances were unequal (Levene's $p < 0.05$), Welch's t-test was used. All tests were two-sided. Boxplots illustrating the distribution of dabigatran trough levels and clotting time across genotype groups for all nominally associated SNPs are provided in the Supplementary Material Figure 3 and Figure 4.

Results:

Baseline patients' characteristics

Out of the 384 patients recruited, the first 200 who provided consent for genetic testing were consecutively selected for sequencing, of which 180 passed quality control filtering and were included in final analysis (Table 1). The mean age was 66.7±9.3 years old and the majority (65.6%) were males. The Mean CHA_2DS_2-VASc and HASBLED score were 3.9±1.7 and 1.2 ±0.8 respectively. Chinese made up approximately half of the samples (48.9%), followed by Malay (21.7%), Bidayuh (16.1%), Iban (10.0%), and other ethnic minorities (3.3%). The mean Dabigatran trough drug level was 34.74±45.40ng/ml (Range: <LLOQ-284.60 ng/ml), Coefficient of variation (CV), 130%. The mean clotting time was 374.63±207.89s (Range: 77-1216s), CV: 55.5%. At one year, there was one stroke event, 0.6%. Overall, the 1-year MACCE was 11.6% and bleeding events, 2.2%.

More patients were prescribed with Dabigatran 150mg BD (58.9%) compared to 110mg BD (41.1%). In the group of lower daily doses (110mg BD), the subjects were older, more females, higher CHA_2DS_2-VASc Score and HAS-BLED scores, and lower creatinine clearance. However, mean trough drug levels and clotting times did not differ significantly between the two dosing groups (35.0±43.8 vs 34.5±46.7 ng/mL, p=0.940; 376.8±211.8 vs 373.1±206.1 s, p=0.909). Both trough drug level and clotting time are significantly inversely correlated with plasma patients' creatinine clearance (r=-0.183, p=0.026 and r=-2.777, p<0.001)

Age was inversely correlated with trough drug level (r=0.196, p=0.008) and clotting time (r-0.248, p<0.001). There was no difference in trough drug level and clotting time among the ethnic groups.

When divided into groups of <65 years old or more or equal to 65 years old, the older group had higher plasma levels (41.15±50.17 vs. 18.95±24.70, p<0.001) and CT (400.80±1218.92 vs. 310.21±162.46,p=0.001). Female subjects had higher plasma levels (48.22±51.81 vs. 27.65±40.09, p=0.004) and clotting times (456.53±277.57 vs. 331.60±143.28, p<0.001) compared to their male counterparts.

Trough DL is significantly correlated with clotting time (r=0.663, p<0.001). Figure 1 shows the scatter plot of log CT vs. log DL. Below Left Lower Quadrant (BLQG) group was defined as, when divided into quartiles, both the DL and CT were less than first quartile of the variables respectively which were DL</=9 ng/ml, and CT </= 239seconds. There was a total of 16.1% subjects in the BLQG group. The Kaplan-Meir Analyses on BLQG group vs. stroke had demonstrated a log rank test of p=0.023. There was one 1-year stroke in BLQG group, and no stroke cases in non-BLOG group (3.4% vs 0%, p=0.163). The MACCE in BLOG vs non-BLOG group was 17.2% vs. 10.7%, p=0.346.

ABCB1 and _CES1_ genetic association with Dabigatran Trough Concentrations and Clotting Time

In the 180 samples sequenced, a total of 2654 variants were detected. After removing SNPs with Minor Allele Frequency (MAF) of less than 1%, and missing genotypes of more than 5%, a total of 519 SNPs (436 from *ABCB1* gene and 83 from *CES1* genes) were used for linear regression analysis using PLINK. A Principal Component Analysis (PCA) plot was generated after Linkage Disequilibrium (LD) pruning in which 635 of the 2654 variants were removed. No obvious sample clustering based on ethnicity was observed (Supplementary Figure 1).

Multiple linear regression analysis (adjusted for age, dose, gender, creatinine clearance, PC1, PC2) was performed in order to determine the association between the 519 SNPs and DL and CT values. After false discovery rate (FDR) correction, none of the associations remained statistically significant. Therefore, results are presented as nominal associations (unadjusted p < 0.05) and was interpreted as exploratory. Based on nominal suggestive associations (p<0.05), 35 SNPs were associated with dabigatran trough concentrations and 32 SNPs were associated with clotting time (Tables 2 and 3; Figure 2). Due to the small number of subjects that were homozygous for the minor alleles, we pooled the homozygous and heterozygous individuals with one or two minor alleles together, and reported the absolute values of Dabigatran drug level and clotting time. A total of 19 SNPs from *ABCB1* locus while 16 from *CES1* locus were associated with DL while a total of 18 *ABCB1* and 14 *CES1* SNPs were associated with CT (Figure 2)

***ABCB1* and *CES1* SNPs association with Clinical Outcomes**

A total of 50 unique SNPs were analysed after combining SNPs associated with clinical outcomes, with overlapping variants removed to avoid duplicate reporting (Supplementary material: Each SNPs genotype vs. clinical outcome) For categorical outcomes, associations between genotype groups and clinical events were assessed using Fisher's exact test when expected cell counts were <5. Otherwise, Pearson's chi-square test without continuity correction was applied. Two SNPs (chr7:87179955 and chr7:87147884) had nominal associations with MACCE or stroke. An exploratory univariate Cox proportional hazards analysis was additionally performed for these SNPs. For chr7:87147884, a nominal association with 1-year stroke was observed using Fisher's exact test ($p = 0.0237$). However, Cox regression produced unstable estimates with wide confidence intervals due to the very small number of events. In contrast, chr7:87179955 showed a nominal association with 1-year MACCE, and exploratory Cox analysis demonstrated an increased hazard (HR 9.9, 95% CI 3.3–30.1; $p < 0.001$) for the wildtype. Given the limited number of clinical events, these findings was regarded as exploratory.

Table 1: Demographic Table of Patients with Atrial Fibrillation Prescribed with Dabigatran. Percentages are in parentheses.

	Total n = 180	Dabigatran 110mg BD n=74 (41.1)	Dabigatran 150mg BD n=106 (58.9)	P value
Age; years old	66.7±9.3	73.1±9.4	65.6 ±7.9	<0.001
Gender (male); n(%)	118 (65.6)	41 (55.4)	77 (72.6)	0.017
Risk Factors; n(%)				
Hypertension	157 (87.2)	66 (89.2)	91 (85.8)	0.509
Congestive Heart Failure	61 (33.7)	22 (29.7)	39 (36.8)	0.325
Diabetes Mellitus	73 (40.3)	30 (40.5)	43 (40.6)	0.997
Family history of AF	20 (11.1)	8 (10.8)	12 (11.3)	0.915
Smoking				
Former or current smoker	44 (24.9)	16 (21.6)	28 (26.4)	0.509
None	136 (75.1)	58 (78.4)	78 (73.6)	
CHA_2DS_2-VASc Score	3.9±1.7	4.4±1.5	3.4±1.5	P<0.001
HAS-BLED	1.2 ±0.8	1.3±0.8	0.9±0.8	0.001
Race; n(%)				
Malay	39 (21.7)	18 (24.3)	21 (19.8)	0.110
Chinese	88 (48.9)	41 (55.4)	47 (44.3)	
Iban	18 (10.0)	3 (4.1)	15 (14.2)	
Bidayuh	29 (16.1)	11 (14.9)	18 (17.0)	
Others	6 (3.3)	1 (1.4)	5 (4.7)	
Trough Drug Level(ng/ml)	34.74±45.40 (Range: 0-284.60)	35.0±43.8	34.5±46.7	0.940
Clotting Time(s)	374.63±207.89 (Range: 77-1216)	376.8±211.8	373.1±206.1	0.909
Creatinine Clearance (ml/min)	62.56±23.11	50.46±15.53	71.05±23.83	0.025
Event at 1-year; n(%)				
Ischemic Stroke	1 (0.6)	1 (1.4)	0 (0)	0.404
All cause mortality	9 (5.0)	5 (7.0)	4 (3.8)	0.488
Cardiac related mortality	8 (4.4)	4 (5.6)	4 (3.8)	0.716
Arrythmia	4 (2.2)	1 (1.4)	3 (2.8)	0.648

Congestive Heart Failure	12 (6.6)	5 (6.9)	7 (6.6)	1.000
Acute Coronary Syndrome	1 (0.6)	0 (0)	1 (0.9)	1.000
Total MACCE	21 (11.6)	10 (13.9)	11 (10.4)	0.487
Bleeding	4 (2.2)	2 (2.8)	2 (1.9)	1.000

Figure 1: Scatter Plot of Log-Clotting Time vs. Log-Plasma Concentration

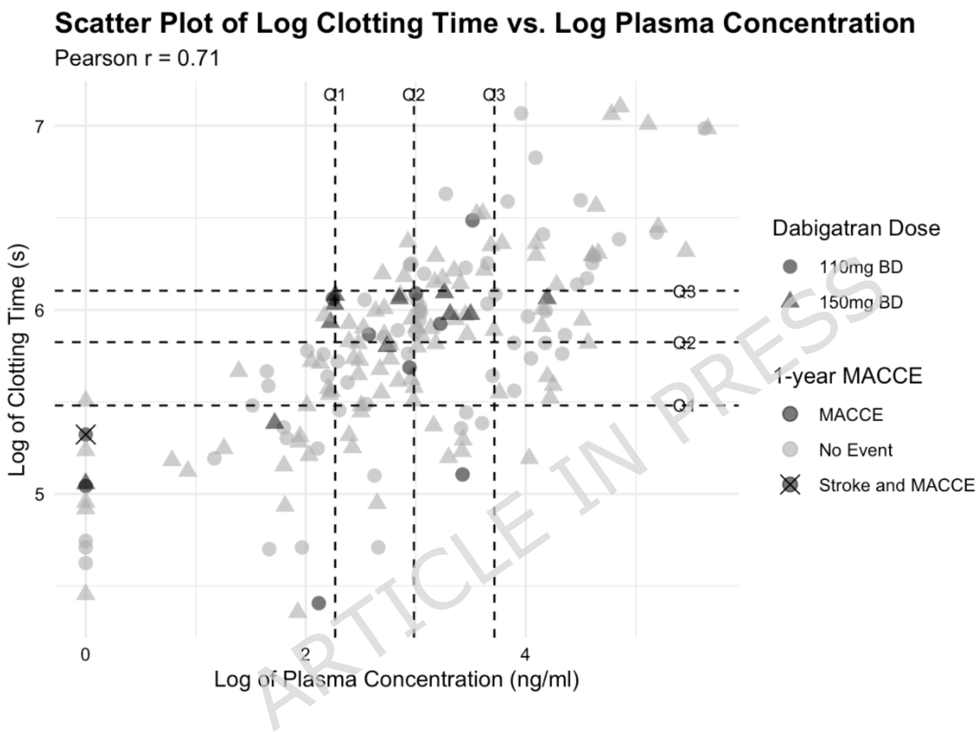

Figure 2: Gene-focused Manhattan plots displaying −$\log_{10}$(p) values of variants across *ABCB1* and *CES1*. The dashed line denotes the nominal significance threshold (p = 0.05). The five variants with the smallest p-values within each gene are labelled for visual reference.(a)Trough Drug Level and (b) Clotting Time.

(a)


ABCB1
CES1
−log10(p)
7:87179955
7:87188337
rs369135400
rs372195275
7:87147884
rs76059198
rs57764933
16:55852381
rs369616432
rs3893204
87,150,000
87,200,000
87,250,000
87,300,000
87,350,000
55,850,000
55,860,000

(b)

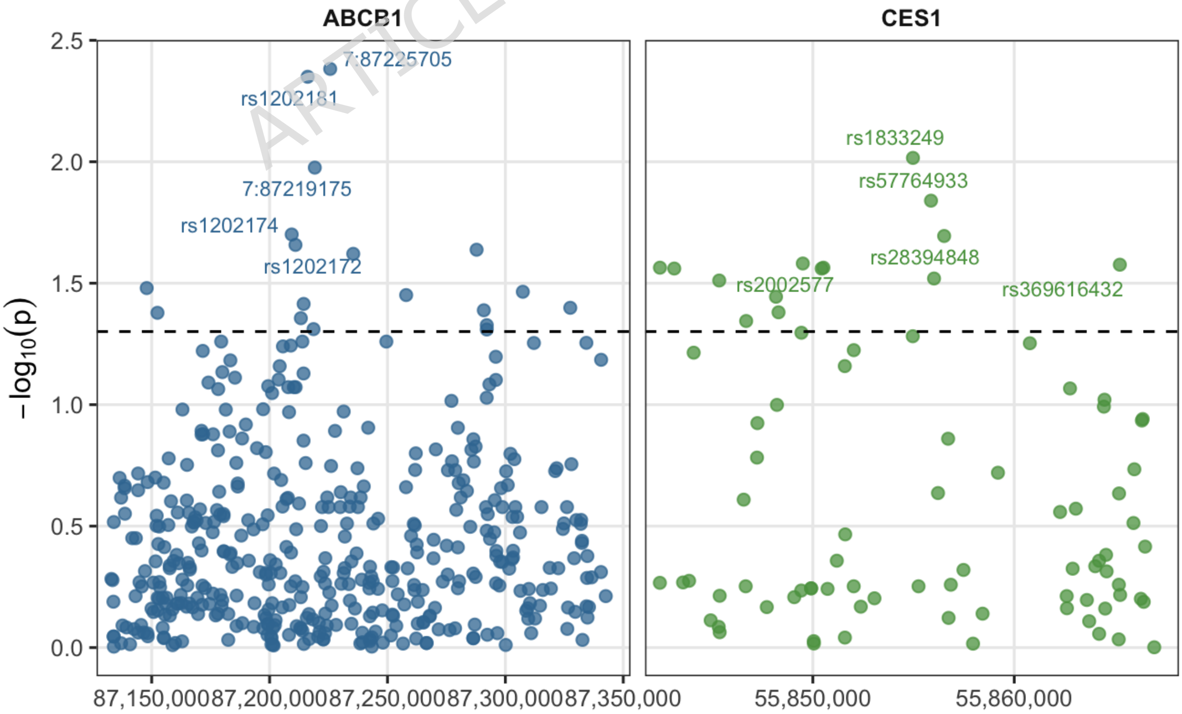
ABCB1
CES1
−log10(p)
7:87225705
rs1202181
7:87219175
rs1202174
rs1202172
rs1833249
rs57764933
rs28394848
rs2002577
rs369616432
87,150,000
87,200,000
87,250,000
87,300,000
87,350,000
55,850,000
55,860,000

Table 2: Suggestive Genetic Associations with Dabigatran Drug Level

SNP	Chromosome	Position, bp	Locus	Function	GENO COUNT	A1	A2	Drug Level A1A1 or A1A2	Drug Level A2A2	Drug Level p	Beta (to change to newest)	MAF (Allele)	H-W-P	P value	Fold Change per Minor Allele (95%CI)	upper fold change	Lower fold change	FDR_BH
7:87179955	7	87179955	ABCB1	intronic	0/6/169	C	T	22.42 (20.58)	35.56 (46.55)	0.493	-1.543	0.01714	1	0.003845	0.21373892	0.07643482	0.59768997	0.6444
7:87188337	7	87188337	ABCB1	intronic	0/5/171	C	T	114.81 (109.24)	31.63 (40.34)	0.164	1.503	0.0142	1	0.00547	4.49515433	1.58323756	12.7627167	0.6444
rs76059198	16	55865920	CES1	intronic	1/21/152	C	G	50.60 (66.75)	32.77 (42.13)	0.09	0.7288	0.06609	0.5406	0.007339	2.072592	1.2263241	3.50285671	0.6444
rs369135400	7	87307405	ABCB1	intronic	0/4/176	T	G	6.24 (5.99)	35.38(45.71)	0.205	-1.607	0.01111	1	0.00741	0.20048818	0.06289655	0.63907331	0.6444
rs372195275	7	87327524	ABCB1	intronic	1/6/169	C	T	6.51 (5.88)	36.08 (46.38)	0.792	-1.064	0.02273	0.07807	0.008755	0.34507276	0.15755196	0.75578374	0.6444
rs57764933	16	55855867	CES1	intronic	38/83/56	C	T	33.47 (44.00)	37.90 (49.18)	0.55	-0.4201	0.4492	0.5431	0.009073	0.65698112	0.48134663	0.89670139	0.6444
rs369616432	16	55865244	CES1	intronic	0/4/176	T	C	97.65 (125.68)	33.06 (41.78)	0.381	1.782	0.01111	1	0.01029	5.941728	1.55140757	22.756194	0.6444
7:87147884	7	87147884	ABCB1	intronic	0/4/167	G	A	9.48 (14.12)	35.69 (46.73)	0.265	-1.523	0.0117	1	0.01196	0.21805673	0.06757356	0.70365891	0.6444
16:55852381	16	55852381	CES1	intronic	0/11/164	A	G	46.14 (32.74)	33.86 (46.30)	0.389	1.042	0.03143	1	0.01432	2.83488111	1.244602	6.45712518	0.6444
7:87200227	7	87200227	ABCB1	intronic	0/11/161	C	T	33.83 (53.48)	34.07 (41.42)	0.986	-1.062	0.03198	1	0.01515	0.34576359	0.14840615	0.8055762	0.6444
rs3893204	16	55848296	CES1	intronic	38/77/59	A	C	34.91 (44.90)	36.54 (48.43)	0.826	-0.3893	0.4397	0.2175	0.01516	0.67753098	0.49684658	0.92392349	0.6444

rs115971798	7	87290865	ABCB1	intronic	2/31/141	C	T	38.72 (35.16)	34.26 (48.30)	0.618	-1.071	0.1006	0.6827	0.0158	0.34266568	0.14521322	0.80860248	0.6444
7:87225705	7	87225705	ABCB1	intronic	0/5/171	A	T	8.53 (7.45)	36.09 (46.17)	0.185	-1.284	0.0142	1	0.01726	0.27692737	0.09750849	0.78648301	0.6444
rs7790722	7	87293223	ABCB1	intronic	1/37/141	T	A	35.48 (33.86)	34.73 (48.24)	0.929	-1.005	0.1089	0.6987	0.01833	0.36604463	0.16037829	0.83545394	0.6444
rs1968753	16	55845351	CES1	intronic	38/78/63	G	A	34.92 (44.93)	34.63 (46.94)	0.967	-0.3669	0.4302	0.1309	0.01895	0.69287893	0.51180244	0.93802057	0.6444
7:87144239	7	87144239	ABCB1	intronic	0/4/176	T	A	9.6 (13.10)	35.31 (45.72)	0.264	-1.638	0.01111	1	0.01986	0.19436839	0.0497531	0.75933101	0.6444
7:87257920	7	87257920	RUNDC3B	UTR5	1/2/174	C	T	111.93 (149.61)	33.86 (41.93)	0.462	2.753	0.0113	0.01697	0.02289	15.6896302	1.50378429	163.696681	0.663
rs10280686	7	87269582	ABCB1	intronic	2/25/152	T	A	39.70 (35.43)	34.03 (47.10)	0.552	-0.7504	0.08101	0.1397	0.02614	0.47217764	0.24544967	0.90833988	0.663
rs1833249	16	55854971	CES1	intronic	33/83/61	G	T	33.44 (44.14)	37.57 (48.88)	0.569	-0.3596	0.4209	0.6441	0.02827	0.69795545	0.50787786	0.9591712	0.663
rs12539395	7	87279856	ABCB1	intronic	1/36/140	T	C	36.13 (34.08)	34.95 (48.34)	0.89	-0.9537	0.1073	0.6973	0.03239	0.38531273	0.16230242	0.91474851	0.663
rs79252647	16	55851190	CES1	intronic	1/6/171	G	T	23.88 (32.26)	35.42 (46.08)	0.513	-1.514	0.02247	0.07721	0.03453	0.2200281	0.05480896	0.88329296	0.663
rs2168610	16	55842408	CES1	intronic	33/92/54	C	T	33.31 (43.61)	37.74 (49.94)	0.551	-0.3581	0.4413	0.65	0.03494	0.69900317	0.50277162	0.97182382	0.663
rs2302721	16	55850524	CES1	intronic	34/90/56	G	T	33.12 (43.72)	38.32 (49.15)	0.478	-0.3581	0.4389	0.8807	0.03494	0.69900317	0.50277162	0.97182382	0.663
rs2002577	16	55849502	CES1	intronic	33/93/54	C	G	33.45 (43.46)	37.74 (49.94)	0.563	-0.3555	0.4417	0.6499	0.03811	0.70082294	0.50245424	0.97750753	0.663
7:87226063	7	87226063	ABCB1	intronic	0/8/164	T	G	17.80 (20.38)	35.46 (46.81)	0.291	-1.151	0.02326	0.04543	0.03884	0.31632029	0.10726246	0.93283822	0.663

rs2188531	7	87328003	ABCB1	intronic	2/31/143	C	T	37.25 (33.54)	34.10 (48.07)	0.721	-0.8579	0.09943	0.6796	0.0398	0.42405166	0.18857608	0.95356636	0.663
rs2214101	7	87269497	ABCB1	intronic	2/25/153	C	T	39.70 (35.43)	33.86 (46.99)	0.539	-0.6886	0.08056	0.3148	0.03999	0.50227877	0.26193362	0.9631599	0.663
rs56695111	16	55850436	CES1	intronic	33/88/58	G	A	32.74 (43.36)	39.29 (49.77)	0.369	-0.3483	0.4302	1	0.04077	0.70588708	0.50717974	0.98244573	0.663
rs8192949	16	55843118	CES1	intronic	34/88/58	A	T	33.86 (44.08)	36.58 (48.42)	0.708	-0.3395	0.4333	1	0.04085	0.7121263	0.51587365	0.9830389	0.663
rs2157926	7	87270500	ABCB1	intronic	2/36/142	A	T	35.48 (33.86)	34.54 (48.13)	0.91	-0.7994	0.1111	1	0.04167	0.44959864	0.20982657	0.96336196	0.663
rs77079297	7	87312097	ABCB1	intronic	0/10/169	G	T	96.00 (109.71)	30.91 (36.15)	0.094	0.8439	0.02793	1	0.04244	2.32541845	1.03692679	5.21499783	0.663
rs1965658	16	55846689	CES1	intronic	32/81/65	C	T	33.44 (44.79)	35.27 (46.17)	0.796	-0.3232	0.4073	0.4404	0.04426	0.72382907	0.52980028	0.98891705	0.663
rs139019792	16	55843555	CES1	intronic	0/4/176	A	G	80.55 (136.39)	33.69 (41.72)	0.542	1.7	0.01111	1	0.04576	5.47394739	1.04835495	28.582018	0.663
rs28431211	16	55849432	CES1	intronic	33/85/60	A	G	34.26 (44.45)	36.69 (47.95)	0.737	-0.3255	0.4242	0.7604	0.048	0.72216618	0.52451069	0.99430575	0.663
7:87178123	7	87178123	ABCB1	intronic	0/5/174	T	C	76.98 (59.95)	33.53 (44.69)	0.035	1.666	0.01397	1	0.04993	5.29096157	1.01527628	27.5730606	0.663

Table 3: Suggestive Genetic Associations with Dabigatran Clotting Time

SNP	Chromosome	Position, bp	Locus	Function	GENO COUNT	A1	A2	Clotting Time A1A1 or A1A2	Clotting Time A2A2	Clotting Time p value	Beta	MAF (Allele)	H-W-P	P value	Fold Change per Minor Allele (95% CI)	Upper fold change	Lower fold change	FDR BH

7:8722 5705	7	8722 5705	ABCB 1	intro nic	0/5/ 171	A	T	218.80 (121.34)	381.01 (209.65)	0.08 7	- 0.6 51	0.01 42	1	0.00 4147	0.521 52399	0.336 69695	0.807810 33	0.7 044
rs1202 181	7	8721 6150	ABCB 1	intro nic	1/7/ 164	G	A	195.88 (80.55)	380.56 (207.82)	0.01 3	- 0.4 346	0.02 616	0.10 18	0.00 4468	0.647 52362	0.482 32256	0.869307 98	0.7 044
rs1833 249	16	5585 4971	CES1	intro nic	33/8 3/61	G	T	364.67 (210.21)	396.16 (207.13)	0.34 2	- 0.1 754	0.42 09	0.64 41	0.00 9641	0.839 12131	0.736 11764	0.956538 09	0.7 044
7:8721 9175	7	8721 9175	ABCB 1	intro nic	0/4/ 167	G	A	693.25 (312.60)	365.14 (202.31)	0.00 2	0.6 462	0.01 17	1	0.01 057	1.908 27559	1.171 09342	3.109500 63	0.7 044
rs5776 4933	16	5585 5867	CES1	intro nic	38/8 3/56	C	T	373.85 (219.23)	383.00 (186.34)	0.78 7	- 0.1 641	0.44 92	0.54 31	0.01 446	0.848 65715	0.745 31449	0.966328 94	0.7 044
rs1202 174	7	8720 9372	ABCB 1	intro nic	2/18 /155	T	C	286.55 (184.08)	388.00 (211.32)	0.04 2	- 0.2 913	0.06 286	0.13 42	0.01 992	0.747 29146	0.586 4676	0.952217 18	0.7 044
rs2839 4848	16	5585 6520	CES1	intro nic	34/8 6/60	T	G	371.84 (221.83)	380.22 (178.40)	0.8	- 0.1 562	0.42 78	0.76 15	0.02 021	0.855 3881	0.750 86237	0.974464 59	0.7 044
rs1202 172	7	8721 0974	ABCB 1	intro nic	2/21 /157	C	A	289.00 (172.19)	387.18 (210.17)	0.03 4	- 0.2 776	0.06 944	0.19 85	0.02 199	0.757 5998	0.598 97873	0.958226 78	0.7 044
rs1808 09886	7	8728 7794	ABCB 1	intro nic	0/12 /167	T	C	277.67 (115.51)	383.19 (211.05)	0.08 9	- 0.3 696	0.03 352	1	0.02 302	0.691 01068	0.504 24124	0.946958 96	0.7 044
7:8723 5474	7	8723 5474	ABCB 1	intro nic	0/8/ 165	T	C	497.13 (260.43)	369.81 (206.51)	0.09 4	0.4 375	0.02 312	1	0.02 393	1.548 8303	1.064 0285	2.254521 66	0.7 044
rs2002 577	16	5584 9502	CES1	intro nic	33/9 3/54	C	G	372.92 (216.97)	378.63 (186.85)	0.86 6	- 0.1 578	0.44 17	0.64 99	0.02 623	0.854 02057	0.744 20045	0.980046 62	0.7 044
rs3696 16432	16	5586 5244	CES1	intro nic	0/4/ 176	T	C	574.25 (343.82)	370.10 (203.10)	0.05 2	0.6 407	0.01 111	1	0.02 653	1.897 80888	1.083 92379	3.322815 28	0.7 044
rs2168 610	16	5584 2408	CES1	intro nic	33/9 2/54	C	T	366.53 (205.58)	378.63 (186.85)	0.71 1	- 0.1 553	0.44 13	0.65	0.02 729	0.856 15829	0.746 96595	0.981312 5	0.7 044
rs2302 721	16	5585 0524	CES1	intro nic	34/9 0/56	G	T	368.19 (215.31)	388.91 (191.52)	0.53 7	- 0.1 553	0.43 89	0.88 07	0.02 729	0.856 15829	0.746 96595	0.981312 5	0.7 044
rs8192 949	16	5584 3118	CES1	intro nic	34/8 8/58	A	T	372.86 (218.20)	378.36 (186.09)	0.86 9	- 0.1 515	0.43 33	1	0.02 751	0.859 41788	0.752 18236	0.981941 53	0.7 044

rs56695111	16	55850436	CES1	intronic	33/88/58	G	A	364.74 (205.99)	397.60 (212.90)	0.324	-0.1552	0.4302	1	0.02753	0.85624391	0.74692334	0.98156477	0.7044
rs59132516	16	55856018	CES1	intronic	33/84/60	C	T	365.71 (206.49)	382.20 (185.82)	0.604	-0.1488	0.4237	0.7582	0.03024	0.86174145	0.75424846	0.98455397	0.7044
rs1968753	16	55845351	CES1	intronic	38/78/63	G	A	377.93 (223.52)	368.94 (179.00)	0.784	-0.1403	0.4302	0.1309	0.03082	0.86909747	0.76618957	0.98582706	0.7044
7:87147884	7	87147884	ABCB1	intronic	0/4/167	G	A	198.00 (69.53)	376.89 (207.26)	0.087	-0.537	0.0117	1	0.03311	0.58449912	0.3584873	0.95300231	0.7044
rs369135400	7	87307405	ABCB1	intronic	0/4/176	T	G	231.00 (98.02)	377.90 (208.71)	0.163	-0.5292	0.01111	1	0.03429	0.58907604	0.36264124	0.95689774	0.7044
7:87257920	7	87257920	RUNDC3B	UTR5	1/2/174	C	T	653.33 (371.95)	372.90 (202.86)	0.02	1.043	0.0113	0.01697	0.03538	2.83771741	1.08435559	7.42619874	0.7044
rs8192947	16	55848171	CES1	intronic	33/85/61	T	C	374.65 (222.07)	377.64 (179.41)	0.928	-0.1432	0.4218	0.7595	0.03595	0.86658074	0.75902735	0.98937432	0.7044
7:87214437	7	87214437	ABCB1	intronic	0/4/174	C	T	650.00 (316.20)	369.06 (202.53)	0.007	0.5269	0.01124	1	0.03848	1.69367377	1.03331666	2.7760424	0.7044
rs372195275	7	87327524	ABCB1	intronic	1/6/169	C	T	241.43 (85.43)	381.59 (211.65)	0.083	-0.3481	0.02273	0.07807	0.03992	0.70602827	0.50810546	0.98104814	0.7044
rs115971798	7	87290865	ABCB1	intronic	2/31/141	C	T	404.70 (258.08)	367.21 (198.33)	0.359	-0.3784	0.1006	0.6827	0.04086	0.68495646	0.47827982	0.98094324	0.7044
rs3893204	16	55848296	CES1	intronic	38/77/59	A	C	379.28 (222.60)	374.02 (183.92)	0.876	-0.1337	0.4397	0.2175	0.04166	0.87485248	0.77020756	0.99371507	0.7044
7:87152442	7	87152442	ABCB1	intronic	0/5/170	A	G	288.00 (159.17)	376.70 (207.10)	0.344	-0.511	0.01429	1	0.04186	0.59989538	0.36838952	0.97688573	0.7044
rs1202186	7	87213258	ABCB1	intronic	1/10/166	C	T	285.73 (230.37)	381.33 (207.14)	0.143	-0.2784	0.0339	0.0655	0.04405	0.75699396	0.57872062	0.99018393	0.7044
rs1965658	16	55846689	CES1	intronic	32/81/65	C	T	367.33 (211.51)	371.58 (178.48)	0.891	-0.1334	0.4404		0.04524	0.87511498	0.76886673	0.99604546	0.7044

7:87292111	7	87292111	ABCB1	intronic	0/6/172	T	A	391.00 (406.56)	375.19 (200.71)	0.856	-0.4554	0.01685	1	0.04706	0.63419424	0.40624405	0.99005102	0.7044
7:87218633	7	87218633	ABCB1	intronic	0/11/164	T	A	449.45 (223.38)	365.68 (202.07)	0.188	0.3364	0.03143	1	0.04886	1.39989887	1.00474927	1.95045362	0.7044
7:87292115	7	87292115	ABCB1	intronic	0/6/166	C	G	391.00 (406.56)	376.68 (202.19)	0.871	-0.4521	0.01744	1	0.04904	0.63629054	0.40726904	0.99409875	0.7044

Discussion

In this study, we explored potential pharmacogenomic contributors to inter-individual variability in dabigatran plasma concentration, clotting time, and 1-year clinical outcomes in a multiethnic Malaysian cohort. Using targeted next-generation sequencing of the *ABCB1* and *CES1* genes, we identified several variants demonstrating nominal associations with dabigatran plasma concentration and clotting time. Multiple-testing correction was applied using the false discovery rate (FDR). However, no associations remained statistically significant after adjustment. Therefore, in this exploratory study, we reported variants that showed suggestive associations at a nominal significance threshold (unadjusted $p < 0.05$).

Specifically, 19 *ABCB1* and 16 *CES1* variants showed nominal associations with dabigatran trough plasma concentrations, while 18 *ABCB1* and 16 *CES1* variants demonstrated nominal associations with clotting time. Seventeen variants overlapped between the two analyses, showing suggestive associations with both plasma concentration and clotting time.

To our knowledge, this study represented one of the first exploratory pharmacogenomic analyses in Asia Pacific region that investigated Dabigatran's pharmacokinetics (drug level), pharmacodynamics (clotting time), and 1-year clinical outcomes, in a multiethnic cohort of atrial fibrillation patients. Although there was no difference in Dabigatran levels or clotting times observed across ethnic groups, our cohort represents multiple ethnicities representative of the population in Sarawak, Malaysia. This study used targeted next-generation sequencing, allowing sequencing of the full gene regions of *ABCB1* and *CES1* rather doing the candidate gene analysis with the predefined genotyping arrays. This approach enabled detection of variants not well represented in public databases, including several without rsIDs. However, these variants should not be interpreted as confirmed novel discoveries, but rather as suggestive SNPs requiring further validation in our population.

Here, our drug level ranged from LLOQ-284.60 ng/ml with Coefficient variation of 130%. In previous European Heart Rhythm guideline in 2021, the reported range of trough level was 28-215ng/ml. Our trough drug level was measured at least ten hours after the preceding dose and before the next due dose of Dabigatran. The sampling timing could be one of the factors contributing for the variations as well. They also reported drug activities on PT, APTT, ACT, and TT. None was reported on clotting time. We observed that the prescribed doses of Dabigatran, was not associated with the trough DL and clotting time. This is contrary to the RELY trial of 1,490 atrial fibrillation patients treated with Dabigatran, those on the 150 mg twice daily dosage had higher peak and trough concentrations compared to the 110 mg twice daily group(8).

The RELY trial reported that *CES1* SNPs rs2244613 and rs8192935 were associated with reduced dabigatran trough levels but not with bleeding or ischemic outcomes. In contrast, Ji et al. found in 198 Chinese NVAF patients that these same variants were associated with increased trough levels and prolonged APTT, with rs2244613 also linked to minor bleeding. No significant associations were observed for *ABCB1* SNPs rs4148738 and rs1045642. A separate 2022 Chinese study (n = 86) confirmed the association of *CES1* rs8192935 with increased trough levels and found that *ABCB1* rs4148738 and rs1045642 were linked to major bleeding, highlighting inconsistencies across populations. Dimatteo et al. reported higher trough levels in women—a finding echoed in our study—and found that minor alleles of *CES1* rs2244613, rs8192935, and *ABCB1* rs4148738 were associated with lower trough levels. Finally, Xie et al. used whole-exome sequencing and GWAS in healthy Chinese individuals and identified novel variants in *SLC4A4*, *FRAS1*, and *SULT1A1* associated with dabigatran metabolism and APTT response. These findings highlight the population-specific nature of pharmacogenomic effects and the need for studies in diverse ethnic groups.

None of the previously reported SNPs showed associations with Dabigatran pharmacokinetics in our cohort. For example, *ABCB1* SNP rs4148738 reported by Ji et al. had p=0.1577 and 0.1047 for DL and CT, respectively. SNPs rs8192935, rs2244613, and rs2244614 in *CES1* reported in the RELY study were excluded from analysis in this cohort due to having genotype missingness rates exceeding 5%. Other studies in Chinese, Kazakh and Italian cohorts also provided contrasting results (10, 22, 23), further highlighting the population specific nature of pharmacogenomic associations.

Most of the SNPs that are nominally associated with Dabigatran pharmacokinetics and pharmacodynamic in this study lack rsIDs, indicating that they have not been previously reported in existing pharmacogenomic literature, making direct comparisons difficult. The SNPs with rsIDs have been reported to be associated with pharmacological effects or drug related phenotypes in other clinical contexts. For example, rs1202181 in *ABCB1* has previously been associated with persistent chemotherapy-induced alopecia (pCIA) in patients treated with docetaxel-based therapies for breast cancer (24). Other *ABCB1* variants of interest include rs115971798 (T>C; MAF = 0.1006) and rs180809886 (C>T; MAF = 0.03352). However, the suggestive SNPs with rsIDs have not been previously linked to Dabigatran or other DOAC responses, to the best of our knowledge. The observed genotype and clinical events associations were regarded as exploratory, as the very low number of clinical events limits reliable time-to-event modeling and effect size estimation.

It should be noted that most variants identified in this study were non-coding, locating within either intronic or in untranslated regions. While non-coding SNPs may not appear to have direct influence on the coded protein, they may play important regulatory roles by affecting transcription factor binding, transcript stability or alternative splicing. Such variants could be key factors to inter-individual differences in drug response and disease risk. Notably, trough concentration and clotting time were positively correlated in our cohort, and 17 SNPs were nominally associated with both phenotypes. This overlap suggests that shared regulatory mechanisms may simultaneously influence dabigatran pharmacokinetics and pharmacodynamics. Two of those SNPs (rs2302721 and rs139019792) had RegulomeDB ranks of 3, providing moderate evidence of potential transcriptional regulatory activity. These findings highlight the importance of investigating non-coding variation as contributors to clinically relevant drug response phenotypes.

This study has several notable limitations that should be considered. Firstly, due to logistical constraints, we were only able to measure trough levels of Dabigatran, as extending patient clinic visits to capture peak levels was not feasible. Secondly, the study was limited to a single time point for both drug level and clotting time measurements, which prevents the assessment of intra-individual variability. Thirdly, information on concomitant medications known to influence dabigatran pharmacokinetics, such as P-glycoprotein (P-gp) inhibitors or inducers, was not available and therefore could not be accounted for in the analyses. Additionally, the low incidence of clinical outcomes such as ischemic stroke and bleeding limited our ability to establish associations between candidate SNPs and these clinical endpoints. The sample size of 180 patients further constrained our statistical power, particularly for detecting small effect sizes or rare variant associations, which may have led to potential false negatives. While many pharmacogenomic studies adopt multiple testing corrections (e.g., Bonferroni or FDR), we employed a nominal significance threshold ($p < 0.05$) in this exploratory analysis, therefore the results remain suggestive. Finally, as this was a single-centre study conducted in a specific Malaysian population, the findings may not be generalizable to other ethnically diverse populations. Future studies should aim to replicate these findings in larger cohorts and include functional validation of key variants, particularly those overlapping pharmacokinetic and pharmacodynamic traits. Integration with expression data, regulatory genomics, or in vitro assays may help clarify the biological significance of the non-coding variants reported here.

(8) (8) (19) (20) (10, 21) (22) (23) (24) (24-26) (24, 25) (24, 25) (27, 28)

Conclusion

Our study provides exploratory insights into pharmacogenomic variability in dabigatran response within a multiethnic Malaysian population, with a focus on genetic variation in *ABCB1* and *CES1*. We observed nominal associations between selected polymorphisms in these genes and measures of dabigatran pharmacokinetics and pharmacodynamics.

However, the clinical implications of these findings remain uncertain. In particular, the extent to which genetic variation translates into differences in clinical outcomes could not be established in this cohort. The observed inter-individual variability highlights the potential limitations of fixed-dose dabigatran regimens, especially in genetically and ethnically diverse populations. While these findings suggest that genetic factors may contribute to variability in drug exposure and anticoagulant effect, they should be interpreted as hypothesis-generating rather than confirmatory. The modest sample size limited statistical power, particularly for rare variants and clinical outcomes. Given that no associations remained significant after false discovery rate correction, all reported SNPs were considered exploratory. At present, the role of pharmacogenomic-guided dosing for dabigatran remains unclear, with significant gaps evident in the current research. Future studies involving larger, multi-centre cohorts with more comprehensive clinical and pharmacological data are required to validate these associations and to investigate their potential relevance for individualized anticoagulation therapy.

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Competing interests

The author(s) declare no competing interests.

Data Availability Statements

The raw sequencing data (fastq files) for the 180 samples analysed in this study have been submitted to the DNA Data Bank of Japan (DDBJ) Sequence Read Archive (DRA) under the BioProject accession no. PRJDB35758 and are currently under review. Individual accession numbers will be made available upon completion. For additional data requests, please contact the corresponding author at **tsnshirley@gmail.com**.

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