

# ABCG2 Gene Polymorphisms May Affect the Bleeding Risk in Patients on Apixaban and Rivaroxaban

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**Purpose:** Direct oral anticoagulants (DOACs) are widely used for stroke prevention in atrial fibrillation. However, they have a bleeding complication. Breast cancer resistance protein, encoded by *ABCG2*, is known to be an efflux transporter of apixaban and rivaroxaban among DOACs. This study aimed to investigate the association between gene variants and bleeding complications during treatment with *ABCG2* substrates (apixaban and rivaroxaban).

**Patients and Methods:** Patients treated with apixaban and rivaroxaban were enrolled from June 2018 to December 2021. Five single nucleotide polymorphisms (SNPs) of *ABCG2* were selected. Previously studied genes (*ABCB1*, *CYP3A4*, and *CYP3A5*) were further analyzed as possible confounders. Finally, a total of 16 SNPs were examined in this case-control study. The outcome was defined as major bleeding and clinically relevant non-major bleeding. Two models were constructed using the multivariable analysis.

**Results:** Among 293 patients, 64 were cases. The mean age of the patients was 68.8 years, and males comprised 62.5% of the study population. Model I revealed that a history of bleeding, concurrent use of proton pump inhibitor (PPI), *ABCG2* rs3114018, and *ABCB1* rs1045642 were significantly associated with bleeding complications; the AORs (95% CI) were 6.209 (2.210–17.442), 2.385 (1.064–5.349), 2.188 (1.156–4.142), and 3.243 (1.371–7.671), respectively. Model II showed that modified HAS-BLED score, concurrent use of PPI, *ABCG2* rs3114018, and *ABCB1* rs1045642 were significantly associated with bleeding complications.

**Conclusion:** The modified HAS-BLED score, a history of bleeding, concurrent use of PPI, *ABCG2* rs3114018, and *ABCB1* rs1045642 were significantly associated with the risk of bleeding complications in patients on apixaban and rivaroxaban, after adjusting for other confounders. These findings can be used to develop individualized treatment strategies for patients taking apixaban and rivaroxaban.

**Keywords:** *ABCG2*, apixaban, bleeding complications, polymorphism, rivaroxaban

## Introduction

Anticoagulants are essential for treating and preventing thromboembolism in various fields, including cardiology, neurology, and orthopedics. Previously, vitamin K antagonists (VKAs) were widely used oral anticoagulants, a well-known example being warfarin. However, the need to develop new oral anticoagulants has emerged because VKA therapy requires frequent monitoring of the international normalized ratio and dose adjustment.

Direct oral anticoagulants (DOACs) are more convenient than warfarin because of the fixed-dose administration and the unnecessary of routine blood monitoring. DOACs directly block factor Xa (apixaban, edoxaban, and rivaroxaban) or block thrombin (dabigatran).

Although DOACs offer safety advantages over warfarin due to a lower rate of life-threatening and intracranial bleeding,<sup>1</sup> they still have bleeding complications, including gastrointestinal bleeding, hemorrhage, hematochezia, hematuria, and epistaxis.<sup>2</sup> In 2021, more than 3700 bleeding cases by DOAC use were reported according to the FDA Adverse Events

Reporting System. Given the higher risk of stroke and intracranial bleeding in Asians, the issue of individualization of DOACs has received much attention.<sup>3,4</sup>

Changes in drug exposure might affect the efficacy and safety of DOACs. Several metabolizing enzymes and transporters contribute to the pharmacokinetics of DOACs. Efflux transporters, such as multidrug resistance protein 1 (MDR1, P-gp, ABCB1) and breast cancer resistance proteins (BCRP, ABCG2), affect the pharmacokinetics of DOACs. BCRP is located in the apical membrane in the epithelia of the intestine and reduces drug absorption by extruding chemical compounds from the cell.<sup>5</sup> Moreover, BCRP is located in the brain, liver, and kidney and limits brain penetration of substrate drugs in addition to secreting substrates actively into the biliary or renal excretion route.<sup>6–8</sup> Among DOACs, apixaban and rivaroxaban are substrates of BCRP.<sup>9</sup>

Although there have been several pharmacogenetic studies on DOAC pharmacokinetics, there have been few conducted in the area of clinical safety.<sup>10</sup> Moreover, little is known about the association between ABCG2 and bleeding complications in patients treated with ABCG2 substrates such as apixaban and rivaroxaban. Therefore, this study aimed to investigate the impact of ABCG2 gene polymorphisms on bleeding complications in apixaban and rivaroxaban users.

## Materials and Methods

### Patients

For this case–control study, patients had been enrolled in Ewha Womans University Mokdong Hospital, Ewha Womans University Seoul Hospital, Severance Hospital of Yonsei University College of Medicine, and Seoul National University Hospital from June 2018 to December 2021. This study was conducted according to the 1975 Helsinki Declaration and its later amendments approved by the Institutional Review Board (IRB) of each hospital following (IRB number: 2018-04-006, 2019-05-038, 4-2018-0823, and 1811-076-9850, respectively). Written informed consent was obtained from the patients.

Patients treated with apixaban or rivaroxaban for reduction of the risk of stroke and systemic embolism in nonvalvular atrial fibrillation were eligible. Patients who met any of the following criteria were excluded: age under 20 years; experience of thromboembolism or infarction in the follow-up period; experience of bleeding which was minor or not verified by health professionals while on treatment; refused consent; less than three months of treatment in the control group; and insufficient sample for genotyping.

### Outcome

Cases were patients who experienced major bleeding or clinically relevant non-major bleeding (CRNMB). The criteria for major bleeding and CRNMB followed the International Society on Thrombosis and Haemostasis guideline.<sup>11,12</sup> Major bleeding was defined as fatal bleeding, and/or bleeding in a critical organ (eg, intracranial, intraspinal, intraocular), and/or bleeding causing a fall in hemoglobin level of 20 g L<sup>-1</sup> or more, or leading to two or more units of blood transfusion. CRNMB was defined as any bleedings which did not satisfy the criteria for major bleeding, but does meet at least one of the following criteria: i) requiring medical intervention by a healthcare professional, ii) leading to hospitalization or increased level of care, or iii) prompting a face-to-face (ie, not just a telephone or electronic communication) evaluation. Cases were followed up until the bleeding events, while controls were followed up until December 31, 2021.

### Data Collection

Patients' data were collected from electronic medical records. The extracted data were as follows: sex, age, body weight, serum creatinine clearance, systolic blood pressure, diastolic blood pressure, dose of DOAC, alcohol, smoking, comorbidities, history of stroke/transient ischemic attacks (TIA)/thromboembolism, history of bleeding, co-medication, and follow-up period. A modified HAS-BLED (hypertension, abnormal renal or liver function, stroke, bleeding history or predisposition, elderly (age ≥ 65 years), concomitant drug, and alcohol use; range 0–8) score was calculated without labile international normalized ratio (INR) compared to HAS-BLED.<sup>13</sup>

## Single Nucleotide Polymorphism (SNP) Selection

To investigate the relationship between *ABCG2* and bleeding complications in patients treated with apixaban and rivaroxaban, SNPs were searched in NCBI (<https://www.ncbi.nlm.nih.gov/variation/view/>). The SNP was excluded if the minor allele frequency was <0.1 in Asians from the 1000 Genome project.<sup>14</sup> Only one SNP was chosen if SNPs were in perfect linkage disequilibrium ( $r^2=1$ ) with others.<sup>15</sup> Finally, five SNPs in *ABCG2* were selected: *ABCG2* rs2231142 (NC\_000004.11:g.89052323G>T; NM\_004827.3:c.421C>A; missense variant), *ABCG2* rs2231137 (NC\_000004.11:g.89061114C>T; NM\_004827.3:c.34G>A; missense variant), *ABCG2* rs2622604 (NC\_000004.11:g.89078924T>C; NM\_004827.3:c.-20+614A>G; intron variant), *ABCG2* rs3114018 (NC\_000004.11:g.89064581A>C; NM\_004827.3:c.-19-3415T>G; intron variant), and *ABCG2* rs1481012 (NC\_000004.11:g.89039082A>G; NM\_004827.3:c.841+179T>C; intron variant).

As apixaban and rivaroxaban are also substrates of *ABCB1* and *CYP3A*,<sup>9</sup> we additionally analyzed SNPs in *ABCB1*, *CYP3A4*, and *CYP3A5* to adjust for their effects. SNPs were selected in the same way as the selection of *ABCG2* SNPs: *ABCB1* rs1045642 (NC\_000007.13:g.87138645A>G; NM\_001348946.2:c.3435T>C; missense variant), *ABCB1* rs2032582 (NC\_000007.13:g.87160618A>C; NM\_001348946.2:c.2677T>G; missense variant), *ABCB1* rs1128503 (NC\_000007.13:g.87179601A>G; NM\_001348946.2:c.1236T>C; synonymous variant), *ABCB1* rs3789243 (NC\_000007.13:g.87220886A>G; NM\_001348946.2:c.117+4196T>C; intron variant), *ABCB1* rs3213619 (NC\_000007.13:g.87230193A>G; NM\_001348946.2:c.-129T>C; intron variant), *CYP3A4* rs2242480 (NC\_000007.13:g.99361466C>T; NM\_017460.6:c.1026+12G>A; intron variant), *CYP3A4* rs4646437 (NC\_000007.13:g.99365083G>A; NM\_017460.6:c.671-202C>T; intron variant), *CYP3A4* rs12333983 (NC\_000007.13:g.99354114T>A; 500B downstream variant), *CYP3A5* rs776746 (NC\_000007.13:g.99270539C>T; NM\_000777.5:c.219-237G>A; splice acceptor variant, *CYP3A5*\*3), *CYP3A5* rs15524 (NC\_000007.13:g.99245914A>G; NM\_000777.5:c.\*14T>C; non-coding transcript variant), and *CYP3A5* rs4646450 (NC\_000007.13:g.99266318G>A; NM\_000777.5:c.319-1630C>T; intron variant).

## Genotyping

The patient's genomic DNA was extracted from the blood or saliva samples. Blood samples were used to extract DNA with QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). Saliva samples were collected by OraGene-600 kits (DNA Genotek, OTT, Canada) and used to extract DNA using PrepIT reagents (DNA Genotek, OTT, Canada).

Extracted DNA sample was genotyped using the TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA, USA) or SNaPshot Multiplex Kits (Applied Biosystems, Foster City, CA, USA). Genotyping was undertaken by a third party blinded to outcome status.

## Statistical Analysis

An unpaired *t*-test was used for continuous variables. The chi-squared test was used for categorical variables, and Fisher's exact test was used if more than 20% of cells had expected frequencies of less than five. To evaluate the genetic association, we applied both dominant and recessive models and the most appropriate model was selected based on their effect size and statistical significance. The unadjusted odds ratios (ORs) and adjusted ORs (AORs) with 95% confidence intervals (CIs) were calculated via univariate and multivariable analyses, respectively. In the multivariable logistic regression analysis, factors with a *p*-value <0.05 in the univariate analysis were put in addition to well-known confounders (sex, age, weight, and creatinine clearance). Next, backward elimination was employed to remove insignificant variables using the probability of the likelihood-ratio statistic based on the maximum partial likelihood estimates.<sup>16</sup> Regarding the modified HAS-BLED score and its components, we constructed two separate models: Model I included each component of modified HAS-BLED score, if it satisfied the criteria, and Model II included a modified HAS-BLED score instead of components of HAS-BLED score in the Model I. The predictive model fit was also evaluated by Hosmer–Lemeshow goodness-of-fit test. To determine whether the study was sufficiently powered to detect differences, the post-hoc power analysis was performed using Quanto 1.2.4 (<https://pphs.usc.edu/biostatistics-software/#quanto>).

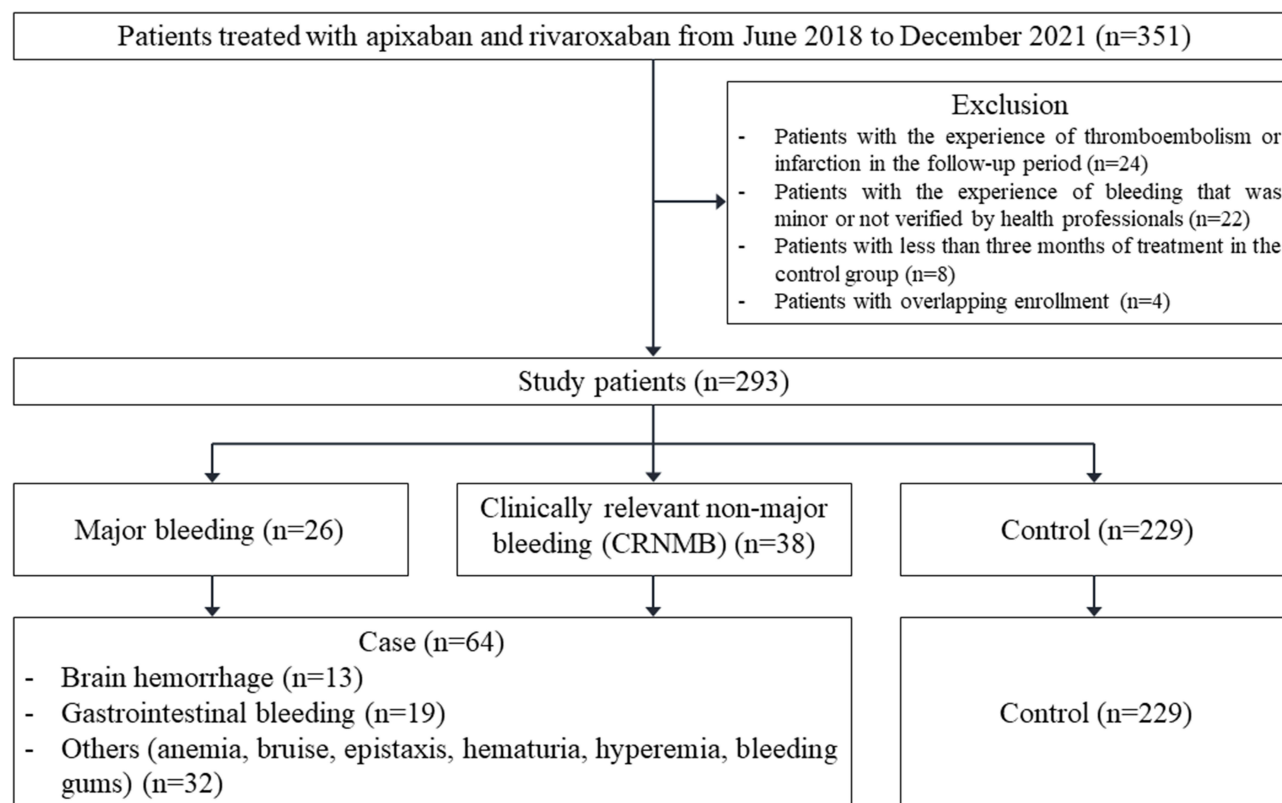
All statistical analyses used SPSS v20.0 (IBM Corp., Armonk, NY, USA), and statistical significance was judged if the  $p$ -value was less than 0.05. Hardy-Weinberg equilibrium and missingness tests were conducted using PLINK.<sup>17</sup> The SNP would be considered to fail the Hardy-Weinberg equilibrium test if the  $p$ -value was  $<0.001$ . Missingness was defined as the loss of  $>10\%$  of data. The outcome of a missingness test was considered a failure when the missingness rate was  $>0.1$  per SNP and per individual.

## Results

Among the 351 patients enrolled, 58 patients were excluded for the following reasons: thromboembolism or infarction in the follow-up period ( $n=24$ ), the experience of bleeding that was minor or not verified by health professionals while on treatment ( $n=22$ ), less than three months of treatment in the control group ( $n=8$ ), and overlapping enrollment ( $n=4$ ). The flow chart of patient selection is shown in Figure 1.

Among 293 patients selected for analysis, cases comprised 64 patients (21.8%), of whom 26 patients experienced major bleeding and 38 patients experienced CRNMB. The baseline characteristics of the study population are detailed in Table 1. Males comprised 62.5% of all the study population. The mean age of the study population was 68.8 years, 201 patients (68.6%) were apixaban users, and 92 patients (31.4%) were rivaroxaban users. Without the factor of labile INRs, the mean of modified HAS-BLED scores was 2.0. The most common comorbidity was hypertension (71.7%), followed by dyslipidemia (29.0%) and diabetes mellitus (28.3%). Eighteen (6.1%) patients had a history of bleeding, and 115 (39.2%) had a history of stroke/TIA/thromboembolism. The most common co-medications were beta-blockers (74.7%), followed by statins (54.5%). Compared to controls, cases showed higher modified HAS-BLED ( $p=0.008$ ), had more histories of stroke/TIA/thromboembolism and bleeding ( $p=0.022$  and  $p<0.001$ , respectively), and received more proton-pump inhibitor (PPI,  $p=0.032$ ).

Genetic differences in candidate SNPs between cases and controls are shown in Table 2. Among 16 SNPs of *ABCG2*, *ABCB1*, *CYP3A4*, and *CYP3A5* genotyped, none of the SNPs failed the Hardy-Weinberg equilibrium test or the



**Figure 1** Flowchart of patient selection.

**Table 1** Baseline Characteristics of the Study Patients

Characteristics	Controls n=229 (%)	Cases n=64 (%)	P
Sex			
Male	146 (63.8)	37 (57.8)	0.385
Female	83 (36.2)	27 (42.2)	
Age (y)			
<65	72 (31.4)	19 (29.7)	0.789
≥65	157 (68.6)	45 (70.3)	
Mean ± SD	68.3 ± 10.1	70.8 ± 9.8	0.076
Body weight (kg)			
<65	103 (46.2)	32 (50.0)	0.590
≥65	120 (53.8)	32 (50.0)	
Mean ± SD	68.3 ± 12.4	66.0 ± 13.8	0.208
Creatinine clearance (mL min <sup>-1</sup> )			
<50	41 (18.6)	14 (22.6)	0.489
≥50	179 (81.4)	48 (77.4)	
Mean ± SD	68.4 ± 24.6	65.1 ± 24.2	0.345
Systolic blood pressure (mmHg)	130.4 ± 21.5	128.7 ± 20.9	0.580
Diastolic blood pressure (mmHg)	76.3 ± 14.6	76.1 ± 12.6	0.919
Modified HAS-BLED <sup>a</sup>			
Mean ± SD	1.9 ± 1.1	2.3 ± 1.0	0.008
Dose of DOAC <sup>b</sup>			
Under & Standard dose	225 (98.3)	61 (95.3)	0.179 <sup>c</sup>
Overdose	4 (1.7)	3 (4.7)	
Alcohol	73 (34.0)	17 (27.9)	0.371
Smoking	35 (16.5)	8 (12.7)	0.465
Comorbidity			
Hypertension	162 (70.7)	48 (75.0)	0.504
Diabetes mellitus	62 (27.1)	21 (32.8)	0.368
Chronic heart failure	49 (21.4)	7 (10.9)	0.060
Dyslipidemia	67 (29.3)	18 (28.1)	0.860
Hepatic abnormality	4 (1.7)	2 (3.1)	0.615 <sup>c</sup>
Renal abnormality	6 (2.6)	2 (3.1)	0.688 <sup>c</sup>
Malignancy	27 (11.8)	3 (4.7)	0.098
Previous history			
Stroke/TIA/thromboembolism	82 (35.8)	33 (51.6)	0.022
Bleeding	7 (3.1)	11 (17.2)	<0.001 <sup>c</sup>
Co-medication			
ACEI/ARB	97 (42.4)	26 (41.3)	0.877
Antiplatelet drugs	26 (11.4)	6 (9.5)	0.681
Beta-blocker	174 (76.0)	44 (69.8)	0.321
Calcium channel blocker	58 (25.3)	15 (23.8)	0.805
Diuretics	63 (27.5)	17 (27.0)	0.934
Statins	121 (52.8)	38 (60.3)	0.291
PPI	24 (10.5)	13 (20.6)	0.032

**Notes:** <sup>a</sup>Modified HAS-BLED (hypertension, abnormal renal or liver function, stroke, bleeding history or predisposition, elderly (age≥65), concomitant drug and alcohol use; range 0–8) score was calculated without the factor of liable international normalized ratio compared to HAS-BLED. <sup>b</sup>Standard dose was defined according to the FDA-approved labeling <sup>c</sup>P-value using the Fisher's exact test.

**Abbreviations:** ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; DOAC, direct oral anticoagulant; PPI, proton pump inhibitor; TIA, transient ischemic attack.

**Table 2** Genetic Factors Associated with Bleeding Complications in Patients Treated with Apixaban and Rivaroxaban

Gene Polymorphism	Minor Allele Frequency (Allele)	Grouped Genotypes	Controls n=229 (%)	Cases n=64 (%)	p
<b>ABCG2</b>					
rs2231142	0.27 (T)	GG GT, TT	116 (50.9) 112 (49.1)	38 (59.4) 26 (40.6)	0.229
rs2231137	0.29 (T)	CC CT, TT	117 (51.5) 110 (48.5)	24 (38.1) 39 (61.9)	0.059
rs2622604	0.16 (T)	TT, TC CC	62 (27.1) 167 (72.9)	28 (43.8) 36 (56.2)	0.011
rs3114018	0.32 (A)	AA, AC CC	115 (50.2) 114 (49.8)	42 (66.7) 21 (33.3)	0.020
rs1481012	0.26 (G)	AA AG, GG	121 (53.1) 107 (46.9)	39 (60.9) 25 (39.1)	0.264
<b>ABCB1</b>					
rs1045642	0.33 (A)	AA AG, GG	19 (8.3) 209 (91.7)	12 (18.8) 52 (81.2)	0.017
rs2032582	0.49 (C)	AA, AC CC	176 (77.2) 52 (22.8)	45 (70.3) 19 (29.7)	0.257
rs1128503	0.41 (G)	AA AG, GG	77 (33.6) 152 (66.4)	25 (39.7) 38 (60.3)	0.372
rs3789243	0.42 (A)	AA, AG GG	154 (67.2) 75 (32.8)	39 (60.9) 25 (39.1)	0.346
rs3213619	0.07 (G)	AA AG, GG	201 (87.8) 28 (12.2)	55 (85.9) 9 (14.1)	0.696
<b>CYP3A4</b>					
rs2242480	0.24 (T)	CC CT, TT	129 (56.8) 98 (43.2)	37 (58.7) 26 (41.3)	0.787
rs4646437	0.07 (A)	GG GA, AA	195 (86.3) 31 (13.7)	56 (88.9) 7 (11.1)	0.588
rs12333983	0.28 (A)	TT, TA AA	213 (93.0) 16 (7.0)	57 (89.1) 7 (10.9)	0.299
<b>CYP3A5</b>					
rs776746	0.26 (T)	CT, TT CC	103 (45.4) 124 (54.6)	31 (49.2) 32 (50.8)	0.589
rs15524	0.29 (G)	AA AG, GG	118 (51.8) 110 (48.2)	31 (48.4) 33 (51.6)	0.639
rs4646450	0.27 (A)	GG GA, AA	122 (53.3) 107 (46.7)	31 (49.2) 32 (50.8)	0.567

**Notes:** There were 1 missing data for rs2231142, rs3114018, rs1481012, rs1045642, rs2032582, rs1128503, rs15524, and rs4646450, 3 missing data for rs2231142, rs2231137, rs2242480, and rs776746, and 4 missing data for rs2231142 and rs4646437.

missingness test. Minor allele frequencies in the selected patients ranged from the smallest 0.07 of CYP3A4 rs4646437 to the largest 0.49 of ABCB1 rs2032582.

The *ABCG2* rs2622604 and *ABCG2* rs3114018 were significantly associated with the risk of bleeding (rs2622604 T allele carriers 31.1% vs CC genotype carriers 17.7%,  $p=0.011$ ; rs3114018 A allele carriers 26.8% vs CC genotype carriers 15.6%,  $p=0.020$ ). In *ABCB1*, patients with the AA genotype of rs1045642 had a higher bleeding risk than G allele carriers (38.7% vs 19.9%,  $p=0.017$ ). Assuming a significance level of 0.05, the post hoc power analysis showed



**Table 3** The Results of Logistic Regression Analysis for Bleeding Complications in Patients Treated with Apixaban and Rivaroxaban

Variables	Unadjusted OR (95% CI)	Model I Adjusted OR (95% CI)	Model II Adjusted OR (95% CI)
Female	1.284 (0.730–2.257)		
Age $\geq$ 65 (years)	1.086 (0.593–1.988)		
Weight < 65 (kg)	1.165 (0.668–2.032)		
Creatinine clearance < 50 (mL min <sup>-1</sup> )	1.273 (0.642–2.527)		
Modified HAS-BLED score <sup>a</sup>	1.425 (1.092–1.859)**		1.347 (1.018–1.782)*
History of stroke/TIA/thromboembolism	1.908 (1.090–3.340)*		
History of bleeding	6.582 (2.436–17.783)***	6.209 (2.210–17.442)***	
Concurrent use of PPI	2.221 (1.057–4.666)*	2.385 (1.064–5.349)*	2.359 (1.079–5.156)*
ABCG2 rs2622604 T allele carrier	2.095 (1.181–3.717)*		
ABCG2 rs3114018 A allele carrier	1.983 (1.105–3.557)*	2.188 (1.156–4.142)*	2.335 (1.250–4.360)**
ABCB1 rs1045642 AA genotype carrier	2.538 (1.159–5.559)*	3.243 (1.371–7.671)**	3.167 (1.349–7.436)**

**Notes:** <sup>a</sup>Modified HAS-BLED (hypertension, abnormal renal or liver function, stroke, bleeding history or predisposition, elderly (age $\geq$ 65), concomitant drug and alcohol use; range 0–8) score was calculated without the factor of labile international normalized ratio compared to HAS-BLED. Model I included variables of female, age, weight, creatinine clearance, history of stroke/TIA/thromboembolism, history of bleeding, concurrent use of PPI, ABCG2 rs2622604, ABCG2 rs3114018, and ABCB1 rs1045642. Model II included variables of female, weight, history modified HAS-BLED score, concurrent use of PPI, ABCG2 rs2622604, ABCG2 rs3114018, and ABCB1 rs1045642. \* $p < 0.05$  \*\* $p < 0.01$  \*\*\* $p < 0.001$ .

**Abbreviations:** CI, confidence interval; PPI, proton pump inhibitor; OR, odds ratio; TIA, transient ischemic attack.

that our study had around 65% statistical power for those three SNPs. None of the *CYP3A4* and *CYP3A5* SNPs were statistically significant.

For the multivariable logistic regression analyses, two models were constructed using variables as follows: female, age  $\geq$  65 years, weight < 65 kg, creatinine clearance < 50 mL min<sup>-1</sup>, modified HAS-BLED score, history of stroke/TIA/thromboembolism, history of bleeding, concurrent use of PPI, and genetic factors, which were significant in univariate analysis (Table 3). Model I included factors except for the modified HAS-BLED score. Model II included a modified HAS-BLED score instead of components of HAS-BLED score in the Model I. After backward elimination, Model I showed that a history of bleeding, concurrent use of PPI, *ABCG2* rs3114018 A allele carriers, and *ABCB1* rs1045642 AA genotype carriers were significantly associated with increased risk of bleeding; the AORs (95% CI) were 6.209 (2.210–17.442,  $p < 0.001$ ), 2.385 (1.064–5.349,  $p = 0.035$ ), 2.188 (1.156–4.142,  $p = 0.016$ ), and 3.243 (1.371–7.671,  $p = 0.007$ ), respectively. In the Model II, modified HAS-BLED score, concurrent use of PPI, *ABCG2* rs3114018 A allele carriers, and *ABCB1* rs1045642 AA genotype carriers were significantly associated with increased risk of bleeding; the AORs (95% CI) were 1.347 (1.018–1.782,  $p = 0.037$ ), 2.359 (1.079–5.156,  $p = 0.031$ ), 2.335 (1.250–4.360,  $p = 0.008$ ), and 3.167 (1.349–7.436,  $p = 0.008$ ). Among the significant SNPs in the univariate analysis, *ABCG2* rs2622604 did not remain as a significant predictor in both models. The Hosmer–Lemeshow test showed that both models were a good fit ( $\chi^2 = 5.121$  and  $p = 0.163$ ;  $\chi^2 = 4.947$  and  $p = 0.763$ , respectively).

## Discussion

In this study, a modified HAS-BLED score, a history of bleeding, concurrent use of PPI, *ABCG2* rs3114018, and *ABCB1* rs1045642 were significantly associated with the risk of bleeding complications in patients on apixaban and rivaroxaban, after adjusting for other confounders.

The finding that the A carriers of rs3114018 was associated with bleeding complications was supported by research by Custodio.<sup>18</sup> Patients harboring the *ABCG2* rs3114018 AA genotype had a higher risk of grade 2–3 oxaliplatin-induced peripheral neuropathy compared to the C allele carriers of *ABCG2* rs3114018, with marginal significance. Meanwhile, ABCG2 protein located in the renal proximal tubular brush border membranes is known to be involved in the pathogenesis of gout by mediating renal urate secretion.<sup>19</sup> The A allele of rs3114018 increased the risk of gout 1.6-fold in the 453 Chinese Han population.<sup>20</sup>

*ABCB1* SNPs are the most commonly documented genetic variants for the alteration of plasma drug levels of apixaban and rivaroxaban.<sup>21,22</sup> An in vitro study showed that the *ABCB1* mRNA expression in the G allele of rs1045642 was significantly higher than that in the A allele.<sup>23</sup> Moreover, A allele carriers in rs1045642 had higher maximum plasma concentration and area under the curve from time 0 to infinity than those of the GG genotype in a meta-analysis involving 535 individuals treated with DOACs.<sup>24</sup> Unusual high drug exposure could raise the risk of drug toxicity. Hence, this could also be a possible explanation for the result of this study, which indicated the association of *ABCB1* rs1045642 with hemorrhage induced by apixaban and rivaroxaban.

PPI is known as a protective factor in gastrointestinal bleeding.<sup>25</sup> Surprisingly, this study found concurrent use of PPI to have a significant relationship with bleeding complications. This result was possibly due to the role of PPI as an ABCG2 inhibitor. A study showed that coadministration of pantoprazole and omeprazole could increase the serum concentrations of methotrexate (ABCG2 substrate), indicating that they inhibited methotrexate transport in Sf9 cells with BCRP vesicles.<sup>26</sup> Another study also demonstrated that coadministration of PPIs, including omeprazole, lansoprazole, and rabeprazole, delayed methotrexate elimination by inhibiting BCRP-mediated transport of methotrexate.<sup>27</sup> Moreover, PPIs are known as substrates and inhibitors of P-gp.<sup>28</sup> Therefore, the inhibition of efflux transporters partly explains the result of this study. However, since our finding showed the association, but did not prove the causality, the possibility remained that patients with higher risk of bleeding may be more likely to be treated with PPI in order to prevent gastrointestinal bleeding.

The history of bleeding showed a 6.3-fold high association with bleeding complications in this study. According to Pisters et al, the percentage of patients with a history of major bleeding was higher in the bleeding group than in the no-bleeding group, from the Euro Heart Survey (17% vs 2%,  $p < 0.001$ ).<sup>13</sup> Further, the hazard ratio of bleeding history on major bleeding was 1.73 in 7411 patients from the Outcomes Registry for Better Informed Treatment of Atrial Fibrillation (ORBIT-AF).<sup>29</sup> Similarly, the history of bleeding was higher in the case group than in the control group in this study.

Among *ABCG2* SNPs, rs2231142 (missense variant) has mainly been studied for apixaban and rivaroxaban. However, the results were controversial. In a previous study, the T allele of rs2231142 predicted higher apixaban concentrations in 358 Caucasian atrial fibrillation patients.<sup>30</sup> The mean of oral clearance of apixaban was also lower in patients with the TT genotype than in those with the G allele carriers.<sup>22</sup> However, no correlations were observed with rs2231142 SNP in the dose-adjusted trough concentrations of rivaroxaban in 86 patients with non-valvular atrial fibrillation.<sup>31</sup> No significant increase in the risk of bleeding complications was found in this study. Therefore, additional studies are required to clarify the role of this SNP in DOAC-induced bleeding.

Regarding CYP-mediated metabolism, apixaban and rivaroxaban are metabolized via *CYP3A4/5* at ~15% and 18%, respectively.<sup>32</sup> One of the noted SNPs among *CYP3A*-encoding gene variants, *CYP3A5* rs776746, is a missense SNP encoding the *CYP3A5*\*3, which is a loss-of-function variant.<sup>33</sup> A positive relationship was reported between the *CYP3A5*\*3 allele and apixaban users' plasma trough concentration/dose ratio in 44 Japanese patients with atrial fibrillation.<sup>34</sup> Moreover, according to FDA-approved drug labeling, strong inhibitors and inducers of *CYP3A* and P-gp should be avoided during treatment with apixaban and rivaroxaban. However, including *CYP3A5* rs776746, none of the *CYP3A* variants had statistical significance in this study. Therefore, further study is needed using populations of various ethnicities.

There are several limitations to this study. First, this study was conducted with a retrospective design, although DNA samples were obtained prospectively. Second, we did not evaluate the pharmacokinetic parameters of DOAC. Moreover, subgroup analysis was not conducted based on the DOAC type (eg, apixaban, rivaroxaban) and bleeding site (eg, brain, gastrointestinal duct) due to the small sample size. Lastly, we did not perform multiple test corrections to avoid the possible loss of the true positives. Therefore, it needs to be validated by further replication studies. However, to the best of our knowledge, this is the first study to discuss the effect of *ABCG2*-related genetic markers on major bleeding and CRNMB in patients receiving apixaban and rivaroxaban.

## Conclusion

In conclusion, this study sought to determine whether demographic and genetic factors had any influence on bleeding in patients treated with apixaban and rivaroxaban. The result of multivariable regression analysis proved the effects of the factors on bleeding complications. The finding of this study, while preliminary, suggested that genotyping could help optimize individualized treatment strategies in patients taking DOACs, especially those who have clinical and genetic



risk factors. These results provide wider support for the hypothesis that genotyping could provide patients with effective and safe anticoagulation therapy.

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## Disclosure

The authors report no conflicts of interest in this work.

## References

- Eikelboom J, Merli G. Bleeding with direct oral anticoagulants vs warfarin: clinical experience. *Am J Med.* 2016;129:S33–40. doi:10.1016/j.amjmed.2016.06.003
- Hellenbart EL, Faulkenberg KD, Finks SW. Evaluation of bleeding in patients receiving direct oral anticoagulants. *Vasc Health Risk Manag.* 2017;13:325–342. doi:10.2147/VHRM.S121661
- Wong KS, Hu DY, Oomman A, et al. Rivaroxaban for stroke prevention in East Asian patients from the ROCKET AF trial. *Stroke.* 2014;45:1739–1747. doi:10.1161/STROKEAHA.113.002968
- Chiang CE, Wang KL, Lip GY. Stroke prevention in atrial fibrillation: an Asian perspective. *Thromb Haemost.* 2014;112:789–797. doi:10.1160/TH13-11-0948
- Staud F, Pavsek P. Breast cancer resistance protein (BCRP/ABCG2). *Int J Biochem Cell Biol.* 2005;37:720–725. doi:10.1016/j.biocel.2004.11.004
- Taskar KS, Yang X, Neuhoof S, et al. Clinical Relevance of Hepatic and Renal P-gp/BCRP Inhibition of Drugs: an International Transporter Consortium Perspective. *Clin Pharmacol Ther.* 2022;112:573–592. doi:10.1002/cpt.2670
- Pauli-Magnus C, Meier PJ. Hepatobiliary transporters and drug-induced cholestasis. *Hepatology.* 2006;44:778–787. doi:10.1002/hep.21359
- Pang KS, Rodrigues AD, Peter RM. *Enzyme-and Transporter-Based Drug-Drug Interactions.* New York: Springer; 2010:301–307.
- Gronich N, Stein N, Muszkat M. Association between use of pharmacokinetic-interacting drugs and effectiveness and safety of direct acting oral anticoagulants: nested case-control study. *Clin Pharmacol Ther.* 2021;110:1526–1536. doi:10.1002/cpt.2369
- Kanuri SH, Kreutz RP. Pharmacogenomics of novel direct oral anticoagulants: newly identified genes and genetic variants. *J Pers Med.* 2019;9:7. doi:10.3390/jpm9010007
- Schulman S, Kearon C. Definition of major bleeding in clinical investigations of antithrombotic medicinal products in non-surgical patients. *J Thromb Haemost.* 2005;3:692–694. doi:10.1111/j.1538-7836.2005.01204.x
- Kaatz S, Ahmad D, Spyropoulos AC, Schulman S. Subcommittee on Control of Anticoagulation. Definition of clinically relevant non-major bleeding in studies of anticoagulants in atrial fibrillation and venous thromboembolic disease in non-surgical patients: communication from the SSC of the ISTH. *J Thromb Haemost.* 2015;13:2119–2126. doi:10.1111/jth.13140
- Pisters R, Lane DA, Nieuwlaat R, et al. A novel user-friendly score (HAS-BLED) to assess 1-year risk of major bleeding in patients with atrial fibrillation: the Euro Heart Survey. *Chest.* 2010;138(5):1093–1100. doi:10.1378/chest.10-0134
- 1000 Genomes Project Consortium. An integrated map of genetic variation from 1092 human genomes. *Nature.* 2012;491:56–65. doi:10.1038/nature11632
- Ward LD, Kellis M. HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease. *Nucleic Acids Res.* 2016;44:D877–81. doi:10.1093/nar/gkv1340
- Field A. *Discovering Statistics Using IBM SPSS Statistics.* 4th ed. London: Sage; 2013.
- Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007;81:559–575. doi:10.1086/519795
- Custodio A, Moreno-Rubio J, Aparicio J, et al. Pharmacogenetic predictors of severe peripheral neuropathy in colon cancer patients treated with oxaliplatin-based adjuvant chemotherapy: a GEMCAD group study. *Ann Oncol.* 2014;25:398–403. doi:10.1093/annonc/mdt546
- McKnight AJ, Currie D, Maxwell AP. Unravelling the genetic basis of renal diseases; from single gene to multifactorial disorders. *J Pathol.* 2010;220:198–216. doi:10.1002/path.2639
- Jiri M, Zhang L, Lan B, et al. Genetic variation in the ABCG2 gene is associated with gout risk in the Chinese Han population. *Clin Rheumatol.* 2016;35:159–163. doi:10.1007/s10067-015-3105-9
- Gouin-Thibault I, Delavenne X, Blanchard A, et al. Interindividual variability in dabigatran and rivaroxaban exposure: contribution of ABCB1 genetic polymorphisms and interaction with clarithromycin. *J Thromb Haemost.* 2017;15:273–283. doi:10.1111/jth.13577
- Ueshima S, Hira D, Kimura Y, et al. Population pharmacokinetics and pharmacogenomics of apixaban in Japanese adult patients with atrial fibrillation. *Br J Clin Pharmacol.* 2018;84:1301–1312. doi:10.1111/bcp.13561
- Wang D, Johnson AD, Papp AC, et al. Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435C> T affects mRNA stability. *Pharmacogenet Genomics.* 2005;15:693–704. doi:10.1097/01.fpc.0000178311.02878.83
- Xie Q, Xiang Q, Mu G, et al. Effect of ABCB1 genotypes on the pharmacokinetics and clinical outcomes of new oral anticoagulants: a systematic review and meta-analysis. *Curr Pharm Des.* 2018;24:3558–3565. doi:10.2174/1381612824666181018153641
- Lee JY. Risk Factors of Gastrointestinal Bleeding in Patients Receiving New Oral Anticoagulants. *Korean J Helicobacter Up Gastrointest Res.* 2018;18:219–224. doi:10.7704/kjhgr.2018.18.4.219
- Breedveld P, Zelcer N, Pluim D, et al. Mechanism of the pharmacokinetic interaction between methotrexate and benzimidazoles: potential role for breast cancer resistance protein in clinical drug-drug interactions. *Cancer Res.* 2004;64:5804–5811. doi:10.1158/0008-5472.CAN-03-4062
- Suzuki K, Doki K, Homma M, et al. Co-administration of proton pump inhibitors delays elimination of plasma methotrexate in high-dose methotrexate therapy. *Br J Clin Pharmacol.* 2009;67:44–49. doi:10.1111/j.1365-2125.2008.03303.x

28. Pauli-Magnus C, Rekersbrink S, Klotz U, et al. Interaction of omeprazole, lansoprazole and pantoprazole with P-glycoprotein. *Naunyn Schmiedeberg's Arch Pharmacol*. 2001;364:551–557. doi:10.1007/s00210-001-0489-7
29. O'Brien EC, Simon DN, Thomas LE, et al. The ORBIT bleeding score: a simple bedside score to assess bleeding risk in atrial fibrillation. *Eur Heart J*. 2015;36:3258–3264. doi:10.1093/eurheartj/ehv476
30. Gulilat M, Keller D, Linton B, et al. Drug interactions and pharmacogenetic factors contribute to variation in apixaban concentration in atrial fibrillation patients in routine care. *J Thromb Thrombolysis*. 2020;49:294–303. doi:10.1007/s11239-019-01962-2
31. Nakagawa J, Kinjo T, Iizuka M, et al. Impact of gene polymorphisms in drug-metabolizing enzymes and transporters on trough concentrations of rivaroxaban in patients with atrial fibrillation. *Basic Clin Pharmacol Toxicol*. 2021;128:297–304. doi:10.1111/bcpt.13488
32. Gong IY, Kim RB. Importance of pharmacokinetic profile and variability as determinants of dose and response to dabigatran, rivaroxaban, and apixaban. *Can J Cardiol*. 2013;29:S24–33. doi:10.1016/j.cjca.2013.04.002
33. Schuetz EG, Relling MV, Kishi S, et al. PharmGKB update: II. CYP3A5, cytochrome P450, family 3, subfamily A, polypeptide 5. *Pharmacol Rev*. 2004;56:159. doi:10.1124/pr.56.2.1
34. Ueshima S, Hira D, Fujii R, et al. Impact of ABCB1, ABCG2, and CYP3A5 polymorphisms on plasma trough concentrations of apixaban in Japanese patients with atrial fibrillation. *Pharmacogenet Genomics*. 2017;27:329–336. doi:10.1097/FPC.0000000000000294

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