

Association between genetic polymorphisms in fibrinogen genes and bleeding risk in patients treated with direct oral anticoagulants

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ABSTRACT

Introduction: This study aimed to investigate the association between polymorphisms in fibrinogen genes and bleeding risk in patients receiving direct oral anticoagulants (DOACs).

Method: Patients treated with DOACs from June 2018 to December 2021 were enrolled in the study. Genotyping was done for rs2070011, rs6050, and rs2070022 in fibrinogen alpha chain (*FGA*); rs1800788, rs4220, and rs4463047 in fibrinogen beta chain (*FGB*); and rs2066865 and rs1800792 in fibrinogen gamma chain (*FGG*), along with *F2* rs5896 and *F10* rs5960. Multivariable logistic regression analysis was performed to investigate the risk factors for bleeding and to develop a risk scoring system.

Results: A total of 468 patients were included in the analysis, 14 of whom experienced major bleeding and 36 experienced clinically relevant non-major bleeding. In the multivariable analysis, overdose, anaemia, *F2* rs5896, and *FGG* rs1800792 were found to be significantly associated with bleeding risk. Specifically, patients with the TT genotype of *F2* rs5896 and the CC genotype of *FGG* rs1800792 had 2.1 times (95% confidence interval [CI] 1.1–3.9) and 2.7 times (95% CI 1.2–5.9) higher bleeding risk than the C allele and T allele carriers, respectively. Based on the risk scoring system, patients with 0, 1, 2, 3, 4, and 5 points were predicted to have 5.2%, 10.8%, 22.4%, 32.3%, 42.3%, and 61.8% of bleeding risk, respectively.

Conclusion: To our knowledge, this is the first study to investigate the effects of polymorphisms in fibrinogen genes on DOAC response. After validation, these results will be useful for personalised DOAC therapy.

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INTRODUCTION

Direct oral anticoagulants (DOACs) are widely prescribed for the prevention and treatment of stroke, systemic embolism and venous thromboembolism.¹ Their mechanism of action involves direct binding to and inhibition of activated coagulation factors—factor

Xa and thrombin—thereby preventing excessive blood clotting.² Overall, DOACs have favourable efficacy and safety profiles but are commonly associated with the complication of bleeding.³ Hernandez et al. reported cumulative 1-year incidence rates of intracranial bleeding, gastrointestinal bleeding, and any bleeding

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CLINICAL IMPACT

What is New

- This study reports the effects of fibrinogen gene polymorphisms on bleeding risk in Asian patients taking direct oral anticoagulants.
- We propose a new bleeding risk scoring system that incorporates clinical (anaemia and overdose) and genetic risk factors (*F2* rs5896 and *FGG* rs1800792).

Clinical Implications

- An understanding of pharmacogenomics can prevent bleeding complications associated with direct oral anticoagulants.

event at 1%, 6–11%, and 19–26% in DOAC-treated patients, respectively.⁴ Several recent works have suggested that some individuals with a genetic predisposition are more susceptible to DOAC-related haemorrhage.⁵ For example, Paré et al. showed that the T allele of carboxylesterase 1 (*CES1*) rs2244613 is associated with an increased risk of any bleeding event in patients treated with dabigatran.⁶ However, most studies were limited to pharmacokinetics-related genes.⁵

Fibrinogen is one of the important factors in the coagulation cascade.⁷ After activating coagulation factor X to Xa and subsequently activating prothrombin to thrombin—both targets for DOACs—fibrinogen is cleaved into fibrin monomers, which form fibrin clots to aggregate platelets and promote coagulation.⁸ As fibrinogen is involved in haemostasis, its abnormality leads to prothrombin time prolongation and increased bleeding risk.⁹ Previous clinical studies revealed that plasma fibrinogen concentration is related to coagulation and haemorrhage.^{10,11} Fibrinogen consists of 3 polypeptide chains—A α , B β and γ —which are encoded by fibrinogen alpha chain (*FGA*), fibrinogen beta chain (*FGB*) and fibrinogen gamma chain (*FGG*) genes, respectively.¹² The fibrinogen gene family is clustered on chromosome 4 and spans ~50 kb, comprising *FGB*, *FGA* and *FGG*. As several single nucleotide polymorphisms (SNPs) of fibrinogen genes are reportedly related to fibrinogen levels,¹³ we hypothesised that these genetic features may affect bleeding risk. Therefore, this study aims to investigate the association between polymorphisms in fibrinogen genes and bleeding risk in DOAC-treated patients.

METHOD

Study patients

This retrospective study was conducted using prospectively collected samples; the study cohort has been previously described in detail.^{14,15} Briefly, patients treated with DOACs (direct thrombin inhibitors or factor Xa inhibitors) from June 2018 to December 2021 at Ewha Womans University Mokdong Hospital and Ewha Womans University Seoul Hospital were included in the study. For control groups, we only included patients who had at least a 3-month follow-up. Patients were excluded if they (1) were younger than 20 years old, (2) experienced infarction-related events, (3) had minor or unverified bleeding, and (4) withdrew consent. The primary outcome was any 1-year bleeding event, defined as a combined endpoint of major bleeding and clinically relevant non-major bleeding (CRNMB) by the International Society on Thrombosis and Haemostasis criteria;^{16,17} therefore, we excluded patients who experienced bleeding episodes ≥ 1 year after DOAC treatment. The following demographic and clinical information were collected from electronic medical records: sex, age, body weight, creatinine clearance (CrCl), CHA₂DS₂-VASc score (Congestive heart failure, Hypertension, Age ≥ 75 years, Diabetes mellitus, Stroke, Vascular disease, Age 65–74 years, Sex category),¹⁸ modified HAS-BLED (Hypertension, Abnormal renal/liver function, Stroke, Bleeding history or predisposition, Labile INR, Elderly, Drugs/alcohol concomitantly), excluding the liable INR,¹⁹ type and dose of DOAC, social history (smoking and alcohol status), comorbidity and co-medication.

The study was performed in accordance with the Declaration of Helsinki principles and was approved by the Institutional Review Board (IRB) of Ewha Womans University Mokdong Hospital (IRB number: 2018-04-006) and Ewha Womans University Seoul Hospital (IRB number: 2019-05-038). All patients provided informed consent before participation.

Genotyping

To select SNPs in 3 fibrinogen genes (*FGA*, *FGB* and *FGG*), genetic information was obtained from the dbSNP,²⁰ HaploReg 4.1²¹ and PharmGKB databases.²² Based on the functionality, minor allele frequency and linkage disequilibrium pattern, the following SNPs were selected: rs2070011, rs6050 and rs2070022 in *FGA*; rs1800788, rs4220 and rs4463047 in *FGB*; and rs2066865 and rs1800792 in *FGG*. As thrombin and factor Xa are the main targets of DOACs, rs5896

and rs5960, which are respectively the representative mutations of *F2* and *F10*,^{23,24} were additionally included as potential confounding variables along with the selected SNPs.

Genomic DNA was extracted from blood or saliva samples collected in EDTA vacutainer tubes or Oragene OG-600 kits (DNA Genotek, Ottawa, ON, Canada), respectively. DNA was extracted following manufacturers' protocols from blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN GmbH, Hilden, Germany) or saliva samples using the PrepIT-L2p kit (DNA Genotek, Ottawa, ON, Canada). Genotyping was performed using the TaqMan assay (Applied Biosystems, Foster City, CA, US).

Statistical analysis

Allele frequency was calculated from genotype frequency, and deviation from Hardy–Weinberg Equilibrium (HWE) was tested using chi-squared test. Chi-squared test and unpaired t-test were used to determine differences between case and control groups. Multivariable logistic regression was performed to determine independent predictors for bleeding risk. All variables with *P* values <0.05 in the univariate analysis were included along with sex and age, and backward elimination was performed for variable selection. The crude and adjusted odds ratio (OR) with the 95% confidence interval (CI) were estimated from univariate and multivariable analyses, respectively. The attributable risk was calculated as (adjusted OR–1)/adjusted OR. The Hosmer–Lemeshow goodness-of-fit was checked for model fitness.

A risk scoring system was developed according to the results of the multivariable logistic regression analysis. To assign the score for each risk factor, its OR was divided by the lowest value and rounded to the nearest integer. The area under the receiver operating characteristic (AUROC) curve was used to evaluate the performance of the risk scoring system.

All statistical analyses were performed using SPSS Statistics version 20 (IBM Corp, Armonk, NY, US). A *P* value of <0.05 was considered statistically significant.

RESULTS

A total of 576 patients treated with DOACs were enrolled. Among them, 108 patients were excluded for the following reasons: patients with <3 months of follow-up in the control group (*n*=5), patients with infarction-related events (*n*=25), patients with minor or unverified bleeding (*n*=23), patients with bleeding episodes >1 year after DOAC treatment (*n*=43), a patient who withdrew the consent (*n*=1), and a patient whose

sample was not analysed (*n*=1). Therefore, 468 patients, including 14 patients who experienced major bleeding and 36 who experienced CRNMB, were included in the analysis.

Overall, 37.4% of patients were female (Table 1). The mean (standard deviation) age and weight were 69.2 (10.1) years old and 67.4 (11.7) kg, respectively. Approximately 90% of the patients had ≥ 2 CHA₂DS₂-VASc scores, whereas two-thirds of them had ≥ 2 modified HAS-BLED scores. Atrial fibrillation was the most prevalent comorbidity (98.5%), followed by hypertension (67.5%). Beta-blockers (71.2%) and statins (58.3%) were the most common comedications in the study population. Among clinical factors, the dose of DOAC and anaemia were significantly associated with bleeding risk. Despite not being statistically significant, patients with CrCl <30 (*P*=0.086) and a history of stroke/transient ischaemic attack/thromboembolism (*P*=0.076) tended to have a higher bleeding risk.

The distribution of allelic and genotypic frequencies is presented in Supplementary Table S1, and all SNPs are in HWE. The results of the genetic association analysis are shown in Table 2. The TT genotype of *F2* rs5896 was associated with an increased bleeding risk compared to the CC or CT genotypes. Among the SNPs in fibrinogen-related genes, *FGA* rs2070011 and *FGG* rs1800792 showed a significant association with bleeding risk; patients with variant homozygotes of *FGA* rs2070011 and *FGG* rs1800792 had a higher bleeding risk than patients with the wild-type allele.

Table 3 shows the result of the multivariable logistic regression analysis. Even after the adjustment of confounders, overdose, anaemia, *F2* rs5896, and *FGG* rs1800792 were still found to be significantly associated with bleeding risk. According to the results, a DOAC overdose increased bleeding risk by 6.2 times (95% CI 1.7–23.1), whereas anaemia increased the risk by 2.3 times (95% CI 1.2–4.3). In addition, patients with the TT genotype of *F2* rs5896 and the CC genotype of *FGG* rs1800792 had 2.1 times (95% CI 1.1–3.9) and 2.7 times (95% CI 1.2–5.9) higher bleeding risk, respectively, than the wild allele carriers. The attributable risks of *F2* rs5896 and *FGG* rs1800792 were calculated as 52.0% and 62.6%, respectively. Hosmer–Lemeshow test showed a good fit of the model ($\chi^2=3.161$, *P*=0.367).

For the risk scoring system, overdose (3 points), anaemia (1 point), *F2* rs5896 (1 point), and *FGG* rs1800792 (1 point) were summed up. Theoretically, the risk scores may range from 0 to 6; however, no patient had 6 points. Based on the risk scoring system, patients with 0, 1, 2, 3, 4, and 5 points had approximately 4.6%,

Table 1. Baseline characteristics of the study population.

	Patients with bleeding (n=50)	Patients without bleeding (n=418)	P value
Sex			0.925
Male	31 (62.0)	262 (62.7)	
Female	19 (38.0)	156 (37.3)	
Age (years)			0.760
<65	16 (32.0)	125 (29.9)	
≥65	34 (68.0)	293 (70.1)	
Body weight (kg)			0.925
<60	12 (25.0)	98 (24.4)	
≥60	36 (75.0)	304 (75.6)	
Creatinine clearance (mL/min)			0.086
<30	5 (10.4)	18 (4.5)	
≥30	43 (89.6)	384 (95.5)	
CHA ₂ DS ₂ -VASc			0.147
<2	3 (6.0)	55 (13.2)	
≥2	47 (94.0)	363 (86.8)	
Modified HAS-BLED			0.870
<2	17 (34.0)	147 (35.2)	
≥2	33 (66.0)	271 (64.8)	
Type of DOAC			0.195
Direct thrombin inhibitors	3 (6.0)	51 (12.2)	
Factor Xa inhibitors	47 (94.0)	367 (87.8)	
Dose of DOAC			0.014
Underdose	18 (36.0)	133 (31.8)	
Standard dose ^a	28 (56.0)	278 (66.5)	
Overdose	4 (8.0)	7 (1.7)	
Alcohol	17 (37.0)	135 (35.4)	0.848
Smoking	7 (14.0)	57 (13.6)	0.944
Comorbidities			
Atrial fibrillation	49 (98.0)	398 (98.5)	0.788
Hypertension	34 (68.0)	282 (67.5)	0.939
Diabetes mellitus	14 (28.0)	119 (28.5)	0.945
Heart failure	5 (10.0)	78 (18.7)	0.130
Previous stroke /TIA/thromboembolism	28 (56.0)	179 (42.8)	0.076

Table 1. Baseline characteristics of the study population. (Cont'd)

	Patients with bleeding (n=50)	Patients without bleeding (n=418)	P value
Previous haemorrhage	4 (8.0)	18 (4.3)	0.277
Anaemia	22 (44.0)	107 (25.6)	0.006
Comedications			
Antiplatelet drugs	3 (6.0)	51 (12.2)	0.195
ACEI/ARBs	19 (38.0)	186 (44.5)	0.381
Beta-blockers	38 (76.0)	295 (70.6)	0.424
Calcium channel blockers	13 (26.0)	116 (27.8)	0.793
Diuretics	11 (22.0)	109 (26.1)	0.533
Statins	28 (56.0)	245 (58.6)	0.723

ACEIs: angiotensin converting enzyme inhibitors; ARBs: angiotensin II receptor blockers; DOACs: direct oral anticoagulants; NA: not available; TIA: transient ischaemic attack. ^a According to the FDA-approved labelling.

11.9%, 21.7%, 22.2%, 40.0%, and 100.0% of bleeding risk. Fig. 1 shows the predicted probability of bleeding risk versus the developed risk score; patients with 0, 1, 2, 3, 4, and 5 points were predicted to have 5.2%, 10.8%, 22.4%, 32.3%, 42.3%, and 61.8% of bleeding risk, respectively. The AUROC curve was 0.680 (95% CI 0.601–0.758).

DISCUSSION

In this study, we analysed the polymorphisms of fibrinogen-related genes and investigated whether these mutations, along with other possible confounding variables, can affect bleeding risk in patients treated with DOACs. The main finding of our study is that the DOAC-associated bleeding risk is related to *F2* rs5896 and *FGG* rs1800792, along with anaemia and overdose.

Among the clinical factors, anaemia was one of the predictors for bleeding risk. Anaemia reduces the red blood cell count in the blood and disrupts platelet adherence to endothelial cells, leading to bleeding due to impaired haemostasis.²⁵ Clinically, most bleeding risk assessment tools for patients taking anticoagulants, which were developed before DOACs were introduced, have included anaemia as a risk factor.²⁶ For example, patients received 1 point and 1.5 points for anaemia in the HEMORR₂HAGES (Hepatic or renal disease, Ethanol abuse, Malignancy, Older age, Reduced platelet count or function, Re-bleeding, Hypertension, Anaemia, Genetic factors, Excessive fall risk and

Table 2. Association between genetic polymorphisms and bleeding risk in patients treated with direct oral anticoagulants.

Genetic polymorphisms	Molecular Consequence	Grouped genotypes	Patients with bleeding (n=50)	Patients without bleeding (n=418)	P value
<i>F2</i> rs5896 (C>T)	Missense	CC, CT	26 (52.0)	279 (67.4)	0.030
		TT	24 (48.0)	135 (32.6)	
<i>F10</i> rs5960 (C>T)	Synonymous	CC	6 (12.2)	97 (23.4)	0.076
		CT, TT	43 (87.8)	318 (76.6)	
<i>FGA</i> rs2070011 (T>C)	5'-UTR	TT, TC	35 (70.0)	341 (81.8)	0.047
		CC	15 (30.0)	76 (18.2)	
<i>FGA</i> rs6050 (T>C)	Missense	TT	15 (30.0)	88 (21.1)	0.151
		TC, CC	35 (70.0)	329 (78.9)	
<i>FGA</i> rs2070022 (G>A)	3'-UTR	GG	34 (68.0)	318 (76.6)	0.179
		GA, AA	16 (32.0)	97 (23.4)	
<i>FGB</i> rs1800788 (C>T)	Upstream	CC	8 (16.0)	50 (12.1)	0.428
		CT, TT	42 (84.0)	364 (87.9)	
<i>FGB</i> rs4220 (G>A)	Intronic	GG	34 (68.0)	307 (73.6)	0.397
		GA, AA	16 (32.0)	110 (26.4)	
<i>FGB</i> rs4463047 (T>C)	Downstream	TT	15 (31.3)	90 (22.1)	0.152
		TC, CC	33 (68.8)	318 (77.9)	
<i>FGG</i> rs2066865 (G>A)	Downstream	GG	16 (32.0)	87 (20.9)	0.074
		GA, AA	34 (68.0)	329 (79.1)	
<i>FGG</i> rs1800792 (T>C)	Upstream	TT, TC	40 (80.0)	381 (91.6)	0.019
		CC	10 (20.0)	35 (8.4)	

UTR: untranslated region.

Table 3. Multivariable logistic regression analysis for bleeding risk in patients treated with direct oral anticoagulants.

Predictors	Crude OR (95% CI)	Adjusted OR (95% CI)	Attributable risk (%)
Female	1.03 (0.56–1.88)		
Age ≥65 (years)	0.91 (0.48–1.70)		
Overdose	5.11 (1.44–18.10)	6.19 (1.67–23.05)**	83.86
Anaemia	2.28 (1.25–4.16)	2.29 (1.23–4.28)**	56.43
<i>F2</i> rs5896 TT	1.91 (1.06–3.45)	2.09 (1.13–3.85)*	52.04
<i>FGA</i> rs2070011 CC	1.92 (1.000–3.70)		
<i>FGG</i> rs1800792 CC	2.72 (1.25–5.90)	2.67 (1.20–5.94)*	62.58

OR: odds ratio; CI: confidence interval. * $P<0.05$; ** $P<0.01$. Multivariable analysis was performed with the factors with $P<0.05$ in the univariate analysis along with sex and age.

Stroke) and RIETE (Registro Informatizado Enfermedad TromboEmbolica) risk scoring systems, respectively, whereas ATRIA (AnTicoagulation and Risk factors

In Atrial fibrillation) assigned 3 points for anaemia. Similar results have been observed for anaemia as a bleeding risk factor after DOAC treatment. According

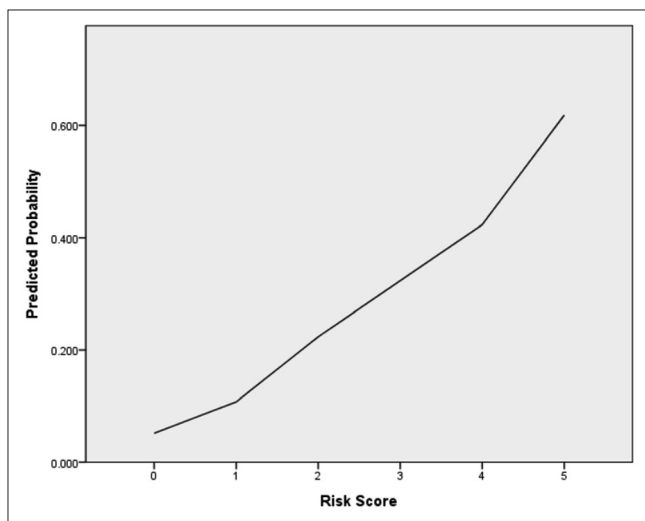


Fig. 1. The predicted probability of bleeding risk versus the developed risk score.

to the post hoc analysis of the RE-LY (Randomised Evaluation of Long-term anticoagulation therapy) trial, which evaluated dabigatran versus warfarin in patients with atrial fibrillation, anaemia was associated with a 2.1-fold higher risk of major bleeding.²⁷ The secondary analysis of ARISTOLE (Apixaban for Reduction In Stroke and Other Thromboembolic Events in atrial fibrillation), a representative randomised controlled trial of apixaban versus warfarin,²⁸ also showed that anaemia increased the major bleeding risk by 1.92 times in patients taking apixaban. Thus, as anaemia is a modifiable risk factor for bleeding, it should be properly evaluated and managed in patients taking DOACs.

DOAC overdose is the most attributable risk factor for bleeding in our study. According to recent meta-analyses for the off-label overdosing of DOAC in patients with atrial fibrillation, patients who received a DOAC overdose were more likely to experience major bleeding than patients who received an on-label dose of DOAC.^{29,30} Thus, tailored dosing of DOAC may be required for each patient for effective and safe treatment.

Among the genetic factors, rs1800792, located in the 5' flanking region of the *FGG*, was the strongest predictor for bleeding risk. This SNP was listed in the GTEx database as a significant expression quantitative trait locus for the *FGG* gene³¹ and was related to fibrinogen levels.³² Previous studies showed that fibrinogen gene haplotypes, including rs1800792, are associated with coagulation-related disorders, such as ischaemic stroke³³ and myocardial infarction.³⁴ Additional effects of rs1800792 have also been suggested; rs1800792 is associated with platelet counts³⁵ and interacts with interleukin levels.³⁶ As

coagulation and fibrinolytic systems are complex and interconnected, many biomarkers can be involved, including coagulation factors, platelets, and inflammatory cytokines.³⁷ Further studies are needed to elucidate the detailed functional mechanisms of this SNP.

In addition to *FGG* rs1800792, the TT genotype of rs5896 in *F2* is associated with an increased bleeding risk. This SNP is a missense mutation of *F2*, which substitutes threonine (Thr) with methionine (Met) at position 165 in prothrombin.²⁰ Similar results were observed in the pharmacogenetic study of warfarin, another oral anticoagulant inhibiting vitamin K-related coagulation factors. According to D'Ambrosio,³⁸ variant allele carriers of rs5896 require a lower adjusted warfarin dose than those with wild-type homozygotes (2.9 mg vs 4.2 mg), which is related to the over-anticoagulation response in variant allele carriers. Shikata et al. also showed that a variant allele of rs5896 is related to increased warfarin sensitivity.³⁹ This effect can be explained by the conformational change of prothrombin. Thr 165 is located in the kringle 1 domain of prothrombin, which is extensively involved in protein–protein interactions with other clotting factors.⁴⁰ At position 165, the substitution of Thr, an amino acid with a polar side chain, with Met, an amino acid with a non-polar side chain, may disrupt hydrogen bond interactions and induce conformational changes of the three-dimensional structure of prothrombin. Although approximately 90% of the patients received Xa inhibitors, the *F2* SNP was identified as a genetic risk factor because the conversion of prothrombin to thrombin is required to complete the coagulation cascade in the presence of coagulation factor Xa.⁷

This study has several limitations. First, only Asian populations living in Korea were included, thereby reducing the generalisability of the results. As genetic variation and its effects can vary across race and ethnic groups, it should be applied with caution. Second, there were possible confounders not considered in this scoring system, which could affect the results. Lastly, the follow-up period was limited to 1 year to exclude the possibility of non-drug-related haemorrhage.

CONCLUSION

Despite these limitations, to our knowledge, this is the first study to investigate the genetic effects of polymorphisms in the fibrinogen genes on DOAC response. Although genotyping is not routinely performed at the initiation of DOAC, this study will be helpful for further personalise DOAC therapy. To generalise the results and apply them to clinical settings, the results should be validated in different populations.

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Conflict of interest

The authors have no conflict of interest to declare.

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