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ORIGINAL RESEARCH

Genetic variations in the transcription factors *GATA4* and *GATA6* and bleeding complications in patients receiving warfarin therapy

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Purpose: GATA4 and GATA6 are known to have potential roles in vascular regulation by affecting vascular smooth muscle cell differentiation and atrial natriuretic peptide levels. The aim of this retrospective study was to investigate the associations between *GATA4* and *GATA6* polymorphisms and bleeding complication risk at a therapeutic international normalized ratio (INR) in patients with mechanical heart valves.

Patients and methods: Study patients were included from the Ewha-Severance Treatment (EAST) Group of Warfarin. It consisted of 229 patients who received warfarin therapy after undergoing mechanical heart valve replacement and maintained a stable INR (INR of 2.0–3.0 for at least three consecutive times). Twenty single-nucleotide polymorphisms including *VKORC1, CYP2C9, GATA4*, and *GATA6* were analyzed. Multivariate logistic regression analysis was employed to investigate the independent risk factors for bleeding complications. To evaluate the potential clinical value of genotyping for preventing bleeding complications in patients with high-risk genotype, the number needed to genotype (NNG) was also calculated.

Results: One hundred forty-two patients were included in this study, 21 of whom had bleeding complications. After adjusting covariates, TT genotype carriers of rs13273672 in *GATA4* and CC genotype carriers of rs10454095 in *GATA6* showed 5.0- (95% CI, 1.6–15.7) and 3.1-fold (95% CI, 1.1–8.7) higher bleeding complications than carriers of C allele and T allele, respectively. NNG for preventing one patient from experiencing bleeding complications in patients with TT genotype of rs13273672 and CC genotype of rs10454095 was 22.2 and 17.5, respectively. Patients with both TT genotype in rs13273672 and CC genotype in rs10454095 showed 8.7-fold (95% CI, 1.7–46.1) higher bleeding complications than those with other genotypes. NNG in patients having both TT genotype in rs13273672 and CC genotype in rs10454095 was calculated to be 40.0.

Conclusions: This study showed that *GATA4* and *GATA6* gene polymorphisms could affect bleeding complications during warfarin treatment in patients with mechanical heart valves.

Keywords: GATA4, GATA6, warfarin, bleeding, polymorphism

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Introduction

Warfarin is a widely used oral anticoagulant for atrial fibrillation, ischemic stroke, deep-vein thrombosis, and pulmonary embolism.[1] Despite the introduction of direct oral anticoagulants, it still remains the first-line anticoagulant therapy for patients with heart valve prosthesis.[2] Because of the narrow therapeutic range and wide inter- and intraindividual variability of warfarin, careful monitoring of international normalized ratio (INR) is required while using it for anticoagulation therapy.[3]

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Bleeding complication is one of the major concerns with warfarin usage.[4] Although an elevated INR level is the most important factor for increasing warfarin-associated bleeding risk, it has been reported that some patients also experienced bleeding complications at therapeutic INR. A study showed that 15.7% of patients suffered from bleeding complications during therapeutic INR control.[5] Some studies showed that in addition to high INR, age, hypertension, and concomitant aspirin use were patient-related risk factors for bleeding complications.[6] However, the genetic effects on bleeding complications during anticoagulation therapy have rarely been investigated.

The GATA family, the zinc finger transcription factor, consists of 6 subtypes in vertebrates. GATA-1/2/3 subfamily is expressed in the hematopoietic cell lineage,[7] whereas GATA-4/5/6 subfamily is expressed in various mesoderm- and endoderm-derived tissues including the heart, blood vessel, lung, and gut.[8] Among GATA 4/5/6 subgroups, GATA4 and GATA6 have similar protein structure and expression pattern. Moreover, both are known to be involved in the regulation of gene expression in cardiomyocytes and vascular smooth muscles.[9] In a study that employed an animal model, *GATA4/GATA6* double-heterozygous mouse showed impaired differentiation of vascular smooth muscle cells.[10]

With respect to the association between polymorphisms of *GATA4* and *GATA6* genes and cell differentiation, *GATA4* mutation p.S335X has been found to pre-terminate its translation, producing a truncated GATA4 lacking a conservative region at C-terminus. Truncated GATA4 delayed the cardiomyocyte differentiation in P19cl6 model and prohibited Bcl2 expression, leading to apoptosis.[11] In addition, GATA4 was found to be a key modifier of sex steroidogenic cell differentiation through conditional loss-of-function mutations in *GATA4* gene.[12]

The *GATA6* mutation p.E386X was identified in a family with bicuspid aortic valves, being transmitted in an autosomal dominant fashion. Cardiac valvular morphogenesis requires accurate regulation of cell proliferation, differentiation, migration, adhesion, and apoptosis. Biological assays revealed that E386X-mutant GATA6 proteins had no transcriptional activity compared with its wild-type counterpart. Furthermore, the E386X mutation led to disrupted synergistic transcriptional activation between GATA4 and GATA6.[13]

Hemostasis is a multiphase process involving blood vessels, platelets, and coagulation factors; an imbalance in any of the steps of hemostasis may result in bleeding.[14] Impaired vascular smooth muscle cell differentiation is involved in vascular malformations,[15] which are known to increase bleeding risks in several organs (eg, gastrointestinal tract, retina, and endometrium).[16–18]

In addition, atrial natriuretic peptide (ANP), the expression of which is regulated by GATA4 and GATA6, plays an important role in vascular function regulation.[19] ANP is also involved in platelet aggregation and lipid metabolism.[20] ANP level is also known to be associated with cardiovascular diseases (eg, hypertension and hyperlipidemia).[21] Although GATA4 and GATA6 have potential roles in vascular regulation, no study has yet investigated the association between *GATA* gene polymorphisms and bleeding complications in patients receiving warfarin.

Therefore, this study aimed to investigate the association between *GATA4* and *GATA6* polymorphisms and the risk of bleeding complications at therapeutic INR during warfarin treatment.

Materials and methods

Study patients and data collection

Study patients were included from the Ewha-Severance Treatment (EAST) Group of Warfarin. It consisted of 229 patients who received warfarin therapy after undergoing mechanical heart valve replacement between January 1982 and December 2009 at Severance Cardiovascular Hospital of Yonsei University College of Medicine. Patients who maintained a stable INR (INR of 2.0–3.0 for at least three consecutive times) were eligible for the study. Patients who had experienced bleeding complications at supra- or subtherapeutic INR were excluded. Patients were also excluded if their complications were not verified by health professionals.

Patients were followed up continuously at the outpatient clinic of Severance Cardiovascular Hospital of Yonsei University Medical Center. Blood samples were collected during the regularly scheduled clinic visit. Patients' first follow-up visits were within 1–2 months after discharge and patients were followed up in 1- to 3-month intervals in accordance with their therapeutic INR. In the case of bleeding occurrences, patients visited the hospital and showed bruises, gum bleeding, and nose bleeding as evidence of bleeding. During the verification of bleeding events by a doctor, INR levels were measured. Data collection was retrospectively done using scanned medical records and electronic medical records of patients from June 1983 to August 2010. Data on sex, age, body weight, height, position of valve prosthesis, valve type, warfarin therapy duration, INR measurements, concurrent medication, comorbidities,

and history of bleeding complications were collected. Bleeding complications were classified as major life-threatening, other major, any major, minor, or minimal using the scheme detailed in Platelet Inhibition and Patient Outcomes trial.[22]

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All patients gave written informed consent for participation. The protocol and informed consent were reviewed and approved by the Institutional Review Board of the Yonsei University Medical Center (approved number: 4-2009-0283).

Genotyping methods

To select single-nucleotide polymorphisms (SNPs) of *GATA4* and *GATA6* that might be associated with warfarin-related bleeding, genetic information concerning *GATA4* and *GATA6* was obtained from the PharmGKB database, Haploreg 4.1, and Database of SNP (dbSNP) from NCBI and previous studies.[23–26] Sixteen SNPs of *GATA4* (rs13273672, rs2645400, rs4841588, rs867858, rs10090884, rs2898292, rs10086064, rs3735814, rs2740434, rs2001470, rs3729849, rs809205, rs2173117, rs62489352, rs2409805, and rs2898293) and 2 SNPs of *GATA6* (rs16964670 and rs10454095) were selected. In addition to the selected SNPs, *VKORC1* rs9934438 and *CYP2C9* rs1057910, which were found to have significant effects on stable doses of warfarin, were also included in the study. Therefore, a total of 20 SNPs were investigated.

Genomic DNA from the patients was isolated from EDTA blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's protocol. Genotyping was performed using a single-base primer extension assay by employing SNaPShot multiplex kits (ABI, Foster City, CA, USA) or TaqMan genotyping assay by employing real-time PCR system (ABI 7300, ABI), according to the manufacturer's recommendation.

Statistical analysis

Continuous variables in patients with bleeding complications and in those without complications were compared using Student's *t*-test. Chi-square test or Fisher's exact test was used to compare categorical variables between the two groups. Multivariate logistic regression analysis was used to examine independent risk factors for bleeding complications. Factors having a p-value <0.05 in univariate analysis along with clinically relevant confounders were included in multivariate analysis. Odds ratio and adjusted odds ratio were calculated through univariate and multivariate analyses, respectively. The time in therapeutic range (TTR) of INR 2.0–3.0 was measured using Rosendaal method. Attributable risk (%) was calculated by (1–1/adjusted odds ratio) □ 100. To test the model's goodness of fit, we performed a Hosmer–Lemeshow test. Discrimination of the model was further assessed using an analysis of the area under the receiver operating curve (AUROC), which assesses the ability of the risk factor to predict bleeding. We calculated the number needed to genotype (NNG) for preventing one patient from experiencing a significantly higher incidence of bleeding complications by 1/absolute risk reduction. Absolute risk reduction was achieved by multiplying the relative risk reduction by genotyping and risk of higher incidence of bleeding complications without genotyping. A p-value of <0.05 was considered statistically significant. All statistical analyses were conducted using IBM SPSS statistics, version 20 software (International Business Machines Corp., Armonk, NY, USA).

Results

Of the 229 patients from the EAST Group of Warfarin, 87 patients were excluded due to the following reasons: 28 patients did not reach a stable INR, 4 patients had bleeding complications at supratherapeutic INR, and 55 patients reported minimal bleeding complications which were not verified by health professionals. Accordingly, data from 142 patients who underwent heart valve replacement were used for the analysis.

The median age of the included patients was 60 years (range, 34–81 years), and there were 52 (36.6%) males. The follow-up periods ranged from 1.0 to 29.7 years (mean 14.3 years). During the follow-up period, one thromboembolic event was observed, and there were no deaths. The mean INR monitoring interval was 2.9 months, and the average number of INR measurements per patient was 23. The TTR of INR 2.0–3.0 was 55.2±12.7%. As shown in Table 1, 21 patients (14.8%) experienced bleeding complications at therapeutic INR. Among them, 11 and 10 patients experienced minor and minimal bleeding complications, respectively. One patient experienced bleeding four times, seven patients twice, and 13 patients once. There was no significant difference between the two groups except for atrial fibrillation. Patients with atrial fibrillation had more bleeding

Table 1 Characteristics of study patients

Characteristics	Bleeding complication, patient number (%)		*p*
	Presence (n=21)	Absence (n=121)	
Sex			0.705
Male	8 (38.1)	44 (36.4)	
Female	13 (61.9)	77 (63.6)	
Age (years)			0.106
<65	11 (52.4)	85 (70.2)	
≥ 65	10 (47.6)	36 (29.8)	
Mean±SD	62.0±11.2	58.7±10.0	0.168
Body weight (kg)			0.989
Mean±SD	58.6±10.7	58.7±10.4	
Body mass index (kg/m^2)			0.756
Mean±SD	22.3±2.3	22.5±2.8	
Comorbidity			
Hypertension	6 (28.6)	33 (27.3)	0.902
Diabetes mellitus	3 (14.3)	10 (8.3)	0.377
Chronic heart failure	7 (33.3)	25 (20.7)	0.199
Atrial fibrillation	17 (81)	70 (57.9)	0.045
Myocardial infarction	2 (9.5)	2 (1.7)	0.104
Co-medication			
Angiotensin-converting-enzyme inhibitor	2 (10.5)	19 (18.8)	0.383
Angiotensin II receptor blocker	4 (21.1)	19 (18.8)	0.820
Antiplatelet drugs	0 (0)	4 (3.8)	0.398
Calcium channel blocker	4 (21.1)	19 (18.8)	0.820
Diuretics	9 (47.4)	35 (34.7)	0.291
Statins	0 (0)	4 (4.0)	0.378
Valve position			0.740
Aortic	6 (28.6)	28 (23.1)	
Mitral	9 (42.9)	66 (54.5)	
Double[a]	5 (23.8)	20 (16.5)	
Tricuspid[b]	1 (4.8)	7 (5.8)	
Valve type			0.418
St. Jude Medical	7 (38.9)	39 (34.2)	
CarboMedics	6 (33.3)	32 (28.1)	
ATS	2 (11.1)	15 (13.2)	
MIRA	1 (5.6)	9 (7.9)	
Duromedics	2 (11.1)	6 (5.3)	
OnX	0 (0)	4 (3.5)	
Others[c]	0 (0)	9 (7.9)	
INR			0.143
Mean±SD	2.41±0.07	2.45±0.10	
Follow-up time (years)			0.886
Median (range)	14.3 (1.4–29.7)	14.7 (1.0–27.7)	
Time in therapeutic range (%)			0.066
Mean ± SD	50.5±13.9	56.0±12.3	

Notes: [a]Aortic plus mitral valve, [b]tricuspid valve with or without other valves, [c]including Sorin, Bjork Shiley, D-ring, and prostheses using two or more different valve types.

complications in therapeutic INR than those without atrial fibrillation (p=0.045).

As shown in Table 2, statistically significant associations between genotypes and bleeding complications were found for rs13273672, rs4841588, and rs2173117 of *GATA4*. For *GATA6*, rs10454095 showed a significant association with bleeding complications. For rs13273672, 8 of 26 patients (30.8%) with TT genotype had bleeding complications, whereas 13 of 116 patients (11.2%) with C allele had bleeding complications (p=0.027). For rs4841588, patients with wild-type homozygote showed a higher bleeding risk than those with variant-allele

Table 2 Factors associated with bleeding complications at therapeutic INR

Gene polymorphism	Allele change	Minor allele frequency	Grouped genotypes	Bleeding complication, number (%)		OR	95% CI for OR		*P*
				Presence (n=21)	Absence (n=121)		Lower	Upper	
VKORC1	C>T	0.113	CC, CT	3 (14.3)	27 (22.3)	1			0.405
rs9934438			TT	18 (85.7)	94 (77.7)	1.723	0.472	6.292	
CYP2C9	A>C	0.043	AA	18 (85.7)	111 (92.5)	1			0.304
rs1057910			AC	3 (14.3)	9 (7.5)	2.056	0.508	8.322	
GATA4	T>C	0.433	TT	8 (38.1)	18 (14.9)	1			0.027
rs13273672			CT, CC	13 (61.9)	103 (85.1)	0.284	0.103	0.782	
GATA4	T>G	0.373	TT, TG	15 (71.4)	106 (87.6)	1			0.054
rs2645400			GG	6 (28.6)	15 (12.4)	2.825	0.951	8.403	
GATA4	G>T	0.384	GG	7 (33.3)	15 (12.4)	1			0.023
rs4841588			GT, TT	14 (66.7)	106 (87.6)	0.283	0.098	0.814	
GATA4	A>C	0.486	AA	8 (38.1)	25 (20.7)	1			0.081
rs867858			AC, CC	13 (61.9)	96 (79.3)	0.423	0.158	1.133	
GATA4	A>C	0.349	AA, AC	14 (66.7)	67 (55.4)	1			0.334
rs10090884			CC	7 (33.3)	54 (44.6)	0.620	0.234	1.646	
GATA4	T>C	0.159	TT, TC	7 (33.3)	33 (28.9)	1			0.390
rs2898292			CC	14 (66.7)	81 (71.1)	0.815	0.302	2.203	
GATA4	C>T	0.134	CC, CT	5 (23.8)	31 (25.6)	1			0.860
rs10086064			TT	16 (76.2)	90 (74.4)	1.102	0.373	3.259	
GATA4	G>A	0.099	GA	4 (19.0)	24 (19.8)	1			0.933
rs3735814			AA	17 (81.0)	97 (80.2)	1.052	0.324	3.413	
GATA4	A>G	0.088	AG	4 (19.0)	21 (17.4)	1			0.851
rs2740434			GG	17 (81.0)	100 (82.6)	0.893	0.272	2.923	
GATA4	C>T	0.310	CC	4 (19.0)	9 (7.4)	1			0.089
rs2001470			CT, TT	17 (81.0)	112 (92.6)	0.342	0.095	1.233	
GATA4	A>G	0.433	AA	7 (33.3)	18 (14.9)	1			0.059
rs3729849			AG, GG	14 (66.7)	103 (85.1)	0.350	0.124	0.985	
GATA4	T>C	0.257	TT	4 (19.0)	7 (5.8)	1			0.059
rs809205			TC, CC	17 (81.0)	114 (94.2)	0.261	0.069	0.987	
GATA4	C>A	0.358	CC	6 (30.0)	14 (11.6)	1			0.040
rs2173117			CA, AA	14 (70.0)	107 (88.4)	0.305	0.101	0.923	
GATA4	C>T	0.257	CC	8 (38.1)	70 (57.9)	1			0.093
rs62489352			CT, TT	13 (61.9)	51 (42.1)	2.230	0.861	5.777	
GATA4	T>C	0.163	TT, TC	20 (95.2)	116 (96.7)	1			0.744
rs2409805			CC	1 (4.8)	4 (3.3)	1.450	0.154	13.649	
GATA4	A>G	0.211	AA, AG	11 (52.4)	43 (35.5)	1			0.142
rs2898293			GG	10 (47.6)	78 (64.5)	0.501	0.197	1.275	
GATA6	G>A	0.135	GG	18 (85.7)	86 (71.7)	1			0.177
rs16964670			GA, AA	3 (14.3)	34 (28.3)	0.422	0.117	1.524	
GATA6	T>C	0.398	TT, TC	9 (42.9)	82 (67.8)	1			0.028
rs10454095			CC	12 (57.1)	39 (32.2)	2.803	1.090	7.210	

carriers (31.8% vs 11.7%, p=0.023). A allele carriers of rs2173117 showed approximately 70% lower bleeding complications than CC genotype carriers (p=0.040). For rs10454095 of *GATA6*, CC genotype carriers experienced more bleeding complications than T allele carriers (23.5% vs 9.9%, p=0.028). The allele frequencies of SNPs used in this study for Koreans and other populations are described in Tables 2 and S1, respectively.

Two models were constructed for conducting multivariate analysis (Table 3). Model I included sex, age, and factors having a p-value <0.05 in the univariate analysis, including atrial fibrillation, rs13273672, rs4841588, and rs2173117 of *GATA4*, as well as rs10454095 of *GATA6*. Model II included sex, age, atrial fibrillation, rs4841588, rs2173117, and a combination of rs13273672 and rs10454095.

As shown in Model I of Table 3, rs13273672 of *GATA4* and rs10454095 of *GATA6* were significantly associated with bleeding complications (p=0.006 and p=0.031, respectively). After adjusting for related covariates, TT genotype carriers in rs13273672 showed approximately 5.0-fold higher bleeding complications than C allele carriers. For rs10454095 of *GATA6*, CC genotype carriers showed approximately 3.1-fold higher bleeding complications than T allele carriers after adjusting for covariates. NNG for preventing one patient with TT genotype in rs13273672 from suffering a higher incidence of bleeding complications was calculated to be 22.2. NNG of rs10454095 in *GATA6* was 17.5. In Model II, patients with both TT genotype in rs13273672 and CC genotype in rs10454095 showed 8.7-fold higher bleeding complications than those with the other genotypes. NNG in patients having both TT genotype in rs13273672 and CC genotype in rs10454095 was calculated to be 40.0.

The AUROC values of Model I and Model II were 0.770 and 0.724, respectively (Figure 1). The Hosmer–Lemeshow test showed that the fitness of the Model I was satisfactory (χ^2=2.396, 7 degrees of freedom, p=0.935) as well as for Model II (χ^2=0.618, 3 degrees of freedom, p=0.892).

Discussion

The main finding of this study is that rs13273672 of *GATA4* and rs10454095 of *GATA6* were associated with bleeding complications at a therapeutic INR during warfarin treatment in mechanical heart valve patients. TT genotype carriers of rs13273672 in *GATA4* and CC genotype carriers of rs10454095 in *GATA6* had 5.0- and 3.1-fold increased risk of bleeding complications compared with the carriers of C allele and T allele, respectively. Patients having a combination of TT and CC genotypes of rs13273672 and rs10454095 experienced 8.7 times higher bleeding complications than those having the other genotypes. The AUROC value of the models constructed for predicting bleeding complications was approximately 0.75.

Stable INR was defined as the INR of 2.0–3.0 for three or more consecutive visits. Although American College of Chest Physicians guidelines 2012 suggest INR of 2.5–3.5 in

Table 3 Multivariate analysis to identify predictors of bleeding complications at therapeutic INR

Variables	Model I		Model II	
	Adjusted OR (95% CI)	Attributable risk (%)	Adjusted OR (95% CI)	Attributable risk (%)
Age ≥ 65 years	2.43 (0.87–6.84)		2.41 (0.88–6.55)	
Atrial fibrillation	3.00 (0.91–9.86)		2.76 (0.85–8.98)	
GATA4 rs13273672, TT	5.01 (1.60–15.72)**	84.3		
GATA6 rs10454095, CC	3.10 (1.11–8.71)*	67.7		
GATA4/*GATA6* rs13273672/rs10454095 TT/CC			8.73 (1.66–46.06)*	88.6

Notes: Logistic regression analyses were carried out with variables such as sex, age, atrial fibrillation, rs13273672, rs4841588, rs2173117, and rs10454095 for Model I, and sex, age, atrial fibrillation, rs4841588, rs2173117, and rs13273672/rs10454095 combination for Model II.
*p<0.05, **p<0.01.

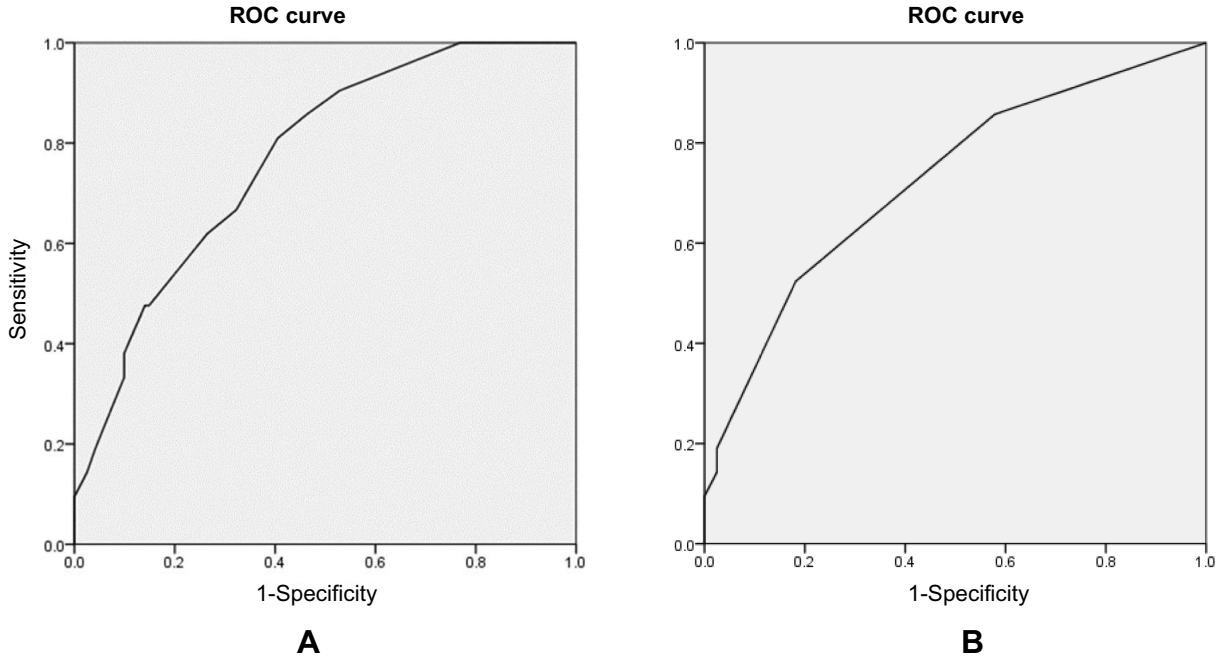

Figure 1 Area under the receiver-operating characteristic (ROC) curve for bleeding complications at a therapeutic INR. (**A**) Area under the curve (AUC) for Model I. AUC is 0.770 (95% CI, 0.672–0.868; $p<0.001$). (**B**) AUC for Model II. AUC is 0.724 (95% CI, 0.602–0.847; $p=0.001$).

patients with mitral valve replacements,[27] many studies of Asian populations suggested considerably lower intensities of warfarin therapy after mechanical valve prostheses including mitral valves.[28–30]

GATA4, a gene located on chromosome 8, is expressed in cardiomyocytes, smooth muscles, and endothelial cells in the heart and blood vessels.[8] It regulates the expression of cardiovascular-related genes, which encode α- and β-myosin heavy chain, cardiac troponin-C, ANP, and brain natriuretic peptide.[8,9]

Among proteins which are regulated by GATA4, the roles of ANP in vasculature is well-established. It exerts vasodilatory effects through the ANP receptor, which is guanylyl cyclase-A.[19] Additionally, it modulates vascular smooth muscle cell proliferation[31] as well as endothelial cell growth and permeability.[32] It is also known that it is reportedly involved in platelet aggregation and energy metabolism processes such as lipolysis.[33,34] It was shown that the ANP gene (*NPPA*) mutation increased platelet aggregation in vitro as well as in clinical settings.[35] In another study, *NPPA* mutation increased residual platelet reactivity in patients with diabetes mellitus who underwent elective percutaneous coronary intervention.

Accordingly, GATA4 is considered to modulate vessel function and platelet aggregation via ANP regulation. In addition to ANP, vascular endothelial growth factor (VEGF) is also known to be regulated by GATA4. VEGF, an angiogenic cytokine, is related to vessel formation and vascular density.[36] Therefore, the effect of *GATA4* polymorphisms on bleeding complications was considered to be partially attributable to an altered vessel formation and function.

Rs13273672 is an introgenic SNP of *GATA4*. In several studies, this SNP was associated with alcohol dependence,[37,38] relapse to alcohol drinking, and treatment response to acamprosate.[39] In Kiefer et al study, patients with G allele of rs13273672 showed low ANP expression; therefore, the underlying mechanism of the rs13273672 effect on alcohol dependence and treatment was explained by the altered ANP expression. We found that rs13273672 of *GATA4* had a significant association with bleeding complications at a therapeutic INR, with homozygous wild-type carriers having increased bleeding risk in both univariate and multivariate analyses. This was also thought to be caused by the alteration in ANP level.

GATA6, which is highly expressed in vascular smooth muscle cells, is known to regulate vascular smooth muscle cell proliferation and its reversible differentiation in vascular injury.[40,41] Similar to GATA4, GATA6 is also an upstream regulator of multiple genes expressed during embryogenesis and cardiac morphogenesis, including the gene that encodes the ANP.[42]

Various studies showed that the *GATA6* mutant demonstrated a significantly decreased transcriptional activity on the ANP promoter.[43–45] Several studies have shown that GATA4 and GATA6 act cooperatively and synergistically to regulate smooth muscle cells, with similar structure and expression patterns.[10,46]

In our study, one SNP of *GATA6* (rs10454095) exhibited a significant association with bleeding complication. Although rs10454095 is rarely studied, polymorphisms of *GATA6* might cause an alteration in transcriptional activity and blood vessel regulation, thereby increasing bleeding risk. When rs10454095 in the *GATA6* gene was combined with rs13273672 in the *GATA4* gene, it showed an additive effect of each SNP on bleeding complications.

A recent study investigating the association between *GATA6* polymorphisms and congenital malformations like bicuspid aortic valve included rs10454095. Although rs10454095 was not associated with bicuspid aortic valve in this study, the authors revealed that three *GATA6* gene variants were associated with bicuspid aortic valve. Since the effects of gene polymorphisms may vary according to target organs or diseases, different SNPs could be found to be associated with different outcomes. Meanwhile, the results indicated the role of *GATA6* polymorphisms on organ formation.[47]

GATA4 showed to play an important role on the regulation of *CYP2C9* gene expression.[48] However, patients with polymorphisms of *VKORC1* or *CYP2C9* received dose adjustment according to INR measurement, and increased risk of bleeding complication was not found in our study.

To evaluate the potential clinical value of SNP genotyping, which was found to be significant in this study, we calculated NNG for preventing bleeding complications in patients with high-risk genotypes. Using the equations, 22.2, 17.5, and 40.0 were determined to be NNG values in patients with high-risk genotypes of rs13273672, rs10454095, and both SNPs, respectively, indicating that prospective SNP genotyping could be cost-effective in clinical practice.

The limitations of our study are its small sample size and retrospective design. Another shortcoming is a lack of detailed mechanisms. Nevertheless, to our knowledge, this is the first study to investigate the effects of genetic variations in *GATA4* and *GATA6* genes on warfarin-associated bleeding complications at a therapeutic INR. In addition, this study provides the prediction models for bleeding risk using various statistical tools (eg, attributable risk, AUROC, and NNG), which can be applied for developing individualized drug therapy with warfarin.

Since this study dealt with patients with INR 2–3, only minimal or minor bleeding events were observed. While there is no doubt that fatal and major hemorrhages are of essential importance, minor bleedings are also important, because they serve as an alert for subsequent major bleedings and may increase the number of visits to clinics and sometimes the emergency room, which results in additional expenditures. They also can result in permanent withdrawal of warfarin therapy, thus depriving patients of the effective therapy available.

In this study, to avoid the possible loss of the true positives, multiple test correction was not performed. It is based on a rigorous follow-up of a cohort of patients with cardiac valve replacements to detect bleeding complications based on objective measurements, followed by a systematic and thorough exploration of polymorphisms in four genes of potential interest to the etiology of bleedings. Bonferroni correction was not applied, as this is considered overly conservative for a hypothesis-generating study. We found possible associations between *GATA* genes and bleeding risks; however, it should be implemented with a caution with the risk of false-positive results and it is needed to be verified by further replication studies.

Conclusion

This study showed that rs13273672 of *GATA4* and rs10454095 of *GATA6* were associated with bleeding complications at a therapeutic INR during warfarin treatment for mechanical heart valve patients. Given the retrospective study design and the relatively small sample size, our hypothesis requires further independent validation using a prospective study design with a large sample size.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 Allele frequencies of 20 single-nucleotide polymorphisms (SNPs) analyzed in the study

Gene	SNP	Allele change	Variant allele frequencies in indicated populations				
			Study patients (Korean)	Africans[a]	Americans[a]	Asians[a]	Europeans[a]
VKORC1	rs9934438	C>T	0.89	0.07	0.44	0.92	0.40
CYP2C9	rs1057910	A>C	0.96	0.01	0.06	0.04	0.06
GATA4	rs13273672	T>C	0.57	0.36	0.32	0.59	0.30
GATA4	rs2645400	T>G	0.63	0.08	0.32	0.31	0.33
GATA4	rs4841588	G>T	0.62	0.22	0.19	0.67	0.14
GATA4	rs867858	A>C	0.51	0.25	0.30	0.55	0.31
GATA4	rs10090884	A>C	0.65	0.30	0.17	0.65	0.09
GATA4	rs2898292	T>C	0.84	0.36	0.18	0.80	0.10
GATA4	rs10086064	C>T	0.87	0.51	0.66	0.80	0.66
GATA4	rs3735814	G>A	0.90	0.53	0.59	0.88	0.47
GATA4	rs2740434	A>G	0.91	0.73	0.71	0.90	0.65
GATA4	rs2001470	C>T	0.69	0.11	0.16	0.67	0.09
GATA4	rs3729849	A>G	0.57	0.17	0.45	0.59	0.47
GATA4	rs809205	T>C	0.74	0.85	0.59	0.75	0.69
GATA4	rs2173117	C>A	0.64	0.26	0.37	0.67	0.31
GATA4	rs62489352	C>T	0.26	0.10	0.32	0.30	0.35
GATA4	rs2409805	T>C	0.16	0.15	0.62	0.21	0.66
GATA4	rs2898293	A>G	0.79	0.13	0.62	0.75	0.65
GATA6	rs16964670	G>A	0.14	0.02	0.20	0.18	0.13
GATA6	rs10454095	T>C	0.60	0.44	0.60	0.61	0.68

Note: [a]Haploreg v4.1.