The β-fibrinogen gene 455G/A polymorphism associated with cardioembolic stroke in atrial fibrillation with low CHA2DS2-VaSc score

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Previous work has suggested that ischemic stroke (IS) may be more likely to occur in individuals with a genetic predisposition. In this study, we investigated the potential association of IS-relevant genetic risk factors with cardioembolic stroke (CES) in atrial fibrillation (AF) patients with low CHA2DS2-VaSc score. Genotyping was performed using the GenomeLab SNPstream genotyping platform for five IS-relevant SNPs (MMP-9 C1562T, ALOX5AP S1314A/T, MTHFR 677 C/T, FGB 455 G/A, and eNOS G298A) in 479 AF patients with CES and 580 age and sex-matched AF patients without CES. The multivariate analysis adjusted for potential confounders and demonstrated that FGB 455 G/A was independently associated with increased risk of CES in AF patients and the significance remained after Bonferroni correction in the additive, dominant, and recessive models with ORs of 1.548 (95% CI: 1.251–1.915, \( P = 0.001 \)), 1.588 (95% CI: 1.226–2.057, \( P = 0.003 \)), and 2.394 (95% CI: 1.357–4.223, \( P = 0.015 \)), respectively. Plasma fibrinogen levels were significantly higher in patients with the A allele compared with patients with genotype of GG (3.29 ± 0.38 mg/dl vs. 2.87 ± 0.18 mg/dl, \( P < 0.001 \)). We found for the first time that the A allele of FGB 455 G/A was a risk factor for CES in AF patients, probably by elevating the level of plasma fibrinogen.

Ischemic stroke (IS) is the most common type of stroke in China, resulting in a heavy socioeconomic burden. Cardioembolic stroke (CES) accounts for approximately one third of all IS and is considered one of the more preventable types of strokes1. Atrial fibrillation (AF) is the most frequent cause of CES, but this risk varies widely among AF patients and depends on the presence of various stroke risk factors2. To date, several clinical risk factors have been identified that contribute to the pathogenesis of IS in AF patients, including age, hypertension, diabetes mellitus, congestive heart failure, vascular disease, and female3,4. These clinical risk factors have been used to formulate stroke risk stratification schemes, such as CHA2DS2 and CHA2DS2-VaSc scores. Patients with CHA2DS2-VaSc score \( \geq 2 \) were defined as high risk and would be recommended to receive oral anticoagulation therapy. Patients with scores of 0 and 1 are defined as low risk, but a proportion of these patients suffer from CES. The risk of CES in AF patients with low CHA2DS2-VaSc score have often been underestimated, especially in Asian people6–7. The American guidelines suggest anti-platelets therapy may be recommended in patients with a CHA2DS2-VaSc score of 18. It would be of great clinical significance to identify individuals at relatively high risk of CES with a lower CHA2DS2-VaSc score of either 0 or 1.

Recent work has suggested that IS may be more likely to occur in individuals with a genetic predisposition9. Previously published reports demonstrated a relationship between the IS and functional variation evidenced by single nucleotide polymorphisms (SNPs) in the matrix metallopeptidas-9 gene (MMP-9)10–11, the arachidonate 5-lipoxygenase-activating protein gene (ALOX5AP)12–14, the methylene tetrahydrofolate reductase...
gene (MTHFR)\textsuperscript{15}, the β-fibrinogen gene (FGB)\textsuperscript{16,17}, and the endothelial nitric oxide synthase gene (eNOS)\textsuperscript{18,19}. However, whether IS-relevant genetic factors convey a risk for CES in AF patients with low CHA\textsubscript{2}DS\textsubscript{2}-VASc score remains unknown. The identification of genes causally related to CES may provide a better understanding of CES pathogenesis and may potentially inform the development of therapies for the prevention of CES in AF patients with low CHA\textsubscript{2}DS\textsubscript{2}-VASc score, thus there is a critical need to identify the genetic risk of CES in this population. Appropriate anticoagulation is the most significant factor that determines the occurrence of CES in AF patients. Therefore, our study subjects were restricted to patients with low CHA\textsubscript{2}DS\textsubscript{2}-VASc score who received no anticoagulation therapy. The aim of this study was to identify genetic factors that can predict CES in non-valvular AF patients with a low CHA\textsubscript{2}DS\textsubscript{2}-VASc score.

**Materials and Methods**

**Study population.** A total of 479 consecutive AF patients (score = 0 or 1) with an initial diagnosis of CES from the Second Affiliated Hospital of Zhejiang University were enrolled in the study. All patients were diagnosed at the hospital from January 2012 to December 2015. Control subjects (n = 580) were AF subjects who underwent a routine medical check-up in the outpatient clinic of the Department of Cardiology at the Second Affiliated Hospital of Zhejiang University during the same period. The controls were frequency-matched to cases on the basis of age and sex. The CHA\textsubscript{2}DS\textsubscript{2}-VASc score was calculated for each patient as follows: two points were assigned for a history of stroke or transient ischemic attack (TIA), or age ≥75 years; and 1 point was assigned for heart failure, hypertension, age 65–74 years, diabetes mellitus, vascular disease, and female sex\textsuperscript{20}.

AF was diagnosed according to the 2014 AHA/ACC/HRS Guideline for the Management of Patients with Atrial Fibrillation\textsuperscript{8}. AF was diagnosed by 12-lead electrocardiogram or 24 h dynamic electrocardiogram and only those patients with documented AF (>6 minutes) were included. Exclusion criteria are as follows: patients with history of cerebral ischemic events; receiving oral anticoagulation therapy; with severe hepatic or renal dysfunction; with congenital heart disease; with rheumatologic disorders; with organic valvular heart diseases; with infective endocarditis; with hyperthyroidism; or with tumors or severe infections.

At admission, data on patient characteristics, including age, gender, body mass index (BMI), the history of hypertension, diabetes mellitus, vascular disease, congestive heart failure, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), plasma fibrinogen level, platelet count, D-dimer, high sensitive C reaction protein (hs-CRP), left atrial diameter (LAD), left ventricle ejection fraction (LVEF), lifestyle (e.g., cigarette smoking and alcohol consumption), and antiplatelet therapy. Fibrinogen levels were measured in blood samples derived from peripheral venous punctures on the day of hospital admission. All participants were unrelated Han Chinese who were consecutively selected from the same geographic region. The protocol in this study conformed to the principles of the Declaration of Helsinki and was ratified by the Human Ethical Committee of the Second Affiliated Hospital of Zhejiang University. Informed consent was obtained from all subjects.

**CES diagnosis.** CES was diagnosed according to the TOAST criteria and based on the clinical findings, neuroimaging data (cranial magnetic resonance imaging (MRI) and/or computed tomography (CT)), and results of diagnostic studies such as cardiac imaging (echocardiography), ECG, duplex imaging of extracranial arteries, and laboratory evaluation\textsuperscript{31}. Unless other investigations (e.g. high-grade internal carotid artery stenosis) showed otherwise, all IS in AF patients were defined as CES.

**Selection of SNPs and genotyping.** Five SNPs were selected based on positive associations in previous studies with a minor allele frequency (MAF) of >5% in the Chinese Han population (http://www.1000genomes.org/) and underlying biological plausibility. The following SNPs were determined: MMP-9 gene C1562T (rs\#3918242), ALOX5AP gene SG13S114A/T (rs\#10507391), MTHFR gene 677 C/T (rs\#1801133), FGB gene 455 G/A (rs\#1800790), and eNOS gene G894T (rs\#1799983) (Table 1).

| Gene    | SNP                      | rs number       | Locus             | Major/minor | Variant class |
|---------|--------------------------|-----------------|-------------------|--------------|---------------|
| MMP-9   | C1562T                   | rs3918242       | 20q13             | C/T         | 5′UTR         |
| ALOX5AP | SG13S114A/T              | rs10507391      | 13q12             | A/T         | Intrinsic     |
| MTHFR   | 677 C/T                  | rs1801133       | 1p36              | G/T         | Exonic (Ala-Val) |
| FGB     | 455 G/A                  | rs1800790       | 4q31              | G/A         | 5′UTR         |
| eNOS    | G894T                    | rs1799983       | 7q35–36           | G/T         | Exonic (Glu-Asp) |

Table 1. Genomic characteristic of studied SNPs. SNP: single nucleotide polymorphism; UTR: untranslated region; MMP-9: matrix metalloproteinase-9 gene; ALOX5AP: arachidonate 5-lipoxygenase-activating protein gene; MTHFR: methylene tetrahydrofolate reductase gene; FGB: the β-fibrinogen gene; and eNOS: endothelial nitric oxide synthase gene.

The genomic DNA was isolated from whole blood samples using the whole blood DNA kit (Tiangen Biotech, Beijing, China). The concentration of DNA was diluted to 20 ng/μl for working solutions and the isolated DNA was stored at −20 °C. SNP genotyping was conducted by Orchid BioSciences using the Genomelab SNPstream genotyping platform (Beckman Statistical analyses) and SNPstream software suite. Two independent research assistants read the results with blindness of cases and controls. For quality control, distilled water was used as a negative control. Ambiguous genotyping results were verified by sequencing analysis.
**Table 2.** Characteristics of the participants. AF: atrial fibrillation; CES: cardioembolic stroke; BMI: body mass index; LVEF: left ventricle ejection fraction; LAD: left atrial diameter; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; hs-CRP: high sensitivity C-reactive protein.

| Variable                  | AF patients with CES (n = 479) | AF patients without CES (n = 580) | P     |
|---------------------------|-------------------------------|----------------------------------|-------|
| Age (years)               | 65.18 ± 5.26                 | 65.11 ± 5.67                     | 0.834 |
| Female, n (%)             | 53(11.1%)                     | 72(12.4%)                        | 0.498 |
| BMI (kg/m²)               | 23.49 ± 2.85                  | 23.33 ± 2.90                     | 0.372 |
| Smoking, n (%)            | 177(37.0%)                    | 210(36.2%)                       | 0.802 |
| Drinking, n (%)           | 161(33.6%)                    | 189(32.6%)                       | 0.724 |
| Non-paroxysmal AF, n (%)  | 232(48.4%)                    | 275(47.4%)                       | 0.741 |
| History of AF (IQR, months) | 33(26–36)                   | 31(26–35.5)                      | 0.376 |
| Hypertension, n (%)       | 100(20.9%)                    | 68(11.7%)                        | < 0.001 |
| Diabetes mellitus, n (%)  | 29(6.1%)                      | 32(5.5%)                         | 0.709 |
| Vascular disease, n (%)   | 19(3.8%)                      | 18(3.3%)                         | 0.671 |
| Congestive heart failure, n (%) | 8(1.6%)                 | 8(1.3%)                          | 0.699 |
| CHA_2DS_2-VaSc score      | 0.94 ± 0.24                   | 0.87 ± 0.34                      | < 0.001 |
| 0                         | 29(6.0%)                      | 77(13.3%)                        |       |
| 1                         | 450(94.0%)                    | 503(86.7%)                       |       |
| LVEF (%)                  | 60.77 ± 5.31                  | 60.87 ± 5.10                     | 0.751 |
| LAD (mm)                  | 38.26 ± 4.90                  | 38.34 ± 4.72                     | 0.015 |
| LDL-C (mmol/l)            | 3.08 ± 0.53                   | 2.99 ± 0.55                      | 0.089 |
| HDL-C (mmol/l)            | 1.36 ± 0.27                   | 1.37 ± 0.32                      | 0.559 |
| Fibrinogen (mg/dl)        | 3.07 ± 0.38                   | 3.00 ± 0.31                      | 0.003 |
| Platelet count (10^9/l)   | 205 ± 3.36                    | 203 ± 3.5                        | 0.488 |
| D-dimer (mg/dl)           | 0.31 ± 0.07                   | 0.30 ± 0.08                      | 0.320 |
| hs-CRP (mg/l)             | 3.92 ± 0.56                   | 3.01 ± 0.56                      | 0.011 |
| Antiplatelet therapy, n (%) | 226(47.2%)                | 267(46.0%)                       | 0.710 |

**Statistical analyses.** Mean ± SD, median, and interquartile were separately used to describe the continuous variables with normal and skewed distribution. Student’s t test, or Mann-Whitney U test were applied to compare demographic and clinical data between groups as appropriate. Categorical variables were represented by frequencies and percentages, and were compared using chi-squared tests. Allele case-control comparisons were analyzed by Pearson’s chi-square test or Fisher’s exact test. The Hardy-Weinberg equilibrium (HWE) was independently evaluated for each polymorphism. Logistic regression was performed to assess the association between the presence of a particular genotype and CES. The following analytical methods were used to compare the subjects from two groups: allelic frequency distribution of the two groups (allele A versus allele B, A as the major allele, B as the minor allele, this also applied to the following methods); additive model (BB versus AB versus AA); dominant model (BB versus AB versus AA); and recessive model (BB versus AA + AB). All the genetic models of the minor allele were performed with or without adjustment for confounding risk factors. All odds ratios (ORs) were given with the 95% confidence interval (CI). Furthermore, the Bonferroni correction was used to define the effective number of independent marker loci. Statistical analyses were performed using SAS Version 9.1 (SAS Institute, Cary, North Carolina, USA). A two-sided P value < 0.05 was considered to be statistically significant.

**Data Availability.** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

**Results**

**Characteristics of the included subjects.** A total of 1059 AF patients (479 CES patients and 580 controls) participated in the study. The age and sex of the participants from the two groups were matched (P = 0.834 and 0.498, respectively). Compared with the control group, patients with CES had significantly higher prevalence of hypertension (20.9% vs. 11.7%, P < 0.001), larger LAD (38.26 ± 4.90 mm vs. 38.54 ± 4.72 mm, P = 0.015), higher LDL-C level (3.08 ± 0.53 vs. 2.99 ± 0.55, P = 0.009), higher plasma fibrinogen level (3.07 ± 0.38 mg/dl vs. 3.00 ± 0.31 mg/dl, P = 0.003), higher hs-CRP level (3.92 ± 0.56 mg/l vs. 3.11 ± 0.56 mg/l, P = 0.011), and higher CHA_2DS_2-VaSc score (0.94 ± 0.24 vs. 0.87 ± 0.34, P < 0.001). There was no significant difference between the two groups for BMI, smoking, drinking, AF type, history of AF, diabetes mellitus, vascular disease, congestive heart failure, LVEF, HDL-C, platelet count, D-dimer, and antiplatelet therapy (Table 2).

**Genotypic and allelic distributions of the five SNPs.** The genotyping success rates of the five SNPs ranged from 99.6–100%. The veracity of the results was confirmed by direct sequencing of PCR products amplified from randomly selected samples. The direct sequencing results were consistent with all corresponding
genotyping results. All genotypes were distributed in concordance with Hardy-Weinberg equilibrium (HWE) with a value of \( P > 0.05 \) in control group, minimizing the possibility of selection bias.

Of the five SNPs, significant differences in genotypic and allelic distribution were only identified for the FGB455 G/A polymorphism between the CES and control groups (\( P = 0.008 \) and \( P < 0.001 \), respectively) (Table 3).

There were more A allele carriers of the FGB455 G/A polymorphism in CES group compared with the number in the control group (25.5% vs. 18.9%, \( P < 0.001 \)).

**Table 4.** Association between FGB 455 G/A polymorphism and risk of CES in AF patients. *Crude ORs were calculated by univariate logistic regression analysis. **Adjusted ORs were obtained from multivariate logistic regression additionally adjusted by age, sex, BMI, smoking, drinking, hypertension, diabetes mellitus, heart failure, vascular disease, LAD, AF type, LDL, and CRP. Additive model (AA vs. GA vs. GG). Dominant model (AA + GA vs. GG). Recessive model (AA vs. GA + GG). OR: odds ratio; CI: confidence interval.***

| Gene     | SNP       | Genotype | AF patients with CES (n = 479) | AF patients without CES (n = 580) | \( P \)     | \( P_{\text{HWE}} \)   | Crude OR (95% CI) | Adjusted OR (95% CI) | Adjusted \( P \) | \( P_{\text{Bonferroni}} \) |
|----------|-----------|----------|-------------------------------|----------------------------------|-------------|------------------------|-------------------|---------------------|-----------------|------------------------|
| MMP9     | C1562T    | CC       | 293                           | 357                              | 0.963       | 0.921                  | 1.452 (1.183–1.783) | 1.548 (1.251–1.915) | <0.001          | 0.001                  |
|          |           | CT       | 160                           | 194                              |             |                        | 1.481 (1.154–1.900) | 1.588 (1.226–2.057) | <0.001          | 0.003                  |
|          |           | TT       | 24                            | 27                               |             |                        | 2.163 (1.245–3.758) | 2.394 (1.357–4.223) | 0.003           | 0.015                  |
| ALOX5AP  | SG13S114A/T | AA        | 147                           | 205                              | 0.116       | 0.500                  | 0.75:0.25          | 0.81:0.19           | <0.001          |                        |
|          |           | AT       | 256                           | 273                              |             |                        | 1.76:0.24          | 1.74:0.26           | 0.195           |                        |
|          |           | TT       | 76                            | 102                              |             |                        | 1.59:0.22          | 1.59:0.21           | 0.886           |                        |
|          |           | A:T      | 0.57:0.43                     | 0.59:0.41                       |             |                        |                   |                     |               |                        |
| MTHFR    | 677 C/T   | CC       | 285                           | 321                              | 0.045       | 0.298                  | 0.75:0.25          | 0.81:0.19           | <0.001          |                        |
|          |           | CT       | 162                           | 213                              |             |                        | 1.57:0.43          | 1.59:0.41           | 0.058           |                        |
|          |           | TT       | 32                            | 44                               |             |                        | 1.57:0.43          | 1.59:0.41           | 0.058           |                        |
|          |           | C:T      | 0.75:0.25                     | 0.76:0.26                       |             |                        |                   |                     |               |                        |
| FGB      | 455 G/A   | GG       | 271                           | 382                              | 0.001       | 0.929                  | 0.73:0.27          | 0.81:0.19           | <0.001          |                        |
|          |           | GA       | 172                           | 177                              |             |                        | 0.75:0.25          | 0.81:0.19           | <0.001          |                        |
|          |           | AA       | 36                            | 21                               |             |                        | 0.75:0.25          | 0.81:0.19           | <0.001          |                        |
| eNOS     | G894T     | GG       | 338                           | 418                              | 0.437       | 0.058                  | 0.70:0.30          | 0.72:0.30           | 0.058           |                        |
|          |           | GT       | 129                           | 140                              |             |                        | 0.71:0.29          | 0.72:0.29           | 0.058           |                        |
|          |           | TT       | 12                            | 20                               |             |                        | 0.71:0.29          | 0.72:0.29           | 0.058           |                        |
|          |           | G:T      | 0.84:0.16                     | 0.84:0.16                       |             |                        |                   |                     |               |                        |

**Table 3.** Association analysis of 5 genotyped SNPs of the MMP9, ALOX5AP, MTHFR, FGB, and eNOS genes with CES. AF: atrial fibrillation; CES: cardioembolic stroke; SNP: single nucleotide polymorphism; MMP-9: matrix metalloproteinase-9 gene; ALOX5AP: arachidonate 5-lipoxygenase-activating protein gene; MTHFR: methylene tetrahydrofolate reductase gene; FGB: the β-fibrinogen gene; and eNOS: endothelial nitric oxide synthase gene.

**Association between FGB 455 G/A polymorphism and risk of CES in AF patients.** In univariate analysis, we detected significant association between FGB 455 G/A and risk of CES in the additive model (OR = 1.452, 95% CI: 1.183–1.783, \( P < 0.001 \)), dominant model (OR = 1.481, 95% CI: 1.154–1.900, \( P = 0.002 \)), and recessive model (OR = 2.163 95% CI: 1.245–3.758, \( P = 0.006 \)). Similar results were obtained after adjusting for confounding factors such as age, sex, BMI, smoking, drinking, hypertension, diabetes mellitus, heart failure, vascular disease, LAD, AF type, LDL, and CRP. After Bonferroni correction, the significance remained in the additive, dominant, and recessive models with ORs of 1.548 (95% CI: 1.251–1.915, \( P_{\text{Bonferroni}} = 0.001 \)), 1.588 (95% CI: 1.226–2.057, \( P_{\text{Bonferroni}} = 0.003 \)), and 2.394 (95% CI: 1.357–4.223, \( P_{\text{Bonferroni}} = 0.015 \)), respectively (Table 4).

**FGF 455 GA polymorphism and plasma fibrinogen levels.** Plasma fibrinogen level for the FGB gene GG genotype, GA genotype, and AA genotype were 2.87 ± 0.18 mg/dl, 3.22 ± 0.33 mg/dl, and 3.73 ± 0.43 mg/dl, respectively. There was a trend towards increasing plasma fibrinogen between FGB 455 G/A genotype. The plasma fibrinogen level was significantly higher in GA + AA genotype (3.29 ± 0.38 mg/dl) compared with the level in the GG genotype group (2.87 ± 0.18 mg/dl, \( P < 0.001 \), unpaired t test; Fig. 1).
Figure 1. Plasma fibrinogen levels in patients with GA + AA genotype compared with those with GG genotype. Plasma fibrinogen level was significantly higher in GA + AA genotype (3.29 ± 0.38 mg/dl) compared with GG genotype (2.87 ± 0.18 mg/dl, P < 0.001, unpaired t test). The box represents the limits of the second and third quartiles; the horizontal band is the median, and the small cross is the mean. Whiskers represent minimum and max values.

Discussion
In this contemporary case-control study, we investigated the association of five common genetic variants with CES in AF patients based on a Chinese Han population. We found that the FGB 455 G/A polymorphism was independently associated with increased risk of CES in AF patients with low CHA₂DS₂-VASc score. Our current study supports an important role of genetic predisposition in the pathogenesis of CES in AF patients.

Earlier studies explored relationships between the FGB 455 G/A polymorphism and IS in different populations. Kessler et al. reported that the AA genotype of the FGB 455 G/A polymorphism occurred significantly more frequently in patients with large vessel infarcts. Nishiuma et al. found that the A allele of the 455 G/A polymorphism was an independent risk factor of IS in hypertensive patients in a Japanese population. Martiskainen et al. demonstrated that the A allele of this polymorphism may predispose people to multiple lacunar infarcts. Similarly, Zhang et al. reported that this polymorphism appears to be a genetic risk factor for IS in the Chinese population. Several large meta-analyses confirmed the association of the FGB 455 G/A polymorphism with IS in Chinese or Asian population. Although this polymorphism has been extensively studied for its association with IS, no study has demonstrated its genetic impact on the pathogenesis of CES in AF patients. To our knowledge, this is the first study demonstrating that a functional SNP in FGB is linked to an increased risk of CES in AF patients.

The exact mechanism by which the FGB 455 G/A polymorphism may affect CES pathology remains unknown. Promoter elements have primary roles in regulating gene transcription. A promoter variant may alter transcription factor binding sites or transcription initiation rates. Experimental studies have reported that the FGB 455 G/A polymorphism has a substantial stimulatory effect on both the basal and stimulated rate of transcription of the FGB gene, and the A allele was associated with a significant increase in promoter activity. Based on epidemiological and biochemical studies, the FGB 455 G/A polymorphism is one of the strongest genetic variations associated with an increase in plasma fibrinogen. Being homozygous for the A allele is associated with increased levels of fibrinogen of approximately 0.30 g/l compared with G allele homozygotes. Consistent with previous studies, we report here that the patients with presence of A allele of FGB 455 G/A polymorphism had a significantly higher fibrinogen level. Fibrinogen is an important component of the coagulation cascade and a major determinant of platelet aggregation and blood viscosity. Elevated fibrinogen levels induce a state of hypercoagulability that may contribute to the progression of thrombosis and increases the number of emboli and the duration of embolization. Additionally, fibrinogen is a key component of inflammation, triggering a variety of inflammatory processes which could cause fluctuations of thrombus plaque and result in IS. These effects collectively could lead to hemorheological impairments, thus contributing to CES.

It is important to note that the functional effect of the FGB 455 G/A polymorphism remains controversial and other SNPs in the fibrinogen gene have also been implicated in causing higher fibrinogen concentrations. Haplotype analyses have shown that other SNPs in the FGB promoter region are functional SNPs but the 455 G/A may not be functional. Additionally, the association of the 455 G/A polymorphism with higher fibrinogen concentrations may actually be due to linkage disequilibrium between the 455 G/A polymorphism and other causal polymorphisms. Additionally, several studies have failed to provide evidence to support the association between 455 G/A polymorphism and thrombotic events. We propose that the discrepancies in the results between studies may result from differences in ethnic background, sample size, and other factors.

The CHA₂DS₂-VASc score is a well validated and widely used clinical risk prediction tool for IS in non-valvular AF, however, so far such division of IS patients related to AF has never been performed according to the genetic risk factors. The risk of IS in Asian people is quite different from that in Western people, especially in patients with...
a low CHA<sub>2</sub>DS<sub>2</sub>-VaSc score of either 0 or 1. Our results provided evidence of the significance of FGB variants in the future genotype-specific risk stratification of CES in AF patients with low CHA<sub>2</sub>DS<sub>2</sub>-VaSc score, which should allow improved decision support for the care of these relatively high risk patients.

This study has several limitations that should be acknowledged. First, this study is a cross-sectional study and is therefore subject to the limitations of this type of clinical analysis. The conclusions may be more precise if it is prospectively validated in an external large cohort of AF populations. Second, limited sample size needs to be considered as a potential source of heterogeneity; since studies with large sample size are more robust to random error and tend to reach a more objective result. Therefore, Bonferroni correction was performed in our study to show credibility of genetic association. Third, we cannot formally exclude the possibility that there are other loci in the FGB gene (or in other genes that have not yet been identified) that are linked with CES in AF patients.

In conclusion, our study provides evidence for a potential association of FGB 455 G/A polymorphism with increased risk of CES in AF patients with low CHA<sub>2</sub>DS<sub>2</sub>-VaSc score. However, it is unclear whether this finding will be reproduced in other populations. Therefore, future well-designed large-scale studies in larger populations are still warranted to validate this finding.

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**Author Contributions**

L.H.C., X.F.H. and J.J.W. designed the study; S.H.X., S.Q. and Y.G.L. were responsible for recruitment of subjects; X.F.H., J.W., L.H.C. and J.H. performed experiments and conducted data management; X.F.H., L.H.C., J.J.W. and J.H. performed statistical analyses and interpreted results; X.F.H. and L.H.C. wrote the manuscript. All authors reviewed the manuscript.

**Additional Information**

**Competing Interests:** The authors declare that they have no competing interests.

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