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GRK5 polymorphisms and Postoperative Atrial Fibrillation following Coronary Artery Bypass Graft Surgery

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Postoperative atrial fibrillation (POAF) is a serious yet common complication after coronary artery bypass grafting (CABG) surgery. Previous study have identified multiple genetic susceptibility loci for POAF susceptibility after CABG, although some studies are under-powered. However, none of these studies have been conducted among Asians. In current study, we aim to systematically evaluated the previous positive findings for POAF susceptibility after CABG among Chinese population, using a large population-based, two-stage, case-cohort study. From a discovery cohort of 1,348 patients, a total of nine independent loci were evaluated. Six significant SNPs were then assessed in a separately collected validation cohort of 2,000 patients. After adjustment for clinical predictors of POAF, two variants in GRK5 gene (rs4752292, and rs11198893) were replicated with significance were replicated in the validation cohort. The ORs for each additional copy of minor allele were 1.32 (95% CI: 1.15–1.50, P $= 5.82 \times 10^{-5}$) and 1.47 (95% CI: 1.28–1.69, P $= 1.16 \times 10^{-7}$), respectively. In this two-stage independently collected cardiac surgery cohorts, genetic variations in the GRK5 gene are independently associated with POAF risk in patients who undergo CABG surgery in Asians.

Postoperative atrial fibrillation (POAF) is a serious yet common complication, also a known predictor of in-hospital morbidity and short-term survival after coronary artery bypass grafting (CABG) surgery, occurring in 25% to 40% of patients[1–5]. Although POAF after on-pump CABG is benign and self-limiting in most cases, in some cases, it can cause stroke or congestive heart failure and may lead to prolonged hospital stay, increased cost, additional concomitant treatment, and worsened prognosis[6]. Some risk factors and possible preventive strategies for POAF have been identified, like echocardiographic parameters, age, number of vessels bypassed, vessel location, net fluid balance on the operative day, POAF score and so on[7–10]. However, little is known about the molecular basis of POAF susceptibility.

Identification of patients who are at higher risk of POAF using genetic markers might be an important step for prevention of this type of operative complication and may provide important insight into the pathogenesis of AF and new therapeutic strategies for individual patients according to relative risk. Druing last 20 years, researchers have evaluated multiple genetic susceptibility loci for POAF susceptibility after CABG, although some studies are under-powered[11–16]. However, all of these studies have been conducted among people of European ancestry. Considering that race was a surrogate for genetic determinants of POAF susceptibility after CABG due to genetic disparities among racial/ethnic groups[17,18], investigation of previously reported loci in non-European populations may help to evaluate the generalizability of these initial findings and to identify causal variants. Thus, we conducted this study to evaluated the previous positive findings for POAF susceptibility after CABG among Chinese population.

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Materials and Methods

Subjects.   The methods were carried out in "accordance" with the approved guidelines. Patients undergoing primary CABG surgery without planned concurrent valve surgery were enrolled. The exclusion criteria were as follows: prior cardiac surgery; emergency surgical procedure; acute coronary syndrome; prior myocardial infarction; congestive heart failure; significant valvular heart disease; prior implantation of a permanent pacemaker, implantable cardioverter defibrillator, or cardiac resynchronization therapy defibrillator converted to a standard on-pump procedure; and use of class I or class III anti-arrhythmic agents. Also, the patients were excluded if they were not in sinus rhythm during echocardiography. In current study, we first conducted a case-cohort study in 1,348 subsequently patients from the total cohort as the discovering stage. Then, 2,000 individuals of the remaining cohort comprised the replication cohort for our study. Study protocols were approved by respective institutional review boards of Laiwu People's Hospital, and participants were enrolled after informed written consent was obtained.

Data collection and end-point definition.   An uniform questionnaire was used to collect patient demographics, preoperative and procedural factors, perioperative medication use, postoperative outcomes obtained from patient interview and medical records, and staff interviews. Diagnosis of new onset POAF was based on postoperative electrocardiogram or rhythm strip or documented by at least 2 of the following: progress notes, nursing notes, discharge summary, or change in medication[3]. Trained medical record data abstractors reviewed the medical records of all subjects. To avoid investigators-related biases, all the physicians involved in patients' care were blinded to the results of the genetic and biochemical analyses. The in-hospital clinical course of all patients, including major and minor postoperative complications were prospectively recorded.

SNP selection and genotyping.   Through a comprehensive retrieval of Pubmed for previous positive findings for POAF susceptibility after CABG, we identified nine independent loci, including four loci in GRK5 gene (rs3740563, rs4752292, rs11198893, and rs10787959), 3 in 4q25 region (rs2200733, rs13143308, rs10033464), 1 in IL6 gene (rs1800795), and 1 in HSP70 gene (rs2227956). All of the nine SNP, which were not in linkage disequilibrium (LD), were evaluated in the discovering stage. DNA was extracted from white blood cells using standard procedures. Genotyping was performed using validated TaqMan assays (Applied Biosystems). PCR product was amplified utilizing $0.9\,\mu M$ each of the forward primer and reverse primers, $0.2\,\mu M$ each of the FAM and VIC MGB (minor groove binder) sequence-specific probes, 3 ng DNA, 5.0 mM $MgCl_2$, and 1X TaqMan Universal PCR Master Mix containing AmpliTaq Gold DNA Polymerase in a $5.5\,\mu l$ reaction volume. Both the SNPs had a call rate of greater than 99%. QC concordance for samples was 100%.

Statistical Analyses.   All statistical tests were 2-sided and a P value significance threshold of 0.05 was set. All analyses were conducted using SAS, version 9.2 (SAS Institute, Cary, North Carolina). Descriptive statistics of clinical variables are presented as frequency and percentage for categorical variables and mean $\pm$ SD or median (interquartile range) for continuous variables.

Hardy–Weinberg equilibrium (HWE) was tested by comparing observed and expected genotype frequencies among controls (x^2 test). For each of the SNPs, allelic associations with POAF were assessed using logistic regression analyses adjusted for the POAF Risk Index. The same logistic regression model adjusted for the POAF Risk Index was applied in the replication dataset. To assess the overall effect of candidate SNPs, we then conducted a meta-analysis using the weighted Z-score meta-analysis as implemented in METAL (http://www.sph.umich.edu/csg/abecasis/metal).

Results

Characteristics of the discovery and validation cohorts, stratified by the occurrence of POAF, are shown in Table 1. The mean age of the discovery cohort was 68.0 ± 9.8 years; 287 (21.3%) of the subjects were female. Of the 1,348 patients in this cohort, 405 (30.0%) developed POAF. For the validation cohort, the mean age was 67.2 ± 9.8 years with 22.1% being female, and 630 (31.5%) of the total subject developed POAF. People in the two genotyping stages were generally comparable for BMI, medical history of hypertension, diabetes, COPD, hyperlipidemia, hemodialysis, and preoperative medications of ACEI/ARB, calcium channel blocker, β-blocker, statins, and postoperative medications of HMG-CoA reductase inhibitor and β-blocker discontinued.

A total of nine independent loci which were previously reported to be associated with POAF susceptibility after CABG were included in the current study, including four loci in GRK5 gene (rs3740563, rs4752292, rs11198893, and rs10787959), 3 in 4q25 region (rs2200733, rs13143308, rs10033464), 1 in IL6 gene (rs1800795), and 1 in HSP70 gene (rs2227956). Of these 9 markers, none was found to deviate from HWE ($P > 0.05$). The estimates of effect of these variants on POAF susceptibility after CABG in discovery stage, adjusted for age and gender are shown in Table 2. The allelic pattern of six SNPs (GRK5 rs3740563, GRK5 rs4752292, GRK5 rs11198893, GRK5 rs10787959, 4q25 rs13143308, and 4q25 rs10033464) was differentially distributed among the the patients who developed POAF and those who didn't develop POAF, with the p-value being significant (ranged from 6.87×10^{-6} to 0.022). The most signigicant association was detected for GRK5 rs11198893 ($P = 6.87 \times 10^{-6}$). Each additional copy of minor allele A was associated with a 1.54-fold increased risk of developing POAF (OR $= 1.54$, 95% CI: 1.27–1.85).

Demographic	Discovery Cohort			Validation Cohor		
	POAF (N = 405)	No (N = 943)	POAF P	POAF (N = 630)	No (N = 1,370)	POAF P
Age, y	68.5 ± 9.6	67.8 ± 9.9	0.230	67.4 ± 10.3	67.1 ± 9.6	0.526
Gender (% female)	87 (21.6%)	200 (21.2%)	0.911	143 (22.7%)	300 (21.9%)	0.689
BMI, kg/m ^{2}	24.3 ± 4.3	24.8 ± 4.7	0.067	24.3 ± 4.3	24.5 ± 4.7	0.364
Medical history						
Hypertension	324 (80.0%)	725 (76.9%)	0.207	530 (84.1%)	1,140 (83.2.%)	0.608
Diabetes	104 (25.7%)	225 (23.9%)	0.476	158 (25.1%)	326 (23.8%)	0.534
COPD	22 (5.5%)	44 (4.7%)	0.550	37 (5.8%)	78 (5.7%)	0.873
Hyperlipidemia	201 (49.7%)	433 (45.9%)	0.211	290 (46.1%)	629 (45.9%)	0.960
Hemodialysis	53 (13.0%)	110 (11.7%)	0.463	80 (12.7%)	156 (11.4%)	0.398
Preoperative medications						
ACEI/ARB	196 (48.4%)	440 (46.7%)	0.558	299 (47.4%)	638 (46.6%)	0.711
Calcium channel blocker	66 (16.3%)	140 (14.8%)	0.498	102 (16.2%)	216 (15.8%)	0.810
β-blocker	319 (78.8%)	736 (78.1%)	0.770	515 (81.7%)	1,133 (82.7%)	0.603
Statins	96 (23.7%)	234 (24.8%)	0.664	143 (22.7%)	326 (23.8%)	0.591
Postoperative medications						
HMG-CoA reductase inhibitor	304 (75.1%)	730 (77.4%)	0.349	484 (76.8%)	1,059 (77.3%)	0.815
β-blocker discontinued	11 (2.7%)	33 (3.5%)	0.458	22 (3.5%)	53 (3.9%)	0.681

Table 1. Demographic Characteristics of the Discovery and Validation Cohorts.

SNP	Gene/loci	Location	Alleles*	Discovery Cohort			
				MAF (AF)	MAF (No AF)	OR (95% CI)	P
rs3740563	GRK5	Chr10:121095400	C/A	0.27	0.22	1.31 (1.09–1.58)	**0.005**
rs4752292	GRK5	Chr10:121100153	G/T	0.35	0.29	1.32 (1.11–1.57)	**1.98×10^{-3}**
rs11198893	GRK5	Chr10:121107900	G/A	0.29	0.21	1.54 (1.27–1.85)	**6.87×10^{-6}**
rs10787959	GRK5	Chr10:121131313	G/A	0.47	0.42	1.22 (1.04–1.44)	**0.016**
rs2200733	4q25	Chr4:111929618	C/T	0.42	0.39	1.13 (0.96–1.34)	0.145
rs13143308	4q25	Chr4:111933868	T/G	0.27	0.32	0.79 (0.65–0.94)	**9.73×10^{-3}**
rs10033464	4q25	Chr4:111940210	G/T	0.25	0.21	1.25 (1.03–1.52)	**0.022**
rs1800795	IL6	Chr7:22727026	G/C	0.07	0.06	1.18 (0.85–1.64)	0.327
rs2227956	HSP70	Chr6:31810495	T/C	0.21	0.19	1.13 (0.92–1.39)	0.230

Table 2. Logistic Regression Analysis of Genetic Predictors of Postoperative Atrial Fibrillation in the Study Populations (Stage I). *Major allele/minor allele. P value in bold means statistically significant.

Then, the six significant vanriants were evaluated in a validation cohort with 630 subjects with POAF and 1370 subjects without POAF (Table 3). Two variants in GRK5 gene (rs4752292, and rs11198893) were replicated with significance. For rs4752292, each additional copy of minor allele T was associated with a 1.32-fold increased risk of developing POAF (OR = 1.32, 95% CI: 1.15–1.50, P = 5.82×10^{-5}). While for rs11198893, each additional copy of minor allele A was associated with a 1.47-fold increased risk of developing POAF (OR = 1.47, 95% CI: 1.28–1.69, P = 1.16×10^{-7}). Sensitivity analyses were used to evaluated the robustness of these findings by additional adjustments by age, gerder, BMI, medical history, preoperative medications, and postoperative medications were conducted respectively. The results didn't change materially.

Discussion

AF is the most frequently occurring arrhythmia both in ambulatory and postcardiac surgical patients and is associated with significant morbidity. There is strong evidence for heritability of POAF. In this large population-based, two-stage, case-cohort study, we idenfied that GRK5 rs4752292, and GRK5 rs11198893 were significantly associated with increased risk of POAF after CABG. Testing for these genetic markers

SNP	Gene/Loci	Alleles	Stages	OR (95% CI)	P
rs3740563	GRK5	C/A	Discovery	1.31 (1.09–1.58)	0.005
			Validation	1.21 (0.89–1.38)	0.089
rs4752292	GRK5	G/T	Discovery	1.32 (1.11–1.57)	1.98×10^{-3}
			Validation	1.31 (1.07–1.63)	**0.012**
			Total	1.32 (1.15-1.50)	$\mathbf{5.82 \times 10^{-5}}$
rs11198893	GRK5	G/A	Discovery	1.54 (1.27–1.85)	$\mathbf{6.87 \times 10^{-6}}$
			Validation	1.38 (1.01–1.56)	**0.004**
			Total	1.47 (1.28–1.69)	$\mathbf{1.16 \times 10^{-7}}$
rs10787959	GRK5	G/A	Discovery	1.22 (1.04–1.44)	0.016
			Validation	0.97 (0.84-1.21)	0.744
rs13143308	4q25	T/G	Discovery	0.79 (0.65–0.94)	9.73×10^{-3}
			Validation	1.17 (0.99–1.32)	0.032
rs10033464	4q25	G/T	Discovery	1.25 (1.03–1.52)	0.022
			Validation	1.05 (0.87–1.33)	0.652

Table 3. Logistic Regression Analysis of Genetic Predictors of Postoperative Atrial Fibrillation in the Study Populations (Stage I and II).

could improve risk stratification and potentially personalize therapy for preventing POAF. To the best of our knowledge, this study is the first to demonstrate that genetic variations in the GRK5 gene are associated with POAF risk in patients who undergo CABG surgery in Asians.

For more than 2 decades, researchers have attempted to identify predictors of AF after cardiac surgery[19]. In the general population, the pathophysiology of AF has been extensively studied, yet it remains incompletely understood[19,20]. Similarly, in the context of cardiac surgery, numerous risk factors for POAF have been identified, but the pathophysiology of this entity is still to a large extent elusive[21–24]. Identification of genetic markers for POAF might be an important step for prevention of this type of operative complication and may provide important insight into the pathogenesis of AF and new therapeutic strategies for individual patients according to relative risk. Previous researchers have identified multiple genetic susceptibility loci for POAF susceptibility after CABG[11–16], although all of these studies have been conducted among people of European ancestry. In 2003, Gaudino et al.[11] first identified that The −174G/C interleukin-6 polymorphism could influence postoperative interleukin-6 levels and POAF in 110 primary isolated coronary artery bypass patients, which are strong arguments in favor of an inflammatory component of the development of atrial arrhythmias after cardiac surgery. Then many studies evaluated other genetic determinants of POAF[11–16]. These data open new perspectives on a possible genetic determinants of POAF after CABG and suggest a genetic modulation of the postoperative course and outcome after cardiac surgery.

In current study, we first identified genetic variations in the GRK5 gene is associated with POAF risk after CABG in Asians. G protein-coupledreceptor kinase 5 (GRK5), which encodes a member of the guanine nucleotide-binding protein (G protein)-coupled receptor kinase subfamily of the Ser/Thr protein kinase family, was located in chromosome 10q26.11[25]. Phosphorylation of ARRB1 by GRK5 inhibits G-protein independent MAPK1/MAPK3 signaling downstream of 5HT4-receptors and GRK5 potentially acts as a physiological regulator of ű-adrenergic receptor activity[26,27]. GRK5 is abundantly expressed in the normal human heart and involves in many biological processes of cardiovascular system[28–33]. Liggett et al.[31] found that a functional loss-of-function SNP (rs17098707) in the coding region of the GRK5 gene has been associated with blunting the effects of ű-adrenergic receptor agonists via enhanced receptor desensitization. Furthermore, Kertai et al.[16] confirmed that genetic variation in GRK5 is associated with POAF despiteperioperative BB therapy in patients undergoing CABG surgery. All of these findings validated the importance of GRK5 gene in the developmet of POAF after CABG.

A strength of this study was the acceptable statistical power to distinguish relatively small genotype associations. Another strength of the current study was a large population, a two-stage genotyping design to minimize type I error. Limitations for this study should be more larger-scale studies are needed to warranted our findings, which may ultimately lead to a comprehensive understanding of the conceivable roles in POAF. Second, the candidate gene design might bias the real situation.

Conclusively, genetic variants in GRK5 gene are identified to be independently associated with an increased risk of POAF. These findings delineate an important genetic role in the etiology of POAF and provide a detailed genomic landscape in which to examine biological mechanisms.

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Author Contributions

L.L., H.X. and Z.Z. designed the experiments; L.L., Z.L., L.M. and Z.Y. performed the investigations and experiments; L.L. and Z.L. analyzed the data; L.L. and Z.L. wrote the manuscript; all authors reviewed the manuscript.

Additional Information