ORIGINAL RESEARCH

Common Variant in Glycoprotein Ia Increases Long-Term Adverse Events Risk After Coronary Artery Bypass Graft Surgery

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***Background*—**This study was aimed to investigate the clinical relevance between glycoprotein Ia (GPIA) rs1126643C/T polymorphism and the outcome of coronary artery disease after coronary artery bypass graft (CABG) surgery and explore the involved potential mechanisms.

***Methods and Results*—**We genotyped GPIA rs1126643 polymorphism of 1592 patients who underwent CABG and followed up for a median period of 72.8 months. Patients who are GPIA rs1126643 T-allele carriers have a higher major adverse cardiac or cerebrovascular events risk post-CABG than those who are CC homozygotes (hazard ratio [HR]=1.29; P=0.022). The clinical association between the risk allele (T) carriage and major adverse cardiac or cerebrovascular events was confirmed in another cohort study, which included 646 CABG patients from various health centers across China. Meanwhile, rs1126643 T allele was also linked with increased risk of major adverse cardiac or cerebrovascular events (HR=1.73; P=0.019). To explore the underlying mechanisms, we prospectively recruited 131 coronary artery disease patients, assessed their platelet aggregation function, and focused on detecting their GPIA mRNA level and protein expression. Results showed that patients with rs1126643 T allele have elevated platelet aggregation activity (P=0.029) when protein expression is increased (P<0.001) and not affected by glycoprotein Ia mRNA level.

***Conclusions*—**The synonymous common variant, GPIA rs1126643, increases the long-term adverse events risk of CABG by augmenting GPIa protein expression and enhancing platelet aggregation function. This finding can serve as the implication of improving secondary prevention of CABG patients. **(*J Am Heart Assoc*. 2016;5:e004496 doi: 10.1161/JAHA.116.004496)**

Key Words: coronary artery bypass grafting • genetic common variant • glycoprotein Ia • long-term adverse events

As the leading cause of mortality and morbidity worldwide, coronary artery disease (CAD) accounts for 14% of all deaths and is predicted to remain so until 2030.[1] Coronary artery bypass graft (CABG) surgery was one of the most important surgical treatments for CAD, especially in patients with diabetes mellitus, left main disease, multivessel disease, or left ventricular dysfunction.[2] However, adverse events post-CABG still concern both patients and clinicians. Several studies have reported that incidence of 5-year major adverse cardiac and cerebrovascular events (MACCE) post-CABG is around 11.8% to 31.0%,[3–5] indicating that an intensive secondary prevention is necessary for patients who are prone to MACCE after having CABG.

For patients after having CABG, occurrence of short-term adverse events mainly depends on factors such as type of surgery, cardiopulmonary bypass time, and cross-clamp time; long-term adverse events tend to be related to persistent risk factors, such as diabetes mellitus, and left ventricular function.[6] Figuring out the factors that influence the long-term prognosis of CABG and screening patients who are

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Accompanying Data S1, Tables S1 through S4, and Figures S1 through S3 are available at http://jaha.ahajournals.org/content/5/12/e004496/DC1/embed/inline-supplementary-material-1.pdf

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susceptible to adverse events are pivotal for secondary prevention. Genetic background is a persistent factor that affects the prognosis of CABG patients.[7–9] Previous studies demonstrated that common variants in P-selectin and C-reactive protein genes influenced susceptibility to cognitive decline post-CABG,[7] and thrombomodulin polymorphism was associated with long-term mortality post-CABG.[8]

Platelet-dependent thrombogenesis played an important role on the adverse events post-CABG[10] and was, in part, genetically determined.[11] Platelet glycoprotein (GP) Ia-IIa is one of the major platelet membrane receptors, which could adhere to collagen exposed in subendothelial and, subsequently, activate platelets.[12–14] Defections of GP Ia-IIa complex would result platelets to be completely unresponsive to collagen.[15]

Being different from other glycoproteins, GPIa expression levels vary significantly among individuals,[16] and this variation results in a significant impact on platelet function.[17] Previous studies have demonstrated a silent polymorphism GPIA rs1126643C/T (C807T), and this was associated with the density of GPIa on platelet membrane,[18] as well as the risk of CAD.[19–21] As for the prognosis of CAD, a 5-year follow-up study showed that rs1126643 T-allele carriers have higher risk of recurrence after acute coronary syndrome.[22]

This study was designed to find out whether this synonymous common variant GPIA rs1126643 has any influence on the long-term prognosis of CABG and explore the potential mechanisms of this influence.

Methods

Study Subjects

The study protocol was approved by the Review Board of Fuwai Hospital, Peking Union Medical College (Beijing, China). We have complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects and/or animals. All patients were properly informed and have signed all necessary written consent to be involved in the study.

Patients from discovery cohort were recruited from Fuwai Hospital (Beijing, China) between December 2007 and December 2011. The inclusive criteria of the cohort are (1) aged over 18 years; (2) Chinese patients; (3) scheduled for CABG surgery; (4) and consent to involve in the study. The exclusive criteria are (1) emergent surgery and (2) CABG with other concurrent surgery. Data of this cohort were collected by trained clinical research staffs and were subsequently inputted into the computer database by 2 independent investigators. All aspects of in-hospital management were done similarly according to standard protocols in this institute. As one of the standard procedures of Fuwai Hospital, all patients were required to return for a routine outpatient follow-up at 1 month, 6 months, and each year postdischarge. For patients who did not attend the routine outpatient follow-up, a group of research nurses would make telephone calls to patients or contact the communities in which patients live to get follow-up information. Patients were asked to fill a predefined follow-up information collection form at every visit. The medical records of those who reported any adverse events postdischarge were reviewed for further confirmation. For the records of major adverse events reported by other hospitals, patients were requested to mail a copy of all relevant medical information for further confirmation. We recruited 1592 CABG patients with a median follow-up time of 72.8 months. And, among these patients, 48 (3.0%) were lost during follow-up.

In the replication cohort, 646 CABG patients were chosen from a multicenter study named HPS2-THRIVE (Heart Protection Study 2, Treatment of HDL to Reduce the Incidence of Vascular Events; NCT00461630), and it was an institutional review board–approved prospective, randomized, placebo-controlled trial. The inclusive and exclusive criteria of HPS2-THRIVE have been described elsewhere.[23] The original study of replication cohort, HPS2-THRIVE aimed to assess the effect of extended-release niacin on CAD patients, and patients treated by CABG were also included. The criteria to enter our replication cohort include Chinese patients who received isolated CABG surgery after January 2005. Patients who had incomplete information on CABG surgery were excluded from enrollment. Follow-up visits were conducted at 3 and 6 months following randomization and then every 6 months. All serious adverse events were recorded.[23]

For functional study, we assumed the mean platelet aggregation rate of patient carrying T allele was 70%, whereas that of CC patient was 60%; the estimated SD was 20%; the power and type I error rate were set to be 0.80 and 0.05, respectively; sample ratio of CC patients versus T carriers was assumed to be 0.8. Then, we calculated the anticipated sample size of the functional study to be 130 patients.[24,25] We recruited CAD patients who were aged over 18 years and scheduled for isolated CABG, and those who had any medical history of bleeding or coagulation disorders were excluded from enrollment. Finally, 131 patients were prospectively recruited from Fuwai Hospital, Peking Union Medical College, between December 2014 and June 2015. Blood samples from these patients were acquired 1 hour before CABG surgery, which was conducted at least 7 days after the stop of antiplatelet regimen.

Primary Endpoints

The primary clinical endpoint was a composite of MACCE, that is, nonfatal stroke, nonfatal myocardial infarction (MI), repeat revascularization, or death from any cause.[26] A detailed definition of MACCE is available in Data S1.[27]

Table 1. Baseline Information of Discovery Cohort

Variable	All Patients (n=1544)	MACCE (n=328)	Without MACCE (n=1216)	*P* Value
Demographics				
Age, y	61.32 (±8.61)	62.54 (±8.08)	60.99 (±8.71)	0.002*
Female sex	307 (19.9)	58 (17.7)	249 (20.5)	0.261
BMI, kg/m^2	25.75 (±4.89)	25.67 (±2.98)	25.78 (±5.29)	0.716
Medical history				
Smokers	784 (50.8)	179 (54.6)	605 (49.8)	0.121
Hypertension	1026 (66.5)	240 (73.2)	786 (64.6)	0.004*
Hyperlipidemia	1042 (67.5)	221 (67.4)	821 (67.5)	0.962
Diabetes mellitus	514 (33.3)	116 (35.4)	398 (32.7)	0.369
Insulin-treated diabetes mellitus	114 (7.4)	30 (9.1)	84 (6.9)	0.169
Renal dysfunction	11 (0.7)	4 (1.2)	7 (0.6)	0.261
COPD	7 (0.5)	3 (0.9)	4 (0.3)	0.170
Peripheral arterial disease	34 (2.2)	8 (2.4)	26 (2.1)	0.742
Previous MI	586 (32.0)	128 (39.0)	458 (37.7)	0.652
Previous PCI	150 (9.7)	30 (9.1)	120 (9.9)	0.695
LVEF (%)	59.64 (±8.64)	59.26 (±9.06)	59.74 (±8.52)	0.501
No. of diseased vessels				
2	148 (9.6)	26 (7.9)	122 (10.0)	0.250
3	1351 (87.5)	293 (89.3)	1058 (87.0)	0.259
Left main coronary artery	488 (31.6)	104 (31.7)	384 (31.6)	0.965
Biochemical characteristics				
LDL cholesterol, mmol/L	2.56 (±0.70)	2.49 (±0.60)	2.58 (±0.73)	0.037*
HDL cholesterol, mmol/L	1.01 (±0.19)	1.02 (±0.19)	1.01 (±0.19)	0.690
Platelet count, 10^9/L	195 (±59)	197 (±62)	194 (±58)	0.447
Procedural characteristics				
Grafts per patient	3.35 (±0.80)	3.31 (±0.80)	3.36 (±0.80)	0.332
Off-pump procedure	812 (52.6)	179 (54.6)	633 (52.1)	0.418
Complete revascularization	1451 (94.0)	305 (93.0)	1146 (94.2)	0.396
Blood transfusion	1078 (69.8)	236 (72.0)	842 (69.3)	0.524
Secondary prevention medication				
Aspirin	1439 (93.2)	307 (93.6)	1132 (93.1)	0.747
ACE inhibitor	493 (31.9)	102 (31.1)	391 (32.2)	0.716
β-blocker	963 (62.4)	210 (64.0)	753 (61.9)	0.486
Diuretics	83 (5.4)	17 (5.2)	66 (5.4)	0.862
Calcium-channel blocker	437 (28.3)	108 (32.9)	329 (27.1)	0.036*
Statins	1104 (71.5)	243 (74.1)	861 (70.8)	0.243

Values are presented as numbers of patients or means±SD. ACE indicates angiotensin-converting enzyme; BMI, body mass index; COPD, chronic obstructive pulmonary disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; MACCE, major adverse cardiac and cerebrovascular events; MI, myocardial infarction; PCI, percutaneous coronary intervention.

*Statistically significant (P<0.05).

DNA Isolation and Genotyping Analysis

Blood samples for genotyping were collected using vacutainers and transferred to test tubes containing EDTA. Genomic DNA was isolated from whole blood using the Wizard Genomic DNA Purification Kit (Promega, Fitchburg, MA). DNA quality and quantity were assessed by a UV spectrophotometer at 260/280 nm. DNA samples were visualized in gel array and the quality of more than 99.5% DNA samples was high. Genotyping was done by MALDI-TOF MS using the MassARRAY system (Sequenom, San Diego, CA) as previously described.[28] Completed genotyping reactions were spotted using a MassARRAY Nanodispenser (Sequenom) and analyzed by matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry. Genotype calling was done with MassARRAY RT software (version 3.1; Sequenom) and analyzed using MassARRAY Typer software (version 4.0; Sequenom).

Platelet Aggregation Measurements

Samples from the 131-patient cohort underwent platelet aggregation tests. Blood was collected in vacutainer tubes containing 3.2% sodium citrate and inverted 3 to 5 times with gentle mixing before use. Platelet aggregation was assessed within 2 hours after blood sampling. We prepared 500 μL of platelet-rich plasma (PRP) and platelet-poor plasma (PPP) by centrifugation at 88 g or 1237 g for 10 minutes; 1 μL of collagen (2 μL/mL; Chrono-Log Corporation, Havertown, PA) was used to induce aggregation. The experiment was carried out using the turbidimetric method in a Chrono-Log model 700 aggregometer (Chrono-Log Corporation). Light transmission was adjusted to 0% for PRP and to 100% for PPP in each measurement. Curves were recorded for 8 minutes, and platelet aggregation was determined as the maximal percent change in light transmittance from baseline using PPP as a reference.

Quantitative Real-Time Polymerase Chain Reaction and Western Blot Analysis

Blood from 20 patients (8 for CC, 8 for CT, and 4 for TT) randomly chosen from the 131-patient cohort were prepared for quantitative real-time polymerase chain reaction (qRT-PCR) and western blot analysis. Blood for qRT-PCR analysis was collected by Tempus RNA tube, and total RNA was isolated from blood using the Tempus spin RNA isolation kit (Thermo

Table 2. Baseline Information of Replication Cohort

Variable	All Patients (n=646)	With MACCE (n=80)	Without MACCE (n=566)	*P* Value
Age, y	62.54 (±6.85)	63.73 (±7.12)	62.38 (±6.80)	0.099
Female sex	79 (12.2)	11 (13.8)	68 (12.0)	0.657
BMI, kg/m^2	26.83 (±3.21)	27.32 (±3.46)	26.76 (±3.17)	0.144
Smokers	447 (69.2)	56 (70.0)	391 (69.1)	0.868
Hypertension	430 (66.6)	51 (63.7)	379 (67.0)	0.569
Diabetes mellitus	327 (50.6)	36 (45.0)	291 (51.4)	0.283
Insulin-treated diabetes mellitus	34 (5.3)	4 (5.0)	30 (5.3)	1
Renal dysfunction	0 (0.0)	0 (0.0)	0 (0.0)	1
COPD	0 (0.0)	0 (0.0)	0 (0.0)	1
Peripheral arterial disease	29 (4.5)	5 (6.3)	24 (4.2)	0.416
Previous MI	512 (79.3)	67 (83.8)	445 (78.6)	0.29
Previous PCI	125 (19.3)	16 (20.0)	109 (19.3)	0.875
Aspirin	609 (94.3)	71 (88.8)	538 (95.1)	0.023*
ACE inhibitor	162 (25.1)	23 (28.7)	139 (24.6)	0.418
β-blocker	470 (72.8)	56 (70.0)	414 (73.1)	0.554
Diuretics	71 (11.0)	15 (18.8)	56 (9.9)	0.018*
Calcium-channel blocker	210 (32.5)	26 (32.5)	184 (32.5)	0.999
Statins	433 (67.0)	42 (52.5)	391 (69.1)	0.003*

Values are presented as numbers of patients or means±SD. ACE indicates angiotensin-converting enzyme; BMI, body mass index; COPD, chronic obstructive pulmonary disease; LVEF, left ventricular ejection fraction; MACCE, major adverse cardiac and cerebrovascular events.
*Statistically significant (P<0.05).

Fisher Scientific, Waltham, MA). Eluted RNA was used immediately in the next reverse-transcription polymerase chain reaction using the PrimeScript 1st strand cDNA synthesis kit (TaKaRa, Dalian, China). GAPDH was selected as the reference gene in this experiment. Details of the primer information are shown in Table S1. For western blot analysis, the platelet clot was collected from the PRP by centrifugation at 3000*g* for 15 minutes and clots were lysed by cell lysis buffer (Cell Signaling Technology, Danvers, MA) with 1% proteinase inhibitors (Cell Signaling Technology). The BCA Protein assay kit (Thermo Fisher Scientific) was used to quantify total protein. We loaded 40 μg of total protein, which was separated by 4% to 12% NuPAGE Bis-Tris gels (Thermo Fisher Scientific) and transferred onto a PVDF membrane. Protein expression was detected using primary antibodies, Anti-Integrin alpha 2 antibody (1:1000; Abcam, Cambridge, MA), followed by HRP-conjugated secondary antibody (1:10 000; ZsBio, Beijing, China). Signal was detected with the SuperSignal West Pico Chemiluminescent kit (Thermo Fisher Scientific). Image band densitometry was analyzed with ImageJ software (NIH, Bethesda, MD).

Statistical Analysis

We used the Student *t* test for continuous variables or chi-squared test for discrete variables to analyze baseline variables. GPIA rs1126643 polymorphism was classified into 2 genotypic groups, distinguished by the absence or presence of at least 1 copy of the minor allele (homozygote major vs homozygote minor and heterozygote). Associations between overall MACCE and single-nucleotide polymorphisms were estimated using the Kaplan–Meier method and log-rank test. Cox proportional hazard analysis was used for multivariable analysis on the outcome. Differences among 3 groups were compared using 1-way ANOVA. Nonparametric methods Kruskal–Wallis (3 groups) and Mann–Whitney (2 groups) and Bonferroni correction were used in mRNA and protein expression analyses when the number of analytic units per group was quite small. A $P<0.05$ was considered as statistically significant. All the 3-group analyses (among CC, CT, and TT groups) are presented in Figures S1 through S3. All statistical analyses were done with SAS software (version 9.3; SAS Institute Inc., Cary, NC) and SPSS software (19.0 for Windows; SPSS, Inc., Chicago, IL).

Results

Clinical Characteristics

Clinical characteristics of CAD cases with or without MACCE are shown in Tables 1 and 2. In the discovery cohort of 1544 cases with an average follow-up of 72.8 months, there were

Table 3. Associations between GPIA rs1126643 and MACCE in Discovery Cohort and Replication Cohort

GPIA rs1126643	Discovery Cohort						Replication Cohort					
	Frequency, %		HR	*P* Value	Adjusted HR	Adjusted *P* Value	Frequency, %		HR	*P* Value	Adjusted HR	Adjusted *P* Value
	Without MACCE	With MACCE					Without MACCE	With MACCE				
CC	81.2	18.8					90.8	9.2				
CT+TT	76.6	23.4					84.7	15.3				
			1.292 (1.037–1.609)	0.022*	1.272 (1.020–1.587)	0.033*			1.739 (1.094–2.765)	0.019*	1.741 (1.092–2.773)	0.020*

Adjusted risk factors are reported in Table S3. GPIA indicates glycoprotein Ia; HR, hazard ratio; MACCE, major adverse cardiac and cerebrovascular events.
*Statistically significant ($P<0.05$).

no significant differences in sex distribution between patients with or without MACCE. Average age and hypertension rate of MACCE cases were significantly higher than those without MACCE (P=0.002 and 0.004). In addition, calcium-channel blocker users were significantly higher in MACCE cases as compared to patients without MACCE (P=0.036). In the replication cohort with 646 cases with an average follow-up of 46.5 months, rates of aspirin and statins usage in patients with MACCE were significantly lower than those patients without MACCE (P=0.023 and 0.003). In addition, diuretics users were significantly more in MACCE cases as compared with patients without MACCE (P=0.018).

GPIA rs1126643 Genotype Information

Genotype and allele frequency information in both cohorts is displayed in Table S2. The observed genotype distributions of rs1126643 in both cohorts were similar and in Hardy–Weinberg equilibrium (P>0.01 in both cohorts).

Correlation Between GPIA rs1126643 Polymorphism and Clinical Outcomes

In the discovery cohort, MACCE incidence during follow-up period was 18.8% in the CC homozygotes group, whereas it was significantly higher in the T-allele carriers group with 23.4% (P=0.028). GPIA rs1126643 was associated with increase of MACCE using univariate Cox-regression analysis (hazard ratio [HR]=1.292; P=0.022); when adjusted by risk factors (listed in Table S3), this correlation of rs1126643 and MACCE still exist (HR=1.272; P=0.033). Then, this association was validated in the independent replication cohort. MACCE incidence was 9.2% in the CC homozygotes group and increased to 15.3% in the T-allele carriers group (P=0.019). Univariate (HR=1.739; P=0.019) and multivariate (HR=1.741; P=0.020) Cox regression analyses also suggested that rs1126643 T-allele carriers have increased MACCE risk in the replication cohort (Table 3). From the Kaplan–Meier survival curves shown in Figure 1, we have noticed that the different MACCE occurrence between 2 groups manifested since the fourth year in the discovery cohort and the third year in the replication cohort, respectively, indicating that rs1126643 T allele is associated only with long-term MACCE.

Platelet Counts and Functions Between CC Homozygotes and T-Allele Carriers Groups

To investigate the influence of GPIA rs1126643 on platelet function, we recruited 131 CAD patients to test their platelet aggregation rate. The baseline information is presented in Table S4. Genotype distributions of this cohort were similar to the discovery and replication cohorts (GPIA rs1126643 CC: n=61, 46.5%; CT: n=63, 48.1%; TT: n=7, 5.3%; Hardy–Weinberg equilibrium, P>0.01). As shown in Figure 2, we found that T-allele carriers have a higher platelet aggregation rate (CC homozygotes: 58.8±26.9%; T-allele carriers: 68.3±22.1%; P=0.029) whereas the platelet counts show no between-group differences.

Influences of rs1126643 on GPIA mRNA and Protein Expression

Next, we studied the underlying mechanisms of the influence on platelet function. First, we found that expression levels of GPIA mRNA show no difference between CC homozygotes and T-allele carriers (Figure 3A). Second, in order to investigate whether T allele would enhance transcription of GPIA, we

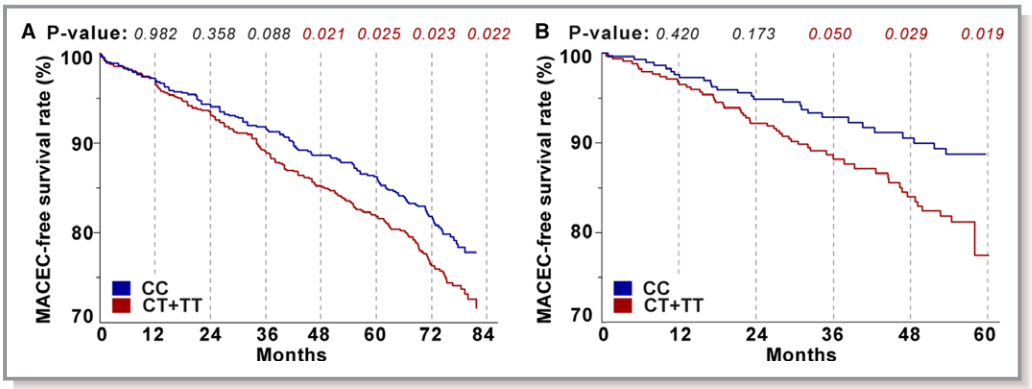

Figure 1. Kaplan–Meier survival curves of GPIA rs1126643 for discovery cohort and replication cohort. A, Survival curve of GPIA rs1126643 for discovery cohort (unadjusted). Four years after CABG in the discovery cohort, rs1126643 T carriers manifested significant low MACCE-free survival rate. B, Survival curve of GPIA rs1126643 for replication cohort (unadjusted). Three years after CABG in the replication cohort, rs1126643 T-allele carriers exhibited significant low MACCE-free survival rate. CABG indicates coronary artery bypass graft; GPIA, glycoprotein Ia; MACCE, major adverse cardiac and cerebrovascular events.

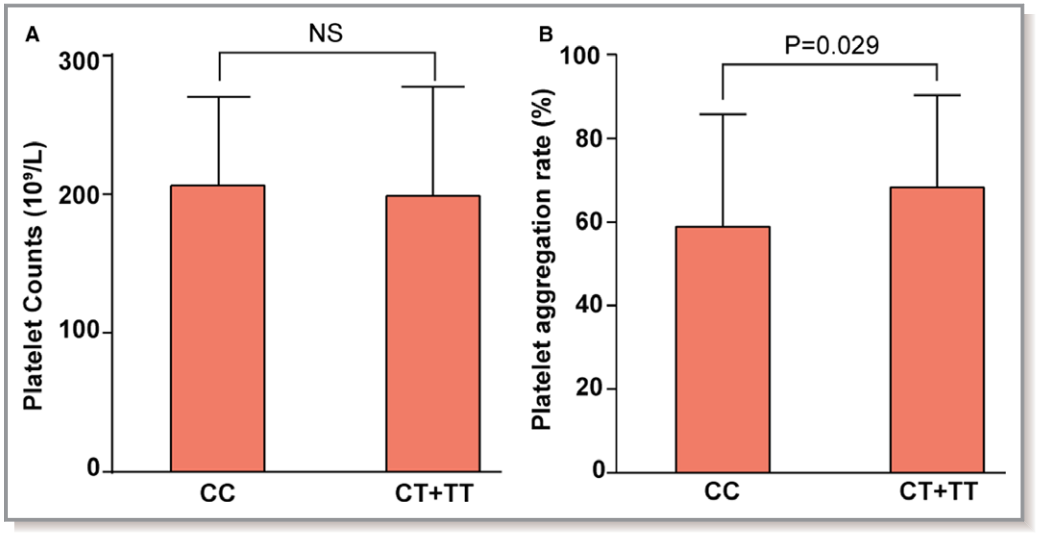

Figure 2. Platelet aggregation rate of CC homozygotes and T-allele carriers. A, Platelet counts of CC homozygotes ($206\pm64\times10^9$/L) and T-allele carriers ($213\pm68\times10^9$/L), P=0.56. B, Platelet aggregation rate of T-allele carriers (68.3±22.1%) was higher than that of CC homozygotes (58.8±26.9%), P=0.029. NS indicates not significant.

monitored GPIA protein expression levels among CAD patients. As shown in Figure 3B, T-allele carriage would increase expression of GPIA protein significantly (P<0.001).

Discussion

This study provides both population- and experiment-based evidence between GPIA rs1126643 and long-term adverse events post-CABG. In a population study, we found that GPIA rs1126643 T-allele carriers have higher MACCE risks 6 years post-CABG than those patients who do not carry any rs1126643 T allele, and this association was validated in another independent group of CABG patients. In an experimental study, we tested the platelet aggregation rate of a 131-patient cohort and found that rs1126643 T allele could enhance platelet function. Further study suggested that rs1126643 T allele augmented GPIA protein expression, but not mRNA level. From this evidence, we demonstrated that GPIA rs1126643 polymorphism is associated with long-term MACCE post-CABG.

There are several types of glycoprotein on the platelet surface, mediating platelet adhesion, activation, and

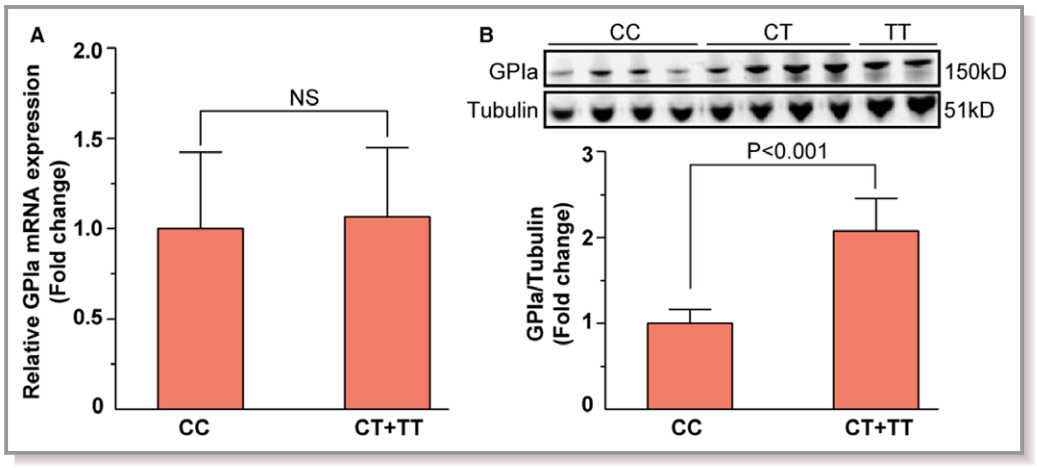

Figure 3. GPIA mRNA and protein expression levels in different genotype groups. A, Relative GPIA mRNA levels of CC homozygotes and T-allele carriers. GPIA mRNA expression levels have no significant differences between CC homozygotes and T-allele carriers. B, GPIA protein expression levels of CC homozygotes and T-allele carriers. GPIA protein expression level of T-allele carriers was 2.08 times higher than that of CC homozygotes, P<0.001. GPIA, glycoprotein Ia; NS, not significant.

aggregation, resulting in a sequential of events of thrombus formation.[29] Among these glycoproteins, GPIIIa polymorphism PIA2 is associated with higher troponin I concentrations following cardiopulmonary bypass surgery[30] and is reported as a hereditary risk factor for graft occlusion, MI, or death in patients post-CABG.[31] Apart from GPIIIa, GP Ia/IIa and GP VI play a vital role to mediate platelet-collagen interactions.[32] Being different from other types of glycoproteins, GP Ia/IIa density varies by up to 10-fold among normal individuals, which is considered to be caused by genetic variations.[33–35] Moreover, platelet attachment to type I collagen at high shear stress in whole blood is more extensive in patients with high GPIa/IIa density.[36] The effect of the extreme case was illustrated in a patient who totally failed to express platelet GPIa, and this patient exhibited complete absence of collagen-induced aggregation and an excessively prolonged bleeding time.[37]

Kunicki et al found that individuals who carry the genotype of rs1126643 TT exhibited much more GPIa/IIa expression on platelets than those bearing CC, and the CT heterozygotes had an intermediate level.[16] Our findings further indicated that mRNA levels between CC patients and T-allele carriers are no different, which indicates that the rs1126643 polymorphism does not influence the transcript process or degrade the transcript mRNA.

Mechanistically, there are several explanations for the regulation role of rs1126643. First, a codon usage bias may account for it. The rate of translation elongation can be enhanced by usage of common codons for several folds compared with the usage of rare ones.[38,39] In GPIA gene, there are 57 codons translate for phenylalanine. Of these 57 codons, 36 (63.1%) are TTT, which is the common codon in the context of GPIA gene, whereas TTC can be defined as the rare codon in GPIA gene. So, it is reasonable that the rate of translation of GPIA gene is promoted in rs1126643 T-allele carriers, which would result in an enhancement of GPIa expression. Second, the synonymous variant may alter the microRNA (miRNA) binding site to impede binding of miRNA-mRNA to promote the translation rate.[40] Although most miRNA targets the 3′ untranslated region of the mRNA, increasing evidence produced in recent years suggested that miRNA also binds in the coding region (CDS),[41] and the miRNA-CDS interaction has a negligible impact on mRNA stability, but is potent in inhibiting protein translation.[42]

To our knowledge, this is the first study to demonstrate that GPIA rs1126643 increases risk of long-term MACCE in CABG patients. Given that the risk allele T is widely distributed (with a frequency of 0.28 among Eastern Asians and 0.40 among Europeans according to data from the 1000 Genome Project), GPIA rs1126643 would be a powerful prediction factor for the prognosis of CABG patients.

However, several limitations should be noted in this study. First, our study is based on Chinese patients; whether the effects of GPIA rs1126643 still exist in other populations should be validated. Second, because the replication cohort was from HPS2-THRIVE study, which was not focused on CABG, several factors involved in CABG procedure and perioperative characteristic are not available, which limited further multivariable analyses in the replication cohort.

Conclusions

In conclusion, the present study provided both population- and experimental-based evidence that GPIa rs1126643 T-allele carriage increases risk of MACCE post-CABG. The underlying mechanisms involve posttranscription regulation of GPIa expression. These findings suggest that GPIa may be a potential target as improvement in long-term prognosis post-CABG. In addition, the genotype of GPIA rs1126643 could be used to recognize patients who are susceptible to MACCE and thus generate an intensive secondary prevention strategy for these patients.

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

Data S1. Revascularization procedures

As part of the standard institutional requirements, all surgeons had to have specialized in congenital or valve heart surgery for more than 3 years prior to undertaking any CABG procedures. With respect to off-pump CABG, the surgeon had to perform at least 100 on-pump CABG procedures before being considered qualified to carry out the off-pump procedure. Once qualified, the choice of off-pump CABG as opposed to on-pump CABG for a particular patient was generally at the discretion of the individual surgeons. Anesthesia was managed by inhalation of isoflurane with the addition of fentanyl or sufentanil, and propofol was administered continuously until the end of the procedure if necessary. Surgical revascularization was performed using standard bypass techniques. For on-pump CABG, a standard cardiopulmonary bypass was established, and moderate systemic hypothermia (28°C to 32°C) and perfusion with antegrade intermittent cold crystalloid cardioplegia were used. Heparin was given to achieve activated clotting times of 480 seconds or above before institution of cardiopulmonary bypass. For off-pump CABG, stabilization devices were used to provide a motionless anastomosis site, and heparin was administered before the start of the first distal anastomosis to achieve an activated clotting time of 300 to 350 seconds. On-pump CABG involved aortic cross-clamping and cardioplegic arrest, while off-pump CABG was performed with a partial occlusion clamp. Whenever possible, complete revascularization was attempted. And the internal thoracic artery was used preferentially for revascularization of the left anterior descending artery. The remaining vessels were to be bypassed either using another arterial conduit or the saphenous vein in the configuration decided by the surgeon. During reperfusion, the bypass grafting was completed with proximal anastomoses to the ascending aorta. The decision to switch to cardiopulmonary bypass during the procedure was based on significant

hemodynamic instability or ventricular arrhythmia. After separation from cardiopulmonary bypass or on completion of all anastomoses, protamine was given to reverse the effects of heparin. Postoperatively, starting within the first 24 hours, aspirin therapy (100 mg/d) is recommended and should be continued indefinitely.

Outcome Definitions:

Death was defined as death from any cause.

Myocardial infarction occurred when there were clinical signs and symptoms of ischemia that were distinct from the presenting ischemic event and meeting at least 1 of the following criteria:

1. Spontaneous (before or without revascularization, >48 h after CABG):

A. New, significant Q waves in at least 2 contiguous leads of an ECG that were not present with the presenting ischemic event;

B. Patients whose most recent cardiac markers measured before reinfarction, which were normal, require an increase in CK-MB or troponin that is above the 99^{th} percentile upper limit of normal and at least ≥20% above the most recent value.

2. Within 48 h after CABG:

A CABG-related MI was defined by elevation of cardiac biomarker values >10 times the 99th percentile upper reference limit in patients with normal baseline cardiac troponin values (≤99th percentile upper reference limit) plus either new pathological Q waves; new left bundle-branch block, angiographically documented new graft, or native coronary artery occlusion; or imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.

Stroke was confirmed by a neurologist on the basis of imaging studies and was defined as follows:

1. A focal neurologic deficit of central origin lasting >72 hours, or

2. A focal neurologic deficit of central origin lasting >24 hours, with imaging evidence of cerebral infarction or intracerebral hemorrhage, or

3. A non-focal encephalopathy lasting >24 hours with imaging evidence of cerebral infarction or hemorrhage adequate to account for the clinical state.

Retinal arterial ischemia or hemorrhage was included in the definition of stroke. ***Repeat revascularization*** was defined as any repeat CABG or percutaneous coronary intervention.

Table S1. Information of primers:

Primer	Sequence(5'to3')
GPIA-F	AAATGTCTCCTCTGTTGAAGGTGG
GPIA-R	CAGCTGCCTTCTCAAAGTATTCAAG
GPIA-RT-F	TGTGGTGAGGACGGACTTTG
GPIA-RT-R	CATCAACCGGCAGGGAGAAT
GAPDH-RT-F	CTATAAATTGAGCCCGCAGCC
GAPDH-RT-R	GCCCAATACGACCAAATCCGT

Table S2. Genotype information and Hardy–Weinberg equilibrium tests for GPIA rs1126643

Cohort	CC (%)	CT (%)	TT (%)	Genotype value	MAF	p for HWE*
Discovery	722 (46.8)	692 (44.8)	128 (8.3)	99.87%	0.31	0.034
Replication	294 (45.5)	281 (43.5)	65 (10.1)	99.07%	0.32	0.858

HWE: Hardy–Weinberg equilibrium.

Table S3. Candidate risk factors

Risk factors		
1. Age*	8. COPD*	15. No. of disease arteries
2. Sex*	9. Peripheral atrial disease*	16. Complete revascularization
3. BMI*	10. Chronic renal failure*	17. Cardiopulmonary bypass
4. Smoker*	11. Previous MI*	18. Blood transfusion
5. Diabetes mellitus*	12. Previous PCI*	
6. Hyperlipidemia	13. LVEF	
7. Hypertension*	14. Left main CAD	

*These risk factors are adjusted in replication cohort.

Table S4. Baseline information of functional study cohort

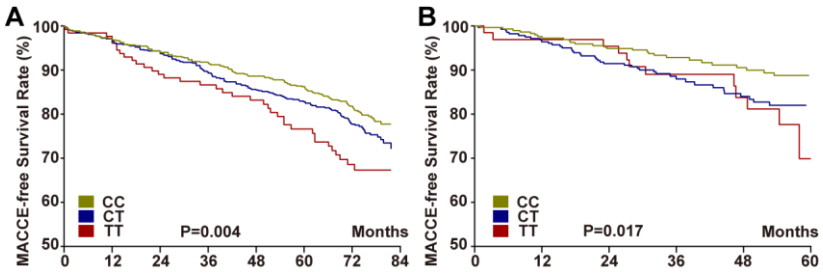
Variable	All patients (n=131)	CC homozygotes (n=61)	T allele carriers (n=70)
Demographics			
Age (years)	61.06 (±8.71)	60.95 (±8.55)	61.16 (±8.91)
Female sex	26 (19.8)	13 (21.3)	13 (18.6)
BMI (kg/m^2)	25.33 (±3.31)	25.19 (±2.87)	25.45 (±3.67)
Medical history			
Smokers	33 (25.2)	14 (23.0)	19 (27.1)
Hypertension	86 (65.6)	39 (63.9)	47 (67.1)
Hyperlipidemia	89 (67.9)	46 (75.4)	43 (61.4)
Diabetes mellitus	27 (20.6)	9 (14.8)	18 (25.7)
Renal dysfunction	0	0	0
COPD	1 (0.8)	0	1 (1.4)
Peripheral arterial disease	7 (5.3)	2 (3.3)	5 (7.1)
Prior MI	25 (19.1)	11 (18.0)	14 (20.0)
Prior PCI	5 (3.8)	2 (3.3)	3 (4.3)
LVEF (%)	59.89 (±9.02)	59.33 (±8.24)	60.37 (±9.69)

Values are presented as numbers of patients or means ± SD; BMI: Body mass index;

COPD: Chronic obstructive pulmonary disease; LVEF: Left ventricular ejection fraction;

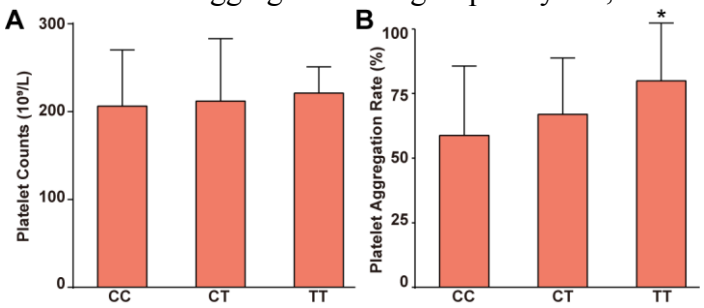
MI: myocardial infarction; PCI: percutaneous coronary intervention.

Figure S1. Kaplan-Meier survival curves of GPIA rs1126643 for discovery cohort and replication cohort grouped by CC, CT and TT genotypes.



(A) Survival curve of GPIA rs1126643 for discovery cohort (grouped by CC, CT and TT genotypes). (B) Survival curve of GPIA rs1126643 for replication cohort (grouped by CC, CT and TT genotypes). Patient with CC genotype has the highest MACCE-free survival rate, while the MACCE-free survival rate of CT genotype is medial and TT genotype is the lowest.

Figure S2. Platelet counts and aggregation rate grouped by CC, CT and TT genotypes.

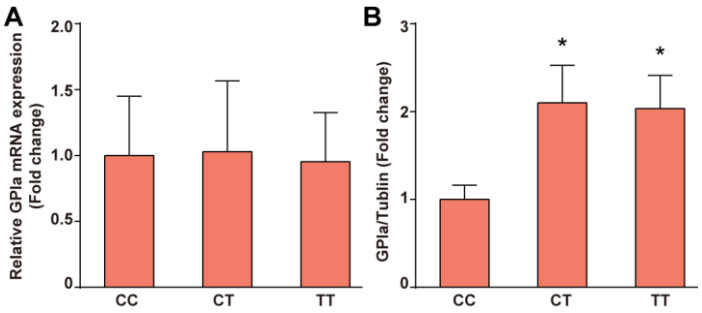


(A) The platelet counts of patients with CC genotype (206 ± 64 $\times 10^9$/L), CT genotype (212 ± 71 $\times 10^9$/L) and TT (221 ± 30 $\times 10^9$/L); p for one-way ANOVA is 0.80.

(B) The platelet aggregation rate of patients with CC genotype (58.8% ± 26.9%), CT genotype (67.0% ± 21.8%) and TT (80.0% ± 22.3%); p for one-way ANOVA is 0.038.

*$p<0.05$ compared to CC genotype.

Figure S3. GPIA mRNA and protein expression levels grouped by CC, CT and TT genotypes.



(A) Relative GPIA mRNA levels of CC homozygotes and T allele carriers grouped by CC, CT and TT genotypes. (B) GPIA protein expression levels of CC homozygotes and T allele carriers grouped by CC, CT and TT genotypes; *$p<0.05$ compared to CC genotype.