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ORIGINAL RESEARCH

Impact of Insulin Receptor Substrate-1 rs956115 and CYP2C19 rs4244285 Genotypes on Clinical Outcome of Patients Undergoing Percutaneous Coronary Intervention

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BACKGROUND: Insulin receptor substrate-1 (*IRS-1*) rs956115 is associated with vascular risk in patients with coronary artery disease and concomitant diabetes. *CYP2C19*2* (rs4244285) modulates clopidogrel response and predicts the outcome of coronary artery disease. This study was designed to explore the association between *IRS-1*, *CYP2C19*2* genotypes, platelet reactivity, and 1-year outcome in patients with coronary artery disease undergoing percutaneous coronary intervention.

METHODS AND RESULTS: Genotyping was performed using an improved multiplex ligation detection reaction technique. Platelet aggregation was assessed by light transmission aggregometry. Major adverse cardiovascular events were defined as a composite of cardiovascular death, myocardial infarction, and ischemic stroke. A total of 2213 consecutive patients were screened and 1614 were recruited. At 1 month, patients with *IRS-1* CG genotype had significantly lower levels of ADP-induced platelet aggregation compared with patients with CC homozygotes. Patients with *IRS-1* CG or GG genotype had a 2.09-fold higher risk of major adverse cardiovascular events compared with those with CC homozygotes (95% CI, 1.04–4.19; *P*=0.0376). By comparison, patients with *CYP2C19*2* GA or AA genotype had higher ADP-induced platelet aggregation compared with patients with GG homozygotes. Although there was no significant difference in risk of major adverse cardiovascular events between patients with GA/AA and GG genotypes, patients with GA genotype had a 2.19-fold higher risk than those with GG homozygotes (95% CI, 1.13–4.24; *P*=0.0200). No interaction between *IRS-1* and *CYP2C19*2* genotypes was observed.

CONCLUSIONS: In patients following percutaneous coronary intervention, *IRS-1* GG/CG and *CYP2C19*2* GA genotypes were associated with 2.09- and 2.19-fold increased cardiovascular risk, respectively, at 1-year follow-up. The association between *IRS-1* genotypes and major adverse cardiovascular events appeared to be independent of known clinical predictors.

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Key Words: coronary artery disease ■ CYP2C19 rs4244285 ■ IRS-1 rs956115 ■ percutaneous coronary intervention ■ platelet reactivity

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CLINICAL PERSPECTIVE

What is New?

- In patients with recent percutaneous coronary intervention, insulin receptor substrate-1 rs956115 G allele was associated with a 2.09-fold higher cardiovascular risk at 1 year.
- The association between the insulin receptor substrate-1 G allele and cardiovascular outcomes was independent of *CYP2C19*2* genotypes and known clinical predictors.

What are the Clinical Implications?

- Insulin receptor substrate-1 genotyping provides further opportunity to improve risk stratification of individual patients undergoing percutaneous coronary intervention.
- The underlying mechanism linking insulin receptor substrate-1 genotype and cardiovascular risk warrants further investigation.

Nonstandard Abbreviations and Acronyms

AA	arachidonic acid
IRS-1	insulin receptor substrate-1
MACE	major adverse cardiovascular events
PL_{AA}	arachidonic acid induced platelet aggregation
PL_{ADP}	ADP-induced platelet aggregation

Insulin receptor substrate-1 (IRS-1), a ligand of insulin receptor tyrosine kinase, plays a central role in the insulin signal transduction system.[1,2] Dysregulation of IRS-1 has been suggested as a common mechanism underlying insulin resistance that may lead to high platelet reactivity and low response to antiplatelet treatment in patients with type 2 diabetes.[3,]

CYP2C19 is one of the isoenzymes of the hepatic cytochrome P450 system, which plays a key role in the bioactivation of clopidogrel.[5,6] Patients with coronary artery disease (CAD) undergoing percutaneous coronary intervention (PCI) who are carriers of *CYP2C19* loss of function *2 (rs4244285) have lower levels of the active metabolite of clopidogrel than wild-type homozygotes, which is associated with lower clopidogrel responsiveness and an increased risk of major adverse cardiovascular events (MACE).[7–9]

This study was designed to examine the association between *IRS-1* rs956115, *CYP2C19*2* genotypes and platelet reactivity as well as MACE in patients with CAD who had undergone PCI and were treated with aspirin and clopidogrel.

METHODS

Ethical Considerations

All protocols for this study were reviewed and approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University (approval number 2011-SRFA099). Written informed consent was obtained from each patient. The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Design

A prospective single-center cohort study was conducted in the First Affiliated Hospital of Nanjing Medical University, Nanjing, China. The inclusion criteria were patients with CAD undergoing urgent or elective coronary stent implantation who were aged >18 years and planning to take dual antiplatelet treatment with clopidogrel 75 mg and aspirin 100 mg once daily for at least 1 year. Patients who met any of the following criteria were excluded: (1) allergic or intolerant to aspirin or clopidogrel; (2) at high risk for bleeding (eg, platelet count $<80\times10^9$/L, known bleeding diathesis, active peptic ulcer, or with a history of cerebral hemorrhage within 1 year); and (3) planning to take drugs that could potentially interfere with the antiplatelet effects of aspirin (eg, NSAIDs) or clopidogrel (eg, CYP3A inhibitors or inducers). Baseline demographic and clinical characteristics as well as medical treatments and procedural details were collected on prespecified case report forms.

Laboratory Sample Collection and Preparation

After receiving >5 days of aspirin and clopidogrel, blood samples were collected 2 hours after the most recent dose (≈10 AM) into one 2-mL BD Vacutainer tube (Becton, Dickinson and Company) containing 3.6 mg of K2 EDTA and into two 2-mL BD Vacutainer tubes containing 0.105 mol/L of buffered sodium citrate (3.2%). Within 1 hour of collection, blood samples were transferred to the central laboratory. EDTA samples were frozen at −80°C for subsequent genotyping, whereas citrated samples were processed immediately for platelet aggregation studies. After centrifuging citrated samples at 200 *g* for 8 minutes at 22°C, platelet-rich plasma was carefully separated. The remaining sample was centrifuged at 2465 *g* for another 10 minutes to obtain platelet-poor plasma. The platelet count in platelet-rich plasma was standardized by adding platelet-poor plasma to achieve a count of

250×10^9/L. Platelet aggregation tests by light transmission aggregometry were performed within 3 hours of platelet-rich plasma preparation.[10] At 1-month follow-up, additional blood samples were collected for repeat platelet aggregation studies.

Platelet Aggregation Studies

Platelet aggregation testing was performed using a Chronolog Model 700 aggregometer (Chronolog Corporation). Immediately after preparation of platelet-rich plasma, 500 µL was transferred into each of the 2 test tubes, with 500 µL platelet-poor plasma as control. Platelet aggregation was induced using ADP or arachidonic acid (AA) as agonists with final concentrations of 5 µmol/L and 1 mmol/L, respectively. ADP and AA-induced platelet aggregation (PL_{ADP} and PL_{AA}, respectively) was recorded using the maximum platelet aggregation within 8 minutes. PL_{ADP} >40% was defined as high on-treatment platelet reactivity.[11]

Genotype Analysis

IRS-1 (rs956115, C>G) and *CYP2C19*2* (rs4244285, G>A) genotyping was performed using a custom-by-design improved multiplex ligation detection reaction technique (Genesky Biotechnologies Inc) based on highly specific double ligation and multiplex fluorescence polymerase chain reaction.[12] For quality control, repeated testing was performed randomly in 5% of samples.

Clinical Follow-up

Patients were followed for 12 months by investigators who were blinded to the results of platelet reactivity testing and genotyping. Patients were reviewed in person or by telephone if they could not attend the clinic. The primary clinical end point was MACE, a composite of cardiovascular death, myocardial infarction (MI), or ischemic stroke within 12 months after PCI. Cardiovascular events were defined according to the 2001 American College of Cardiology criteria.[13]

Statistical Analysis

Assuming a MACE rate of 2.3%,[14] a sample size of 1052 patients was required to detect a hazard ratio (HR) of 2.88[15] with 90% power and a 2-sided α value of 0.05.

Continuous variables were described as mean±SD or median and interquartile range when data did not follow a normal distribution, and the statistical significance of any differences between groups was analyzed using a *t* test or nonparametric test. Categorical variables were expressed as numbers and percentages, and the statistical significance of any differences between groups was analyzed using a χ^2 test or Fisher exact method. One-way ANOVA was used to compare platelet reactivity among different genotypes of *IRS-1* and *CYP2C19*2*. Multivariable Cox proportional hazard model analysis was used to estimate the association between genotypes of *IRS-1* and *CYP2C19*2* and risk of MACE, reported as HRs and 95% CIs. The model was adjusted for clinical covariables including age, previous MI, hypertension, diabetes, smoking status, previous PCI, left ventricular ejection fraction, serum creatinine, low-density lipoprotein, and diagnosis. The date of PCI was set as "time zero" with censoring at the end of study follow-up.

All data analyses were performed using SAS version 9.4 (SAS Institute Inc) and figures were created using R version 3.2.0 (R Foundation for Statistical Computing).[16,17] A 2-tailed *P* value of <0.05 was considered statistically significant.

RESULTS

Between March 2011 and September 2016, 2213 patients were consecutively screened and 1614 patients who fulfilled the eligibility criteria were enrolled. Three patients were excluded from the final analysis because of unsatisfactory blood sample quality. All of the remaining patients completed the genotype assessment and 1-year clinical follow-up. Platelet aggregation testing was performed in 1175 patients at baseline and in 624 patients at 1 month (Figure 1).

Patient Characteristics

Baseline characteristics are summarized in Table 1. Patients who experienced MACE compared with those who did not were older (69.00 [14.50] versus 64.00 [15.00], *P*=0.0069) and more commonly having reduced left ventricular ejection fraction (25.0% versus 7.66%, *P*<0.0001) and diagnoses of non–ST-segment–elevation acute coronary syndromes and ST-segment–elevation MI (63.63% versus 42.44%, *P*=0.0010). There was no significant difference in baseline characteristics between the 602 patients screened but not included and the 1611 patients who were enrolled (Table S1, Figure 1). Of the enrolled patients, 1175 had their platelet reactivities measured at baseline and 602 remeasured at 1 month. There were no significant differences in all baseline characteristics except smoking and previous PCI between patients who underwent reassessment of platelet reactivity at 1 month and those who did not (Table S2, Figure 1).

On-Treatment Platelet Reactivity and Genotypes

The baseline and 1-month PL_{ADP} were 29.88%±14.34% and 26.27%±15.10%, respectively. There was no significant difference in baseline PL_{ADP} according to *IRS-1* genotypes (*F*=0.20, *P*=0.8200) (Figure 2A), but

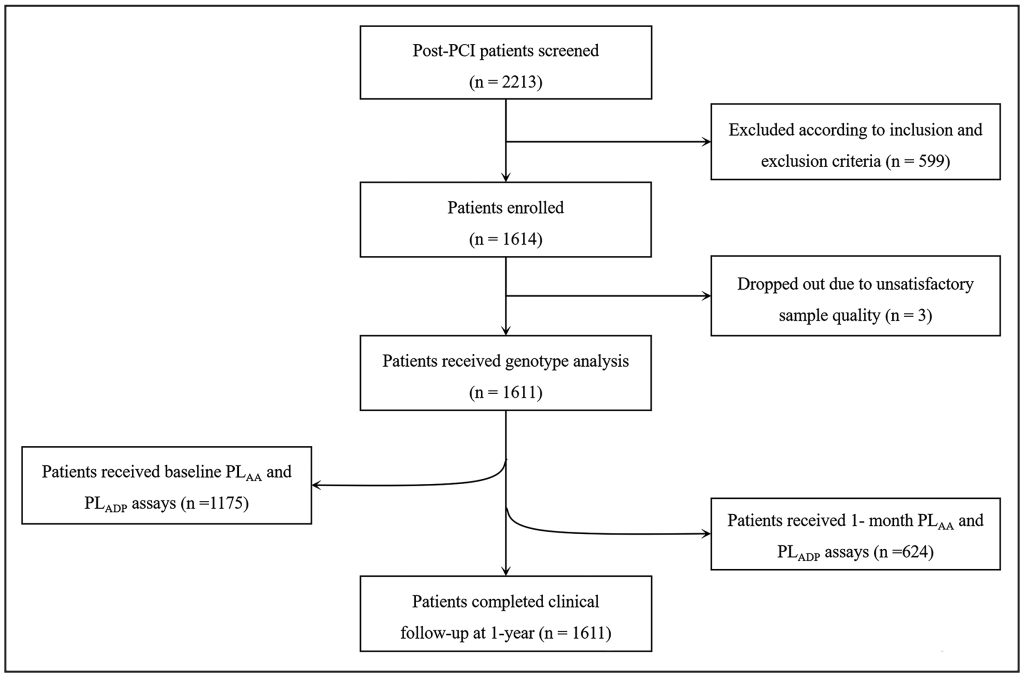

Figure 1. Study flow chart.
PCI indicates percutaneous coronary intervention; and PL_{ADP}, ADP-induced platelet aggregation.

a significant difference emerged at 1 month (F=3.28, P=0.0381) (Figure 2A). CG genotype was associated with a significantly lower PL_{ADP} compared with CC genotype (P=0.0158) (Figure 2A). Regarding PL_{AA}, there was no significant difference among the 3 genotypes of *IRS-1* either at baseline (F=2.73, P=0.0656) (Figure S1A) or at 1 month (F=0.20, P=0.8180) (Figure S1A).

For *CYP2C19*2*, PL_{ADP} were significantly different among the 3 genotypes at baseline (F=53.27, P<0.001) (Figure 2B) and at 1 month (F=12.07, P<0.001) (Figure 2B). By pairwise comparisons, the platelet reactivities corresponding to different genotypes of *CYP2C19*2* were all significantly different except the comparison between GA and AA at 1-month follow-up (P=0.4392) (Figure 2B). As shown in Figure 2B, *CYP2C19*2* GA or AA genotype were associated with higher PL_{ADP} compared with GG genotype. Regarding PL_{AA}, there was no significant difference among the 3 genotypes of *CYP2C19*2* either at baseline (F=0.38, P=0.6870) (Figure S1B) or at 1-month follow-up (F=0.78, P=0.4590) (Figure S1B).

There was no significant difference in the prevalence of high on-treatment platelet reactivity among patients with different genotypes of *IRS-1* at both baseline (CC 22.80% versus CG 19.74% versus GG 8.33%; P=0.3109) (Table S3, Figure S2A) and 1-month follow-up (CC 18.52% versus CG 14.50% versus GG 0.00%; P=0.2655) (Table S3, Figure S2C). However, high on-treatment platelet reactivity was more frequently presented in the A allele carriers of *CYP2C19*2* at baseline (GG 16.41% versus GA 25.45% versus AA 40.00%; P<0.0001) (Table S3, Figure S2B), as well as at 1-month follow-up (GG 11.70% versus GA 21.48% versus AA 25.00%; P=0.0021) (Table S3, Figure S2D).

Association Between *IRS-1/CYP2C19*2* Genotypes and Cardiovascular Outcomes

A total of 44 patients experienced MACE, including 15 cardiac deaths, 16 nonfatal MIs, and 13 ischemic strokes.

For *IRS-1*, patients with CG or GG genotypes had a 1.99-fold higher MACE risk compared with those with CC genotype (dominant model: adjusted HR, 1.99; 95% CI, 1.00–3.98 [P=0.0499]) (Table 2). When further adjusted for *CYP2C19*2* genotypes, patients with CG or GG genotypes had a 2.09-fold higher MACE risk compared with those with CC homozygotes (dominant model: adjusted HR, 2.09; 95% CI, 1.04–4.19 [P=0.0376]) (Table 2 and Figure 3A). There was no significant difference in risk of MACE between CG and CC genotypes (P=0.0586) and between GG and CC genotypes (P=0.1351) (Table 2 and Figure 3C).

For *CYP2C19*2*, there was no significant difference in the risk of MACE between patients with GA or AA genotype and those with GG genotype (dominant model: P=0.0759) (Table 2). However, the risk of MACE was 2.13-fold higher in patients with GA genotype than in GG homozygotes (adjusted HR, 2.13; 95% CI, 1.10–4.12 [P=0.0248]) (Table 2). In the meantime, no significant difference in the risk of

Table 1. Baseline Characteristics of Patients Grouped by the Occurrence of MACE

Variables	MACE (n=44)	MACE free (n=1567)
Age, median (IQR), y	69.00 (14.50)	64.00 (15.00)
Sex, n (%)		
Women	8 (18.18)	393 (25.08)
Men	36 (81.82)	1174 (74.92)
Previous MI, n (%)		
No	42 (95.45)	1499 (95.66)
Yes	2 (4.55)	68 (4.34)
Hypertension, n (%)		
No	12 (27.27)	520 (33.18)
Yes	32 (72.73)	1047 (66.82)
Diabetes, n (%)		
No	30 (68.18)	1165 (74.35)
Yes	14 (31.82)	402 (25.65)
Smoking, n (%)		
No	24 (54.55)	743 (47.42)
Yes	20 (45.45)	824 (52.58)
Previous PCI, n (%)		
No	42 (95.45)	1424 (90.87)
Yes	2 (4.55)	143 (9.13)
LVEF, n (%)		
≥55%	33 (75.00)	1447 (92.34)
<55%	11 (25.00)	120 (7.66)
Serum creatinine, n (%)		
≤133 μmol/L	42 (95.45)	1537 (98.09)
>133 μmol/L	2 (4.55)	30 (1.91)
Low-density lipoprotein, n (%)		
≥1.8 mmol/L	36 (81.82)	1335 (85.19)
<1.8 mmol/L	8 (18.18)	232 (14.81)
Diagnosis, n (%)		
SA	16 (36.36)	902 (57.56)
NSTE-ACS	12 (27.27)	412 (26.29)
STEMI	16 (36.36)	253 (16.15)

Values are presented as median (interquartile range [IQR]) or number of patients (percentage) as appropriate. LVEF indicates left ventricular ejection fraction; MACE, major adverse cardiovascular events; MI, myocardial infarction; NSTE-ACS, non–ST-segment–elevation acute coronary syndromes; PCI, percutaneous coronary intervention; SA, stable angina pectoris; and STEMI, ST-segment–elevation myocardial infarction.

MACE was found between AA and GG genotypes (P=0.4814) (Table 2). When further adjusted for *IRS-1* genotypes, there was still no significant difference in the risk of MACE between patients with GA or AA and those with GG genotype (dominant model: P=0.0666) (Table 2 and Figure 3B). The risk of MACE was 2.19-folder higher in patients with GA genotype than in GG genotype (adjusted HR, 2.19; 95% CI, 1.13–4.24 [P=0.0200]) (Table 2 and Figure 3D), while no significant difference was observed in the risk of MACE between AA and GG genotypes (P=0.4787) (Table 2 and Figure 3D). The entire results with categorical, dominant, additive, recessive models are presented in Table S4.

Interaction Analysis

Among patients with GG genotype of *CYP2C19*2*, those who had CG or GG genotypes of *IRS-1* presented with a 4.85-fold higher MACE risk than those who had CC genotype (adjusted HR, 4.85; P=0.0081) (Figure 4). By comparison, among patients with the non-GG genotype of *CYP2C19*2*, those with CG or GG genotypes of *IRS-1* presented with a 1.40-fold higher risk than those who had CC genotype (adjusted HR, 1.40; P=0.4764) (Figure 4). The interaction between *IRS-1* and *CYP2C19*2* genotypes was nonstatistically significant (P=0.1453) (Figure 4).

Association of *IRS-1* Genotypes With MACE in Subgroup Analysis

We performed multivariable Cox regression analysis for *IRS-1* genotypes in different patient subgroups (Figure S3). The association between *IRS-1* genotypes and MACE remained statistically significant in the subgroup of normal serum creatinine (adjusted HR, 2.09; 95% CI, 1.04–4.18) (Figure S3). Although the adjusted HR between CG or GG and CC genotypes of *IRS-1* did not reach statistical significance in the diabetes subgroup (Figure S3), the dominant model HR of MACE for patients with CG or GG genotypes of *IRS-1* tended to be similar among subgroups. No significant interactions were observed in those subgroups except left ventricular ejection fraction (interaction P=0.0006) (Figure S3).

DISCUSSION

This study examined the association between *IRS-1*, *CYP2C19*2* genotypes and clinical outcomes of patients undergoing PCI and receiving dual antiplatelet treatment, and found that G allele carriers of *IRS-1* had a 2.09-fold higher risk of MACE compared with non-carriers at 1-year follow-up. Patients with *CYP2C19*2* GA genotype had a 2.19-fold higher risk compared with GG homozygotes. The association between *IRS-1* genotypes and MACE was independent of known clinical covariables, while the association between *CYP2C19*2* genotypes and MACE could be mediated by lower clopidogrel response.

Angiolillo et al[15] examined 7 single nucleotide polymorphisms of *IRS-1*. They found that *IRS-1* rs956115 polymorphism was associated with a hyperreactive platelet phenotype and adverse cardiovascular outcomes in White patients with type 2 diabetes who had concomitant CAD. However, uncertainty remains about the association between *IRS-1* genotypes and

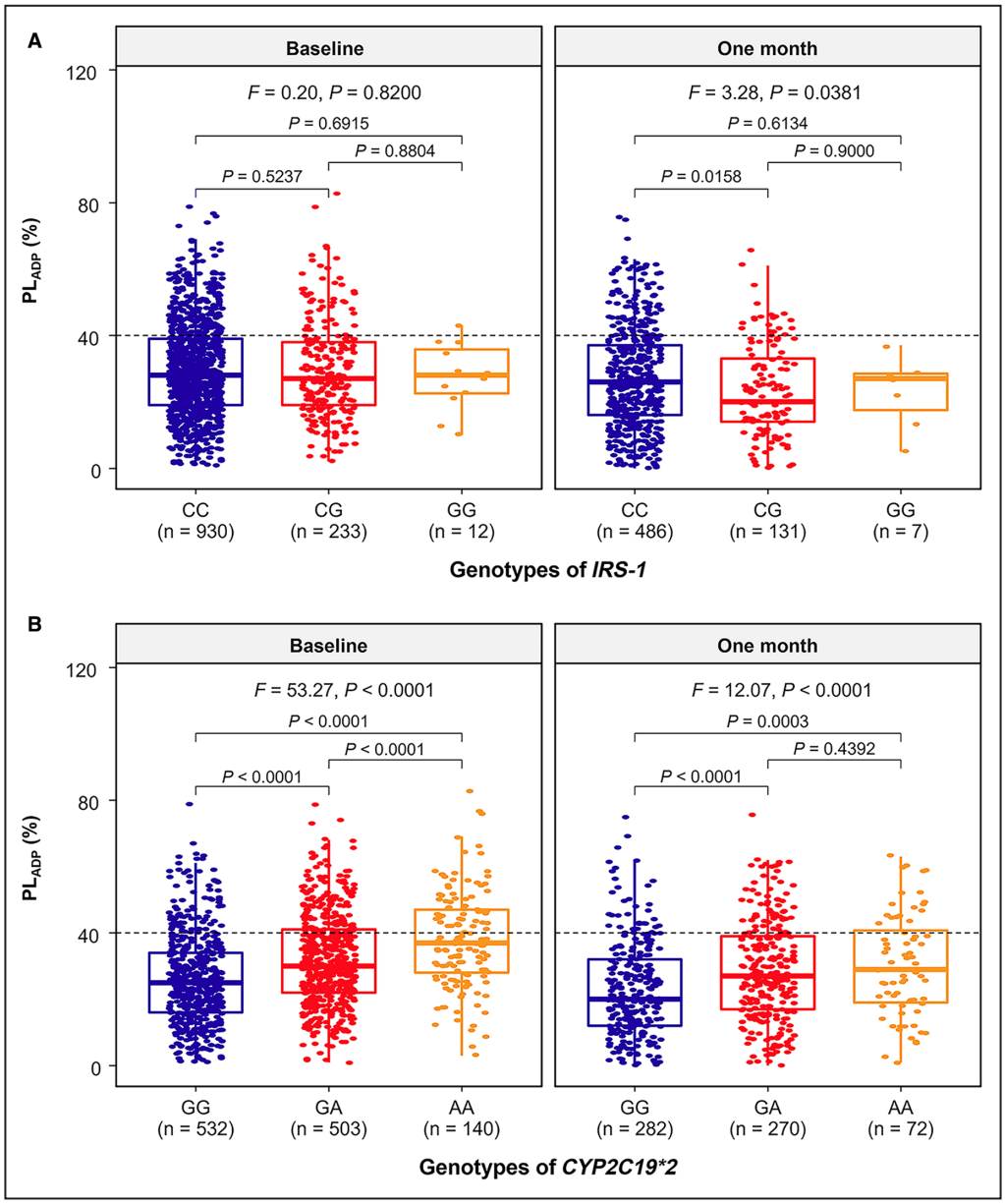

Figure 2. Platelet reactivity (ADP-induced platelet aggregation [PL_{ADP}]) in patients with different genotypes of insulin receptor substrate-1 (*IRS-1*) and *CYP2C19*2*.
A, Boxplot of *IRS-1* and PL_{ADP} at baseline and 1 month; (**B**) Boxplot of *CYP2C19*2* and PL_{ADP} at baseline and 1 month. The dashed line represents the cutoff point for high on-treatment platelet reactivity (PL_{ADP} >40%).

platelet function or cardiovascular outcome in patients with nonselective CAD.

In this study, we found that the *IRS-1* G allele was an independent prognostic factor of adverse cardiovascular outcomes in patients with nonselective CAD, irrespective of *CYP2C19*2* genotype, diabetes, and other known risk factors. Although the *IRS-1* G allele did not show a significant correlation with MACE in the subgroup of diabetes, our results showed the consistent tendency of almost all subgroups, as shown in Figure S3.

Regarding the underlying mechanism, Angiolillo et al[15] suggested that *IRS-1* rs956115 polymorphism was associated with a hyperactive platelet phenotype in White patients with type 2 diabetes. However, in a later study by Zhang et al,[18] no association was observed between *IRS-1* rs956115 polymorphism and platelet function profile. Our results were consistent with that of Zhang and colleagues' in a larger Chinese population, showing no significant difference in AA or ADP-induced platelet aggregation at baseline among different *IRS-1* genotypes. Moreover, ADP-induced

Table 2. MACE Risk Loci by Multi-Cox Regression

SNP	Gene	Genotype	MACE, n	Censored, n	Comparison	Unadjusted model		Adjusted model*		Adjusted model†	
						HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
rs956115	*IRS1*	CC	32	1245		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
		CG	11	305	CG vsCC	1.66 (0.82–3.34)	0.1571	1.91 (0.94–3.88)	0.0751	1.99 (0.98–4.08)	0.0586
		GG	1	17	GG vs CC	2.65 (0.36–19.53)	0.3377	4.23 (0.55–32.29)	0.1643	4.70 (0.62–35.84)	0.1351
					Dominant	1.71 (0.87–3.38)	0.1211	1.99 (1.00–3.98)	0.0499	2.09 (1.04–4.19)	0.0376
rs4244285	*CYP2C19*	GG	14	712		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
		GA	28	666	GA vs GG	2.04 (1.07–3.90)	0.0303	2.13 (1.10–4.12)	0.0248	2.19 (1.13–4.24)	0.0200
		AA	2	189	AA vs GG	0.60 (0.14–2.65)	0.5010	0.58 (0.13–2.61)	0.4814	0.58 (0.13–2.60)	0.4787
					Dominant	1.76 (0.93–3.33)	0.0843	1.81 (0.94–3.49)	0.0759	1.85 (0.96–3.56)	0.0666

HR indicates hazard ratio; MACE, major adverse cardiovascular events; and SNP, single nucleotide polymorphism.
*Model adjusted for clinical covariates, including age, previous myocardial infarction, hypertension, diabetes, left ventricular ejection fraction, serum creatinine, diagnosis, low-density lipoprotein, smoking status, and previous percutaneous coronary intervention.
†Model adjusted for *CYP2C19*2*/insulin receptor substrate-1 (*IRS-1*) genotypes and clinical covariates, including age, previous myocardial infarction, hypertension, diabetes, left ventricular ejection fraction, serum creatinine, diagnosis, low-density lipoprotein, smoking status, and previous percutaneous coronary intervention. Dominant model: *IRS-1* CG and GG vs CC; *CYP2C19*2* GA and AA vs GG.

platelet aggregation was even lower in the *IRS-1* CG genotype compared with the CC genotype at 1-month follow-up. Along with the results of Zhang et al's study, we suggest that the association between *IRS-1* genotypes and the risk of MACE cannot be explained by impaired platelet reactivity to either clopidogrel or aspirin.

Theoretically, IRS-1 is one of the central nodes in the insulin signaling network.[19] It has been reported that IRS-1 is necessary for insulin-stimulated activation of the phosphatidylinositol 3 kinase/AKT pathway and subsequent enhanced production of nitric oxide in endothelial cells,[20] which plays a critical role in maintaining cardiovascular homeostasis.[21] Previous studies have demonstrated that functional variants of *IRS-1* directly impaired insulin-regulated nitric oxide synthesis in cultured human endothelial cells.[22,23] Considering the pivotal role of IRS-1 in the phosphatidylinositol 3 kinase/AKT signaling pathway of insulin, it may be reasonable to assume that *IRS-1* rs956115 polymorphism affects the same process or an unknown pathway and consequently impacts the clinical outcome of patients with CAD.

Our results were consistent with previous reports and further confirmed that *CYP2C19*2* loss of function polymorphism is a strong predictor of impaired clopidogrel response and adverse clinical outcomes.[7–9] This consistency, in turn, enhances the credibility of our results on *IRS-1*. Meanwhile, we did not find a statistically significant interaction between *IRS-1* and *CYP2C19*2* genotypes from the interaction analysis, which proved the *IRS-1* G allele to be an independent risk factor for MACE in patients with CAD after PCI. However, the apparent lack of interaction between genotypes on MACE may also be explained by low power caused by the small number of events. Regarding medication compliance, 42 (2.6%) patients permanently discontinued 1 or 2 antiplatelet agents because of major or minor bleeding events.

Our data indicate that IRS-1 genotyping provides further opportunity to improve risk stratification of individual patients undergoing PCI. We suggest that genotyping of the *IRS-1* gene should be done in patients with high ischemic risks or recurrent ischemic events to predict the prognosis. Ideally, any treatment strategy that involves genotyping of the *IRS-1* gene requires prospective evaluation to confirm that identification of patients at high risk using this approach can improve clinical outcomes.

Study Limitations

This study has potential limitations. First, because of limited funding, we did not evaluate *CYP2C19*3* genotypes, also a determinant of clopidogrel metabolism. A potential interaction between *IRS-1* and *CYP2C19*3* genotypes and their impact on the clinical outcome remains to be investigated. Second, the number of

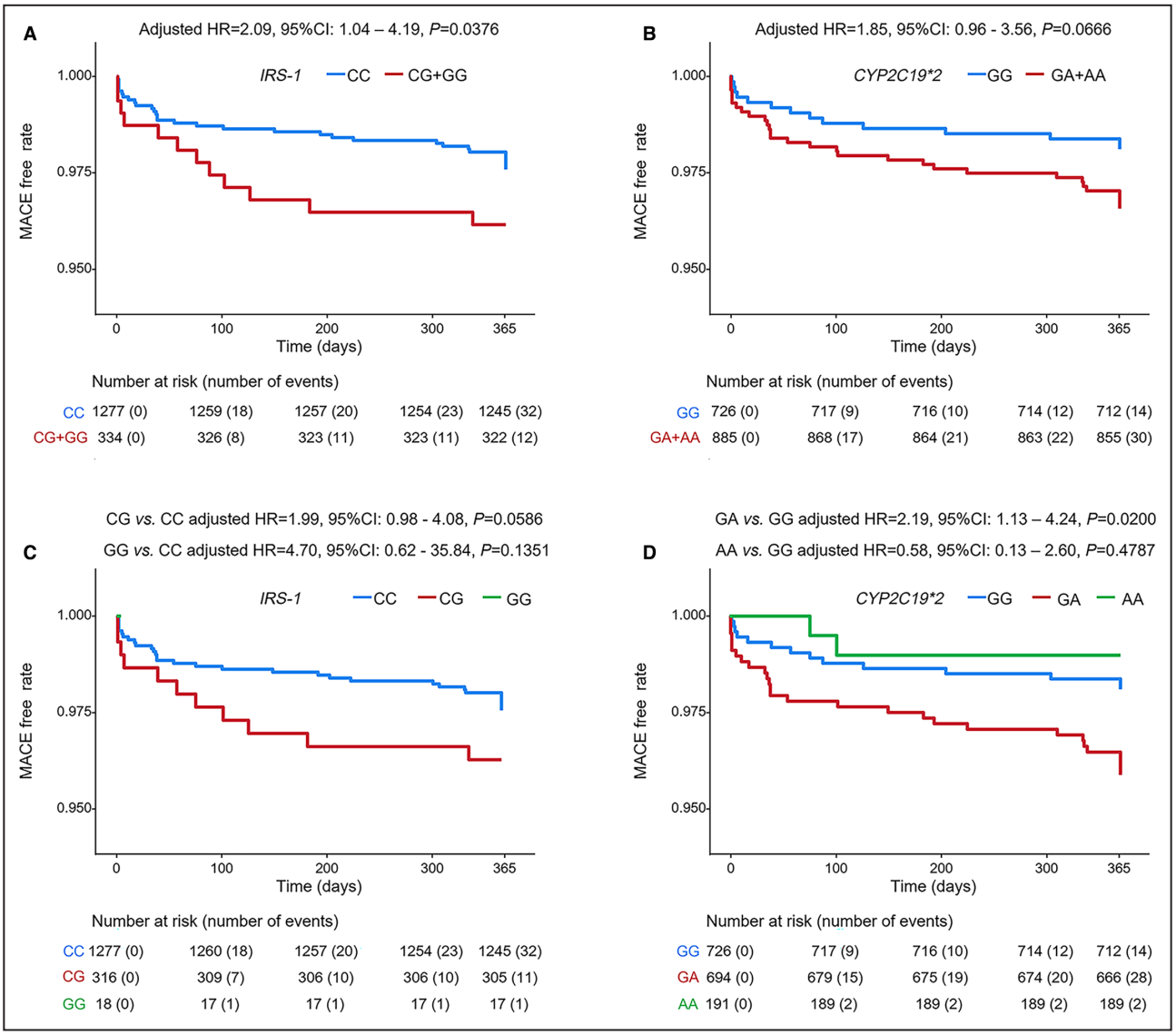

Figure 3. Survival curve of major adverse cardiovascular events (MACE)–free rate and insulin receptor substrate-1 (*IRS-1*), *CYP2C19*2* genotypes.

Cox regression model adjusted for *IRS-1* or *CYP2C19*2* genotypes and clinical covariates including age, previous myocardial infarction, hypertension, diabetes, smoking status, previous percutaneous coronary intervention, left ventricular ejection fraction, serum creatinine, low-density lipoprotein, and diagnosis. **A**, Survival curve of MACE-free rate and dominant model of *IRS-1* genotypes. **B**, Survival curve of MACE-free rate and dominant model of *CYP2C19*2* genotypes. **C**, Survival curve of MACE-free rate and categorical model of *IRS-1* genotypes. **D**, Survival curve of MACE-free rate and categorical model of *CYP2C19*2* genotypes. HR indicates hazard ratio.

MACE was relatively low and there was only 1 event in patients with GG homozygotes of *IRS-1* and 2 events in patients with AA homozygotes of *CYP2C19*2*. Third, despite adjustment for clinical covariates including age, previous MI, hypertension, diabetes, left ventricular ejection fraction, serum creatinine, diagnosis, low-density lipoprotein, smoking status, and previous PCI, we cannot exclude residual confounding as a contributor to our findings. Fourth, only 53.1% of the patients had platelet reactivity remeasured at 1 month, which may impact the generalizability of our results. However, there were no significant differences in all baseline characteristics except smoking and previous PCI between patients who underwent reassessment of platelet reactivity at 1 month and those who did not (Table S2). Furthermore, the pattern of platelet reactivity according to genotype at 1 month were consistent with those seen at baseline (Figure 2, Figure S1), which also makes it less likely that selection bias accounts for our findings.

CONCLUSIONS

IRS-1 rs956115 G allele was associated with an increased cardiovascular risk in patients post-PCI by 2.09-fold at 1-year follow-up, which was independent

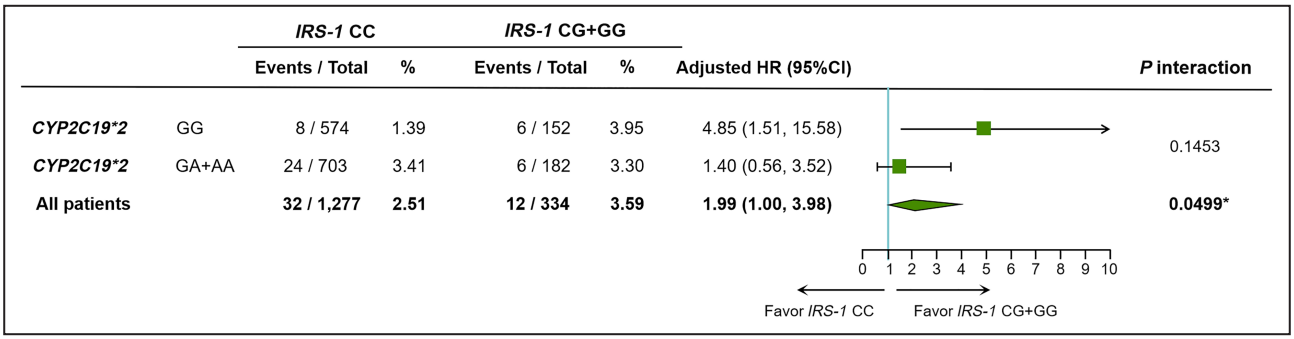

Figure 4. The hazard ratio (HR) of insulin receptor substrate-1 (*IRS-1*) by different genotypes of *CYP2C19*2*.
Model adjusted for clinical covariates, including age, previous myocardial infarction, hypertension, diabetes, smoking status, previous percutaneous coronary intervention, left ventricular ejection fraction, serum creatinine, low-density lipoprotein, and diagnosis. **P* value indicates the association between *IRS-1* genotypes and major adverse cardiovascular events in all patients.

of *CYP2C19*2* genotypes, pharmacological platelet response, and known clinical covariables.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Table S1-S4
Figure S1-S3

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SUPPLEMENTAL MATERIAL

Table S1. Comparison of Baseline Characteristics between the Included and the Excluded/dropped-out Patients

Variables	Included Patients (n=1, 611)	Excluded/dropped-out Patients (n=602)	*P* value
Age, median (IQR), years	63.75 (10.47)	63.71 (10.58)	0.9499
Sex, No. (%)			0.4586
Female	401 (24.9)	140 (23.3)	
Male	1210 (75.1)	462 (76.7)	
Previous MI, No. (%)			0.6894
No	1541 (95.7)	575 (96.2)	
Yes	70 (4.3)	23 (3.8)	
Missing	0 (0.0)	4 (0.7)	
Hypertension, No. (%)			0.7763
No	532 (33.0)	203 (33.8)	
Yes	1079 (67.0)	398 (66.2)	
Missing	0 (0.0)	1 (0.2)	
Diabetes Mellitus, No. (%)			0.9370
No	1195 (74.2)	446 (74.1)	
Yes	416 (25.8)	153 (25.4)	
Missing	0 (0.0)	3 (0.5)	
Smoking, No. (%)			0.6798
No	767 (47.6)	280 (46.5)	

Yes	844 (52.4)	322 (53.5)	
Previous PCI, No. (%)			0.9787
No	1466 (91.0)	546 (90.7)	
Yes	145 (9.0)	55 (9.1)	
Missing	0 (0.0)	1 (0.2)	
LVEF, No. (%)			0.3167
≥ 55%	1480 (91.9)	228 (37.9)	
< 55%	131 (8.1)	26 (4.3)	
Missing	0 (0.0)	348 (57.8)	
Serum creatinine, No. (%)			0.3704
≤ 133μmol/L	1579 (98.0)	569 (94.5)	
> 133μmol/L	32 (2.0)	16 (2.7)	
Missing	0 (0.0)	17 (2.8)	
Low density lipoprotein, No. (%)			0.7309
≥ 1.8mmol/L	1371 (85.1)	490 (81.4)	
< 1.8mmol/L	240 (14.9)	81 (13.5)	
Missing	0 (0.0)	31 (5.1)	
Diagnosis, No. (%)			0.0341
SA	424 (26.3)	129 (21.4)	
NSTE-ACS	918 (57.0)	344 (57.1)	
STEMI	269 (16.7)	120 (19.9)	

Others	0 (0.0)	9 (1.5)

Values are presented as median (IQR) or number of patients (percentage) as appropriate. *P* values were calculated with the use of t test or χ^2 test as appropriate. *P* values were calculated without considering missing data. MI, myocardial infarction; PCI, percutaneous coronary intervention; LVEF, left ventricular ejection fraction; SA, stable angina pectoris; NSTE-ACS, non-ST-segment elevation acute coronary syndromes; STEMI, ST-segment-elevation myocardial infarction.

Table S2. Comparison of Baseline Characteristics between Patients Who Underwent Re-assessment of Platelet Reactivity at 1 Month and Those Who Did Not

Variables	Platelet Reactivity Remeasured at 1 Month (n=624)	Platelet Reactivity Measured only at Baseline (n=551)	*P* value
Age, median (IQR), years	64.00 (15.0)	65.00 (15.0)	0.4423
Sex, No. (%)			0.3115
Female	145 (23.2)	143 (26.0)	
Male	479 (76.8)	408 (74.0)	
Previous MI, No. (%)			0.0565
No	605 (97.0)	521 (94.6)	
Yes	19 (3.0)	30 (5.4)	
Hypertension, No. (%)			0.4348
No	223 (35.7)	184 (33.4)	
Yes	401 (64.3)	367 (66.6)	
Diabetes Mellitus, No. (%)			0.2225
No	454 (72.8)	419 (76.0)	
Yes	170 (27.2)	132 (24.0)	
Smoking, No. (%)			0.0124
No	309 (49.5)	314 (57.0)	
Yes	315 (50.5)	237 (43.0)	
Previous PCI, No. (%)			0.0015

No	580 (92.9)	481 (87.3)	
Yes	44 (7.1)	70 (12.7)	
LVEF, No. (%)			0.8797
≥ 55%	567 (90.9)	503 (91.3)	
< 55%	57 (9.1)	48 (8.7)	
Serum creatinine, No. (%)			0.7630
≤ 133μmol/L	613 (98.2)	539 (97.8)	
> 133μmol/L	11 (1.8)	12 (2.2)	
Low density lipoprotein, No. (%)			0.1836
≥ 1.8mmol/L	533 (85.4)	454 (82.4)	
< 1.8mmol/L	91 (14.6)	97 (17.6)	
Diagnosis, No. (%)			0.5969
SA	156 (25.0)	135 (24.5)	
NSTE-ACS	360 (57.7)	308 (55.9)	
STEMI	108 (17.3)	108 (19.6)	

Values are presented as median (IQR) or number of patients (percentage) as appropriate. *P* values were calculated with the use of t test or χ^2 test as appropriate. *P* values were calculated without considering missing data. MI = myocardial infarction; PCI = percutaneous coronary intervention; LVEF = left ventricular ejection fraction; SA = stable angina pectoris; NSTE-ACS = non-ST-segment elevation acute coronary syndromes; STEMI = ST-segment-elevation myocardial infarction.

Table S3. Association between IRS-1, CYP2C19*2 genotypes and HOPR by Logistic regression

SNP	Gene	HOPR	Unadjusted model		Adjusted model *	
			OR (95%CI)	*P* value	OR (95%CI)	*P* value
rs956115	IRS-1	Baseline	0.80 (0.56, 1.14)	0.2256	0.81 (0.56, 1.14)	0.2382
		1 month	0.70 (0.40, 1.18)	0.1963	0.67 (0.38, 1.12)	0.1413
rs4244285	CYP2C19	Baseline	2.44 (1.82, 3.31)	<0.0001	2.47 (1.84, 3.36)	<0.0001
		1 month	2.16 (1.39, 3.40)	<0.0001	2.21 (1.42, 3.52)	0.0006

Dominant models were adopted in the analysis. * Model adjusted for clinical covariates including age, hypertension, diabetes mellitus, smoking status, serum creatinine, low density lipoprotein and diagnosis. HOPR, high on-treatment platelet reactivity; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence intervals.

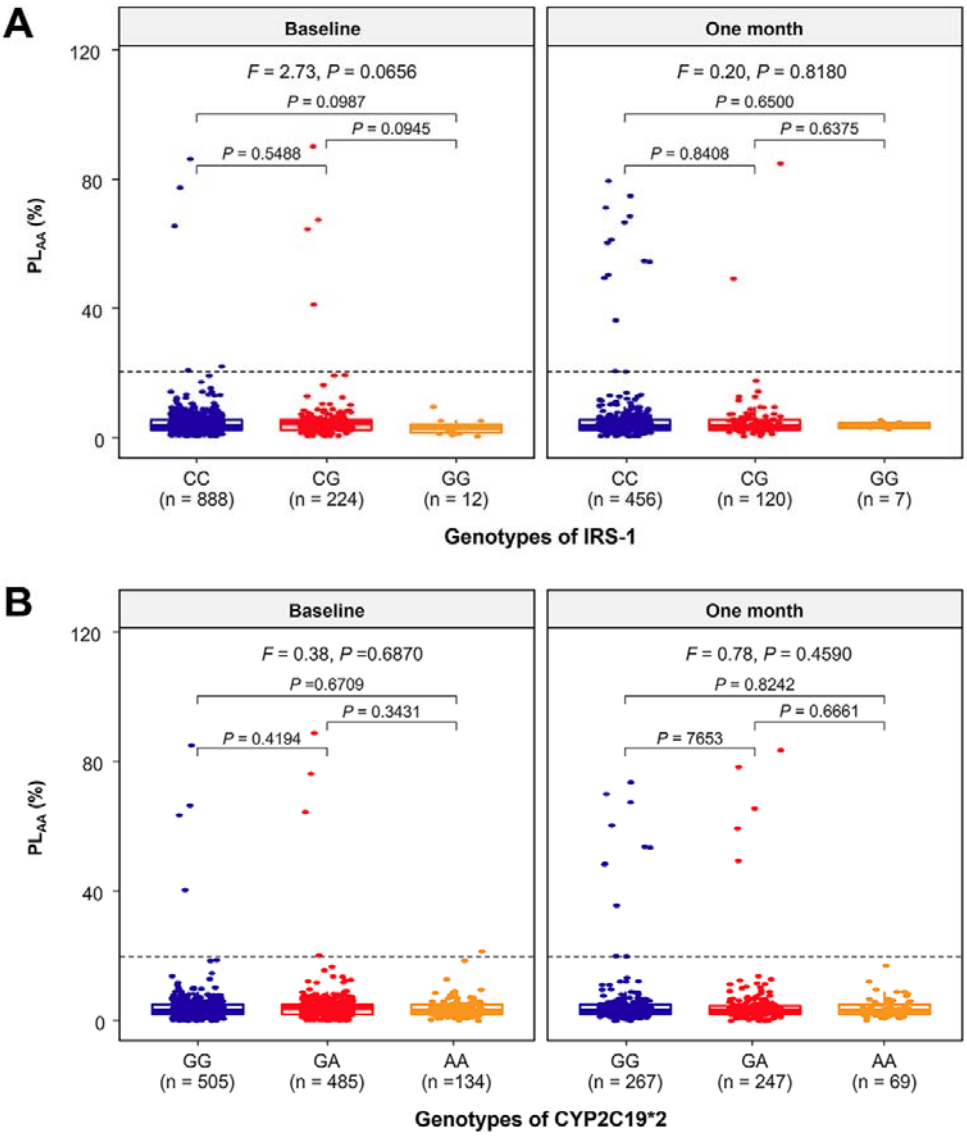
Table S4. MACE Risk Loci by Multi-Cox Regression for Categorical, Dominant, Recessive and Additive Models.

SNP	Gene	Genotype	MACE N	Censored N	Comparison	Unadjusted model		Adjusted model *		Adjusted model †	
						HR (95%CI)	*P* value	HR (95%CI)	*P* value	HR (95%CI)	*P* value
rs956115	IRS1	CC	32	1245		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
		CG	11	305	CG *vs*.CC	1.66(0.82,3.34)	0.1571	1.91(0.94,3.88)	0.0751	1.99(0.98,4.08)	0.0586
		GG	1	17	GG *vs*.CC	2.65(0.36,19.53)	0.3377	4.23(0.55,32.29)	0.1643	4.70(0.62,35.84)	0.1351
					Dominant	1.71(0.87,3.38)	0.1211	1.99(1.00,3.98)	0.0499	2.09(1.04,4.19)	0.0376
					Recessive	2.35(0.32,17.11)	0.3992	3.58(0.48,26.96)	0.2157	3.91(0.52,29.33)	0.1851
					Additive	1.65(0.91,3.00)	0.1013	1.95(1.05,3.61)	0.0341	2.04(1.10,3.81)	0.0244
rs4244285	CYP2C19	GG	14	712		1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
		GA	28	666	GA *vs*.GG	2.04(1.07,3.90)	0.0303	2.13(1.10,4.12)	0.0248	2.19(1.13,4.24)	0.0200
		AA	2	189	AA *vs*.GG	0.60(0.14,2.65)	0.5010	0.58(0.13,2.61)	0.4814	0.58(0.13,2.60)	0.4787
					Dominant	1.76(0.93,3.33)	0.0843	1.81(0.94,3.49)	0.0759	1.85(0.96,3.56)	0.0666

Recessive	0.40(0.10,1.65)	0.2049	0.37(0.09,1.56)	0.1767	0.37(0.09,1.53)	0.1702
Additive	1.17(0.75,1.82)	0.4853	1.16(0.74,1.81)	0.5114	1.17(0.75,1.81)	0.4936

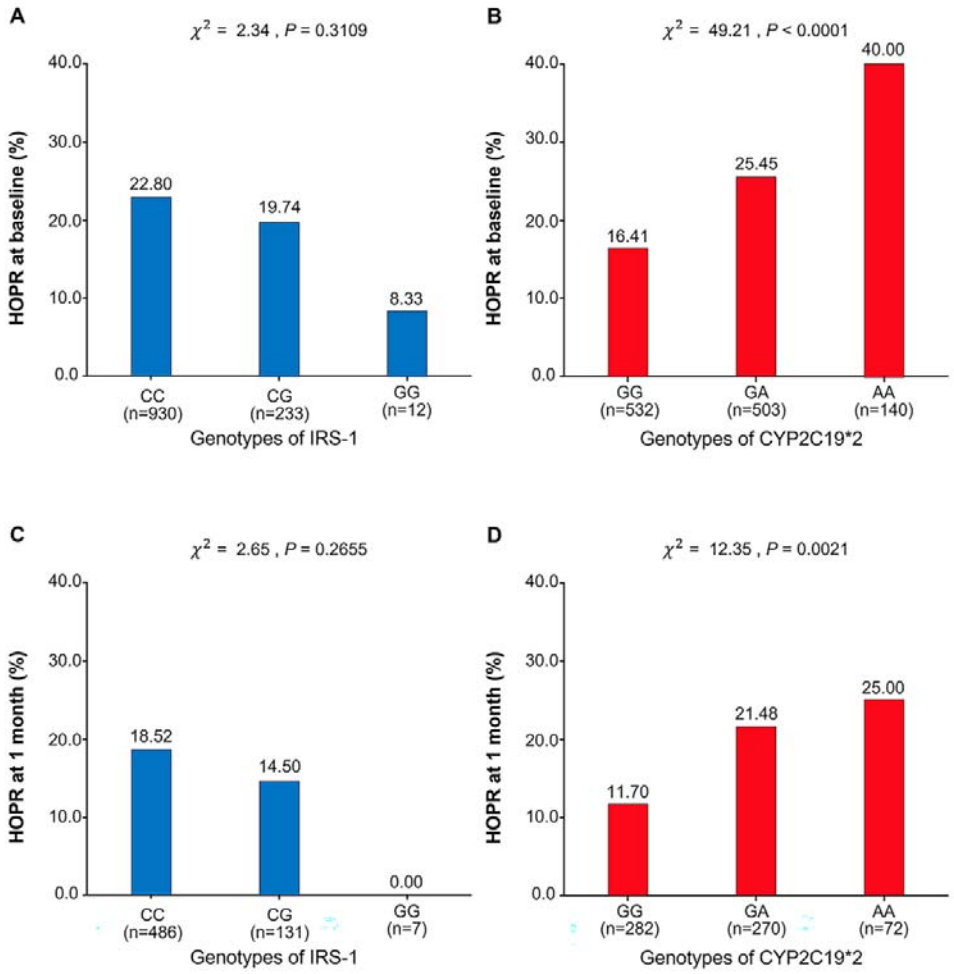
[*] Model adjusted for clinical covariates, including age, previous MI, hypertension, diabetes mellitus, LVEF, serum creatinine, diagnosis, low density lipoprotein, smoking status, previous PCI. [†] Model adjusted for CYP2C19*2/IRS-1 and clinical covariates including age, previous MI, hypertension, diabetes mellitus, LVEF, serum creatinine, diagnosis, low density lipoprotein, smoking status, previous PCI. Dominant model: IRS-1 CG and GG *vs*.CC, CYP2C19 GA and AA *vs*.GG. Recessive model: IRS-1 GG *vs*.CC and CG, CYP2C19 AA *vs*.GA and GG. Addictive model: the number of risk alleles is proportional to the risk of MACE. MACE, major adverse cardiovascular events; SNP, single nucleotide polymorphism; HR, hazard ratio; CI, confidence intervals; MI, myocardial infarction; LVEF, left ventricular ejection fraction; PCI, percutaneous coronary intervention.

Figure S1. Platelet reactivities (PL_{AA}) in patients with different genotypes of IRS-1 and CYP2C19*2.



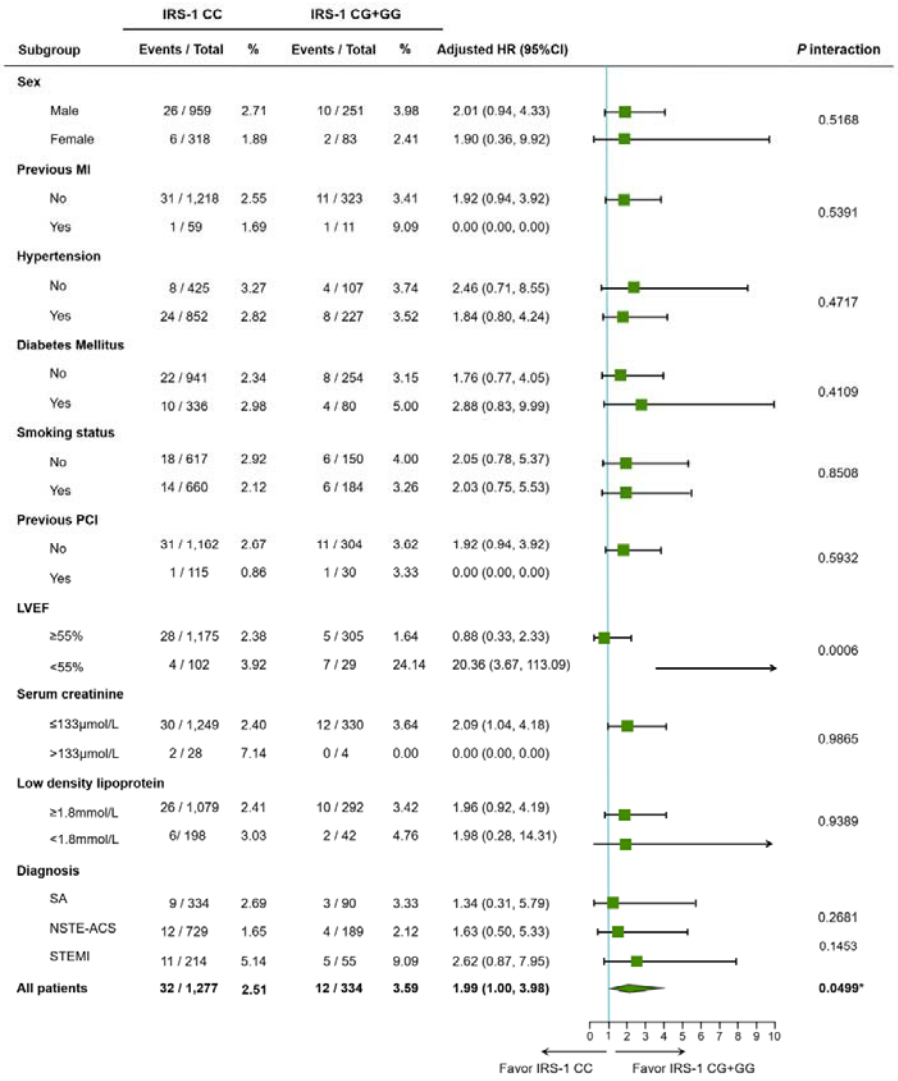
(A) Boxplot of IRS-1 and PL_{AA} at baseline and 1 month; (B) Boxplot of CYP2C19 and PL_{AA} at baseline and 1 month. The dashed line represents the cut-off point for HOPR (PL_{AA} > 20%). PL_{AA}, arachidonic acid induced platelet aggregation; HOPR, high on-treatment platelet reactivity.

Figure S2. Prevalence of HOPR by Genotypes of the IRS-1 and CYP2C19*2.



(A) Prevalence of HOPR in patients with different genotypes of IRS-1 at baseline. (B) Prevalence of HOPR in patients with different genotypes of CYP2C19*2 at baseline. (C) Prevalence of HOPR in patients with different genotypes of IRS-1 at 1 month. (D) Prevalence of HOPR in patients with different genotypes of CYP2C19*2 at 1 month. HOPR, High on-treatment platelet reactivity (PL_{ADP}>40%).

Figure S3. Forest plot of MACE risk in different IRS-1 genotypes.



* *P* value indicated the association between IRS-1 genotypes and MACE. The adjusted HR for LVEF <55% and the upper end of the 95% CI for LVEF<55% and LDL <1.8 mmol/L are not shown because they are >10. MACE, major adverse cardiovascular events; MI, myocardial infarction; PCI, percutaneous coronary intervention; LVEF, left ventricular ejection fraction; SA, stable angina pectoris; NSTE-ACS, non-ST-segment elevation acute coronary syndromes; STEMI, ST-segment-elevation myocardial infarction; HR, hazard ratio; CI, confidence intervals.