 Candidate Gene of NOS3, MMP3, AGT, and AGT1R and Pathway Analyses for Platelet Reactivity and Clinical Outcomes of Repeat Revascularization After First PCI in Chinese Patients

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Abstract

Purpose Major disadvantages of the percutaneous coronary intervention (PCI) are the high occurrence of repeat revascularization due to restenosis and disease progression. The current study aimed to identify indicators that can predict the risk of repeat revascularization.

Methods A total of 143 patients who underwent PCI and had genetic test results were enrolled. We retrospectively reviewed their medical records after the first PCI. P2Y12 reaction unit (PRU) test results were obtained by VerifyNow; 4 candidate genes (NOS3, MMP3, AGT, and AGT1R) and 380 genes related to platelet activation-related processes and clopidogrel activity were selected for analysis. Repeat revascularization and in-stent restenosis (ISR) were used as clinical outcomes, and PRU and ADP aggregation rates were used as platelet function outcomes in analysis.

Results After the first PCI, the incidence of repeat revascularization at 18, 30, and 42 months was 14.1% (20/142), 17.5% (24/137), and 39.7% (31/78), respectively. In the candidate gene analysis, rs7830 (NOS3) was associated with both ADP aggregation rate and 18- and 30-month ISR, and rs 62,275,847 (AGTR1) was associated with both ADP aggregation rate and 30-month ISR. In the pathway, gene-set analysis, the linkage rs471683 and rs7785386 of GNAI1|GNAT3 were associated with PRU and ADP aggregation rate, 18-month and 30-month ISR, and repeat revascularization within 30 months. Rs1715389 of GNAI1|GNAT3 was associated with both PRU and ADP aggregation rate, 18-month and 30-month ISR, and repeat revascularization within 30 months. Rs7313458 of ITPR2 was associated with PRU and ADP aggregation rate, 18-month and 30-month ISR, and repeat revascularization within 18 months.

Conclusions The genetic polymorphisms of rs7830 (NOS3), rs62275874 (AGTR1), linkage rs471683 and rs7785386 (GNAI1|GNAT3), rs1715389 (GNAI1|GNAT3), and rs7313458 (ITPR2) may lead to an increased risk of in-stent restenosis and revascularization after the first PCI in Chinese patients by affecting the efficacy of clopidogrel. The above six SNP may be used as potential genetic biomarkers for high risk of in-stent restenosis and revascularization after the first PCI in Chinese patients.

Keywords Genetic polymorphism · Clopidogrel · Percutaneous coronary intervention · Repeat revascularization · In-stent restenosis

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Introduction

Over the past few years, an exponential increase in percutaneous coronary intervention (PCI) as the common form of myocardial revascularization has led to a significant improvement in the clinical management of coronary artery disease (CAD) patients [1, 2]. Despite advances in stent technology, restenosis continues to be the most frequent cause of target lesion failure following PCI. In-stent restenosis (ISR) has been reported to occur in 32–55% of patients undergoing angioplasty and in 17–41% of patients receiving the bare metal stents (BMS) [3]. Restenosis-related complications include stable angina, unstable angina, acute coronary syndrome, acute myocardial infarction, and even death. Revascularization procedures for ISR could also be hindered by complications from access site bleeding, stent under-expansion, incomplete revascularization, coronary artery dissection, and stent thrombosis [4].

Some extrinsic and intrinsic factors may contribute to ISR. Numerous studies have examined the risk predictors of ISR. The following characteristics have been associated with ISR: age, female gender, diabetes, chronic kidney disease, and multivessel CAD; the lesions’ characteristics include smaller reference artery diameter, ostial lesion, and initial plaque burden [5–7]. Furthermore, the efficacy of clopidogrel as the necessary antiplatelet therapy after PCI is also a relevant factor that should not be ignored. Clopidogrel, with or without aspirin, has been proven to reduce the occurrence of ISR [8]. However, there is inter-individual variability in response to clopidogrel, and a substantial number of ISR still occur despite clopidogrel treatment, even among those treated with dual-antiplatelet agents [9].

Previous studies reported that genetic polymorphisms, such as P2RY12 [10], PON1 [11], ABCB1 [12], and CES1 [13], especially the reduced function of CYP2C19 [14], were associated with interindividual variability in response to clopidogrel. Still, the polymorphisms of these genes do not account for all the individual differences.

Our previous meta-analysis revealed that the polymorphism of NOS3, MMP3, AGT, and AGT1R might increase ISR risk after PCI based on the studies on the relationship between polymorphism and clinical outcomes [15]. However, to the best of our knowledge, no studies investigated the relationship between the polymorphism of these four genes and clopidogrel response. Furthermore, gene set analysis was mainly developed to analyze large-scale genomic data, which facilitates the interpretation of experimental results and helps to identify key biological findings [16, 17]. Candidate gene and gene set analysis has been widely used to determine a cumulative effect on platelet function by modifying basic platelet parameters, altering the expression or activity of key platelet receptors, and influencing downstream effector pathways utilized by these receptors [18, 19].

The present study focused on candidate gene association studies and gene set analysis to further explore the effect of NOS3, MMP3, AGT, and AGT1R as candidate genes on platelet function and repeat revascularization.

Methods

Subjects, Design, and Procedures

This study was a retrospective cohort review from medical records of CAD patients who took antiplatelet drugs and underwent genetic testing at the Peking University First Hospital. All patients were enrolled in a previous study, which was approved by the ethics committee of the Peking University First Hospital (NO. 2013 [634]). The protocol of this study was approved by the Ethics Committee of the Medical University of Peking University First Hospital and was in accordance with the Declaration of Helsinki (ethics approval number: 2017 scientific research ethics No. 81).

In this study, we screened patients according to the following inclusion criteria: (1) PCI or (2) patients who received dual antiplatelet therapy consisting of 100 mg of aspirin daily and 75 mg of clopidogrel for at least 1 year after the first PCI; however, if the endpoint occurred within 1 year, patients must have used dual antiplatelet therapy during the period from the first PCI to repeat revascularization. The exclusion criteria were (1) patients with cancer, viral hepatitis, and other related diseases or (2) incomplete records of the PCI operation. According to the above criteria, a total of 168 patients were enrolled between April 2015 and June 2016. Written informed consent was obtained from all participating patients.

Information was collected from a medical record review, including hospital inpatient, outpatient visits, and telephone contact records with patients or their families. Sociodemographic variables, medical history, therapeutic procedures, smoking status (i.e., current smoker vs. former/no smoker at admission), clinical events, stent implantation, laboratory results, genomics results, and follow-up information were reviewed.

The independent variables in the present study were (1) clinical variables, such as the age when the first PCI was conducted, gender, diabetes mellitus (DM), hypertension, chronic kidney disease (CKD), smoking status, and the time (days) from the first PCI to the repeat revascularization, including target vessel revascularization (TVR), target lesion revascularization (TLR), and non-target vessel revascularization (NTVR), either by repeated PCI or coronary artery bypass graft (CABG). The repeat revascularizations...
due to incomplete initial revascularization were excluded; (2) variables on genetic results; and (3) variables on the platelet aggregation by VerifyNow.

The outcomes of this study consisted of two following parts: the first part was platelet function, including PRU and ADP aggregation rate; the second part was the repeat revascularization and ISR after the first PCI. Either repeated PCI or CABG was determination as repeat revascularization, and the re-narrowing of vessel cross-sectional area of coronary CTA and angiography were determination as ISR. Follow-ups at 18, 30, and 42 months were used to evaluate the relationship between revascularization and potential indicators.

Platelet Function Measurements

In 71 out of 143 enrolled patients, the platelet function testing was performed by VerifyNow measurements. Platelet function was detected after the clopidogrel efficiency reached homeostasis (clopidogrel 75 mg daily for more than 7 days, 300 mg loading dose and 75 mg daily for 5 days, or 600 mg loading dose with 75 mg for 3 days) [20, 21].

The platelet activity of P2Y12 reaction unit (PRU) was assessed using the VerifyNow® P2Y12 (VN-P2Y12) assay (Accumetrics, San Diego, CA, USA) as described by Price MJ [22]. This test constitutes a whole-blood, point-of-care, light transmission-based optical detection assay that measures adenosine diphosphate (ADP)-induced platelet agglutination [23]. In addition to ADP, prostaglandin E1 is incorporated into the VN-P2Y12 assay. Prostaglandin E1 suppresses the intracellular free-calcium levels and thereby reduces the contribution of activation by ADP binding to P2Y1 receptors [24]. The assay was performed according to the manufacturer’s instructions within 10 to 15 min of venipuncture. Data were directly recorded from the VerifyNow® device as percent inhibition of PRU.

The inhibition of ADP-induced platelet aggregation of platelet-rich plasma (PRP) was measured in response to the exposure to 4.918 μmol/L ADP. A 2.7-mL blood sample was drawn from the antecubital vein and was anticoagulated with sodium citrate solution (0.3 mL, 0.109 M). The measurement was performed using a LBY-NJ4 Platelet Aggregation Profiler (Precil, Beijing, China) with a temperature maintained at 37 °C. The observed maximal platelet aggregation was recorded.

Candidate Gene and Pathway Selection

Candidate genes came from a meta-analysis of the relationship between NOS3, MMP3, AGT, AGT1R, and the risk of restenosis after PCI performed by our project team members and colleagues. In this study, 372 SNPs in these four genes were selected for analysis (Appendix Table 1).

Pathways related to platelet activation-related processes and clopidogrel activity were selected from KEGG, BioCarta, and GeneCards. A total of 380 genes from platelet activation pathway (KEGG), platelet amyloid precursor protein pathway (BioCarta), aspirin blocks signaling pathway involved in platelet activation (BioCarta), and clopidogrel related genes (GeneCard) were analyzed in our study (Appendix Table 2).

Genotyping

We collected the information on genetic testing results from enrolled patients. The SNPs were measured by OmniZhongHua-8 (Omni China), a specific genomic SNPs chip for Chinese individuals launched by the Illumina Company. The chip especially covers those frequent and rare variants that are found in the Chinese population, which covers 85% of the common genetic variation in the Chinese population at $r^2 > 0.8$. The optimized 900,000 SNPs tag information was from all three stages of the HapMap and the 1000 genome project (1KGP). OmniZhongHua-8 detection was relegated to the Beijing Yi De PR Technology Development Co. Ltd. After genotyping, samples and genetic markers were subjected to a stringent quality control protocol.

IMPUTE2 [25] was used to impute genotypes for autosomal SNPs using the CHB (Han Chinese in Beijing, China) population as a reference retrieved from the 1000 Genome Project dataset (Phase 3) [26]. Threshold criteria for imputed SNPs were set as pro > 0.9 and info > 0.5, where pro referred to the probability of an imputed genotype and info referred to the overall quality of an imputed SNP. There were 7,802,735 SNPs after genotyping, and only high-quality SNPs (genotyping rate > 0.95, call rate > 0.95, Hardy–Weinberg p > 0.00001) were kept for subsequent analysis.

Statistical Analysis

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) 21.0 software (IBM, Armonk, NY) and R (http://www.R-project.org). For clinical parameters, $P < 0.05$ was considered statistically significant. The continuous and categorical variables were presented as mean ± standard deviation (SD) and frequencies and percentages, respectively. To identify independent variables affecting repeat revascularization at 18 or 30 months, the effect was evaluated and expressed as an odd ratio (OR). Multivariable logistic regression was used to analyze adjusted correlations of SNPs with repeat revascularization and PRU.

PLINK v1.07 software was used to filter relevant SNPs, which were adjusted by smoking statuses. The SNPs of candidate genes and selected pathways were performed. This analysis was repeated 10,000 times in simulated datasets.
SNPs showing a significant ($p < 0.05$) association with the phenotypes under analysis were selected.

**Results**

**Patient Characteristics**

A total of 143 patients who underwent PCI with dual anti-platelet therapy were included in this study. The baseline characteristics of included patients at the first PCI are shown in Table 1.

**Association of SNPs with Platelet Function**

**The SNPs of Candidate Gene Variations**

To analyze the association of SNPs of candidate gene and platelet function, 372 SNPs of 4 genes (15 SNPs of NOS3, 9 SNPs of MMP3, 46 SNPs of AGT, and 302 SNPs of AGT1R) were evaluated in the current study. After adjusting for smoking status, 4 and 7 SNPs were significantly related with PRU values and ADP aggregation rate, respectively (Table 2).

**The SNPs of Pathway Gene Variations**

To analyze the association of SNPs of pathways and PRU or ADP aggregation rate, a total of 380 genes in four pathways (platelet activation pathway, platelet amyloid precursor protein pathway, aspirin blocks signaling pathway involved in platelet activation, and clopidogrel related gene) were analyzed in our study. After adjustment of smoking status, 363 SNPs were significantly related to PRU values, and 529 SNPs were significantly related to ADP aggregation rate (Appendix Table 3). Furthermore, 25 SNPs were associated with PRU value and ADP aggregation rate; details are listed in Table 3. Among these SNPs, the $P$-link values of kglp10633449 were less than 0.01 both for PRU and ADP aggregation rate, and the $P$-link values of PRU and ADP aggregation rate were 0.00606 and 0.003235, respectively.

**Association of SNPs with the Outcome**

**The SNPs of Candidate Gene Variations**

To analyze the association of SNPs of candidate genes and clinical outcomes, 372 SNPs of the four genes were analyzed. After adjusting for smoking status, 10, 20, and 8 SNPs were significantly related with repeat revascularization at 18, 30, and 42 months, respectively. Meanwhile, 24, 35, and 8 SNPs were significantly related with ISR at 18, 30, and 42 months, respectively (Table 4).

**The SNPs of Pathway Gene Variations**

In addition to platelet function, 380 identical genes were used to analyze the association between SNPs and clinical outcomes. After adjusting for smoking status, 462 SNPs were significantly related with repeat revascularization.
within 18 months, 500 SNPs were significantly related with it within 30 months, 273 SNPs were significantly related with it within 42 months, 468 SNPs were significantly related with ISR within 18 months, 428 SNPs were significantly related with ISR within 30 months, and 363 SNPs were significantly related with ISR within 42 months. The details of the 2494 SNPs are shown in Appendix Table 3.

There were 61 SNPs and 2 SNPs significantly associated with repeat revascularization and ISR within 18 months, 30 months, and 42 months (Table 5). There were three linkage SNPs (rs3788367, kgp3029439, and kgp15051272) of CABIN1. The correlation \( P \)-link values between them and repeat revascularization at 18 months, 30 months, and 42 months were 0.002694, 0.0001099, and 0.007271, respectively (\( P < 0.01 \) for all).

### Association of SNPs with Platelet Function and Outcomes

#### The SNPs of Candidate Gene Variations

There were two SNPs associated with both platelet function and clinical outcome. rs7830 of NOS3 was associated with both ADP aggregation rate (Plink-P, 0.013) and 18-month ISR (Plink-P, 0.035) and 30-month ISR (Plink-P, 0.025), respectively. rs 62,275,847 of AGTR1 was associated with both ADP aggregation rate (Plink-P, 0.029) and 30-month ISR (Plink-P, 0.036).

#### The SNPs of Pathway Gene Variations

Among the 588 SNPs associated with PRU, 21, 28, and 12 were associated with ISR at 18, 30, and 42 months, and 36, 33, and 16 SNPs were associated with re-revascularization at 18, 30, and 42 months, respectively. Of the 529 SNPs associated with ADP aggregation, 13, 15, and 8 SNPs were associated with ISR at 18, 30, and 42 months, and 16, 12, and 11 SNPs were associated with repeat revascularization at 18, 30, and 42 months, respectively (Table S5).

There were four SNPs of pathway gene variations associated with both platelet function and clinical outcome. The linkage rs471683 and rs7785386 of GNAI1|GNAT3 were associated with both PRU (Plink-P, 0.016) and ADP aggregation rate (Plink-P, 0.002), 18-month ISR (Plink-P, 0.013), 30-month ISR (0.035), and repeat revascularization within 30 months (Plink-P, 0.030). Rs1715389 of GNAI1|GNAT3 was associated with both PRU (Plink-P, 0.019) and ADP aggregation rate (Plink-P, 0.002), 18-month ISR (Plink-P, 0.046), and repeat revascularization within 30 months (Plink-P, 0.040). Rs7313458 of ITPR2 were associated with both PRU (Plink-P, 0.024) and ADP aggregation rate (Plink-P, 0.036), 18-month ISR (0.043), 30-month ISR (Plink-P, 0.004), and repeat revascularization within 18 months (Plink-P, 0.009).

### Discussion

In the current study, we selected 372 SNPs from 4 genes to analyze their association with the platelet function and clinical outcomes of dual antiplatelet therapy after the first PCI. Furthermore, we explored the relation of the polymorphisms of 380 genes in 9 platelet function pathways with platelet function and clinical outcomes in the included patients. To the best of our knowledge, this is the first study that investigated the association of polymorphism with both platelet function detected by two assays and clinical outcomes during 42-month follow-up in the Chinese cohort after PCI. We intended to capture the polymorphic variations in relation to higher thrombotic risk after PCI in Chinese.
In the candidate gene-associated SNP analysis section, two individual SNPs were significantly associated with the pharmacodynamics of clopidogrel and clinical outcomes. Their polymorphism was significantly associated with both ADP aggregation rate and ISR events during the follow-up. SNP rs7830, which is located in the NOS3 locus on chromosome 7, produces nitric oxide (NO) that is implicated in vascular smooth muscle relaxation through a cGMP-mediated signal transduction pathway. NO mediates vascular endothelial growth factor (VEGF)-induced angiogenesis in coronary vessels and promotes blood clotting through the activation of platelets [27, 28]. While there is good evidence on the clinical importance of NOS3 single nucleotide polymorphisms, the current knowledge is superficial in most clinical settings, and further studies are needed [29]. In addition, rs62275874 of AGTR1 has a similar association with both the ADP aggregation rate and ISR events during the follow-up period of 30 months. Furthermore, by its control functions on the blood pressure and volume in the cardiovascular system, AGTR1 may play a role in the generation of reperfusion arrhythmias following the restoration of the blood flow to the ischemic or infarcted myocardium. In this respect, Azova et al. found that the heterozygotes for AGTR1 rs5186 were more frequent among patients with

| GeneSymbol | SNP | Plink-P (PRU) | Plink-P (ADP aggregation rate) | CHR | BP | Gene type | GENO | GENO (ADP aggregation rate) | GeneLocation |
|------------|-----|---------------|-------------------------------|-----|----|-----------|------|-----------------------------|------------|
| TCF4 | rs8097803 | 0.0405 | 0.02925 | 18 | 53,805,335 | AA/AC/CC | 0/3/68 | 2/6/80 | INTERGENIC |
| TXNL1 | GNAI1 | rs808956 | 0.02125 | 0.04342 | 7 | 80,074,216 | AA/AC/CC | 3/25/43 | 4/36/49 | INTERGENIC |
| GNAI1 | rs7785386 | 0.01622 | 0.002119 | 7 | 80,053,909 | AA/AC/CC | 4/19/48 | 3/28/58 | INTERGENIC |
| GNAI1 | MAPK3 | rs7313458 | 0.0239 | 0.03557 | 12 | 26,763,273 | GG/GA/AA | 7/34/30 | 15/44/29 | INTERGENIC |
| CORO1A | rs6565176 | 0.001379 | 0.01754 | 16 | 30,174,926 | AA/AG/GG | 2/14/55 | 2/12/75 | INTERGENIC |
| F11 | rs6552971 | 0.03955 | 0.04706 | 4 | 187,238,388 | AA/AG/GG | 3/25/43 | 4/36/49 | INTERGENIC |
| MTRN1A | EDN1 | rs5370 | 0.01436 | 0.01492 | 6 | 12,296,255 | AA/AC/CC | 3/23/45 | 6/34/49 | CODING |
| GNAI1 | rs471683 | 0.01622 | 0.013317 | 7 | 80,040,648 | AA/AG/GG | 4/19/48 | 3/28/58 | INTERGENIC |
| GNAI1 | rs330708 | 0.0403 | 0.01392 | 1 | 187,183,135 | AA/AG/GG | 3/31/56 | 3/33/55 | INTERGENIC |
| FAM5C | ADCY8 | rs273429 | 0.01981 | 0.005593 | 8 | 132,479,901 | GG/GA/AA | 3/24/49 | 6/42/41 | INTERGENIC |
| F13A3 | CDH5 | rs2344564 | 0.02326 | 0.0005299 | 16 | 66,413,150 | GG/GA/AA | 6/31/34 | 6/33/50 | INTERGENIC |
| COL4A4 | rs2078635 | 0.03225 | 0.04817 | 2 | 228,020,491 | AA/AC/CC | 15/36/20 | 14/40/35 | INTERGENIC |
| EDN1 | rs2071943 | 0.01436 | 0.013317 | 6 | 12,295,814 | AA/AG/GG | 3/23/45 | 6/34/49 | INTERGENIC |
| COL4A4 | rs1922021 | 0.03246 | 0.013317 | 7 | 80,051,323 | GG/GA/AA | 15/35/21 | 15/38/36 | INTERGENIC |
| GNAI1 | rs17153898 | 0.01947 | 0.002119 | 7 | 80,040,648 | GG/GA/AA | 4/19/47 | 3/28/58 | INTERGENIC |
| NLRP1 | PRKG1 | rs16913596 | 0.0374 | 0.02699 | 10 | 54,023,299 | GG/GA/AA | 12/26/33 | 14/44/31 | INTERGENIC |
| COL4A3BP | HMGC1 | rs16872521 | 0.006834 | 0.04888 | 5 | 74,663,223 | GG/GA/AA | 1/9/61 | 1/7/80 | INTERGENIC |
| COL4A4 | PRKG1 | rs1406477 | 0.02911 | 0.04511 | 10 | 54,019,620 | GG/GA/AA | 11/26/33 | 15/42/32 | INTERGENIC |
| CST2T | PRKG1 | rs10823243 | 0.04954 | 0.04969 | 10 | 53,344,284 | AA/AC/CC | 1/4/57 | 0/14/75 | INTERGENIC |
| COL4A4 | COL4A4 | rs10498217 | 0.03879 | 0.009097 | 2 | 228,010,257 | AA/AG/GG | 0/12/59 | 0/10/79 | INTERGENIC |
| FAM5C | PLA2G4A | rs10494595 | 0.03723 | 0.02745 | 1 | 187,239,230 | CC/CA/AA | 6/23/42 | 8/34/47 | INTERGENIC |
| COL4A4 | ADCY2 | rs10051470 | 0.01634 | 0.04435 | 13 | 110,970,267 | GG/GA/AA | 5/17/49 | 4/26/59 | INTERGENIC |
Table 4 Results of individual polymorphism analysis of candidate genes associated with clinical outcomes

| Gene Symbol | SNP | P value | CHR | BP | Gene type | GENO | Gene Location |
|-------------|-----|---------|-----|----|-----------|------|---------------|
| **Repeat revascularization within 18 months** |
| MMP3 | rs522616 | 0.003671 | 11 | 102,715,048 | GG/GA/AA | 12/52/67 | INTERGENIC |
| FLJ30375 | AGTR1 | rs4308618 | 0.02121 | 3 | 148,337,463 | GG/GA/AA | 18/59/54 | INTERGENIC |
| FLJ30375 | AGTR1 | rs16859648 | 0.02182 | 3 | 147,394,647 | CC/CA/AA | 19/57/55 | INTERGENIC |
| FLJ30375 | AGTR1 | rs11928247 | 0.0224 | 3 | 147,492,839 | GG/GA/AA | 5/33/93 | INTERGENIC |
| FLJ30375 | AGTR1 | rs6777677 | 0.02432 | 3 | 147,443,667 | GG/GA/AA | 1/16/114 | INTERGENIC |
| AGT | rs2148582 | 0.03039 | 1 | 230,849,799 | GG/GA/AA | 5/50/76 | INTRON |
| AGT | rs2493134 | 0.03039 | 1 | 230,849,359 | GG/GA/AA | 5/50/76 | INTRON |
| AGT | rs5051 | 0.03039 | 1 | 230,845,794 | GG/GA/AA | 5/50/76 | UTR |
| AGT | rs699 | 0.03039 | 1 | 230,845,794 | GG/GA/AA | 5/50/76 | CODING |
| FLJ30375 | AGTR1 | rs2873943 | 0.0322 | 3 | 147,403,067 | GG/GA/AA | 17/70/44 | INTERGENIC |
| **Repeat revascularization within 30 months** |
| FLJ30375 | AGTR1 | rs275685 | 0.001839 | 3 | 148,347,742 | GG/GA/AA | 0/18/71 | INTERGENIC |
| FLJ30375 | AGTR1 | rs16859648 | 0.007301 | 3 | 147,394,647 | CC/CA/AA | 12/42/35 | INTERGENIC |
| FLJ30375 | AGTR1 | rs4528908 | 0.01295 | 3 | 148,085,522 | GG/GA/AA | 12/44/33 | INTERGENIC |
| FLJ30375 | AGTR1 | rs2873943 | 0.01663 | 3 | 147,403,067 | GG/GA/AA | 12/43/33 | INTERGENIC |
| FLJ30375 | AGTR1 | rs4681375 | 0.01745 | 3 | 147,496,499 | GG/GA/AA | 22/44/23 | INTERGENIC |
| FLJ30375 | AGTR1 | rs6440539 | 0.01981 | 3 | 148,124,129 | GG/GA/AA | 17/43/29 | INTERGENIC |
| FLJ30375 | AGTR1 | rs6440544 | 0.02098 | 3 | 148,170,986 | GG/GA/AA | 14/42/33 | INTERGENIC |
| FLJ30375 | AGTR1 | rs7610743 | 0.0262 | 3 | 148,170,986 | GG/GA/AA | 14/42/33 | INTERGENIC |
| FLJ30375 | AGTR1 | rs1391796 | 0.03621 | 3 | 147,505,136 | GG/GA/AA | 10/46/33 | INTERGENIC |
| FLJ30375 | AGTR1 | rs79128431 | 0.03703 | 7 | 148,131,545 | GG/GA/AA | 2/35/52 | INTERGENIC |
| FLJ30375 | AGTR1 | rs1808593 | 0.03779 | 7 | 150,708,302 | CC/CA/AA | 1/33/55 | INTERGENIC |
| NOS3 | ATG9B | rs1843056 | 0.03779 | 7 | 148,706,915 | GG/GA/AA | 1/33/55 | INTERGENIC |
| NOS3 | rs743506 | 0.03779 | 7 | 150,708,302 | CC/CA/AA | 1/33/55 | INTERGENIC |
| **Repeat revascularization within 42 months** |
| FLJ30375 | AGTR1 | rs275685 | 0.00856 | 3 | 148,347,742 | GG/GA/AA | 0/8/36 | INTERGENIC |
| FLJ30375 | AGTR1 | rs7627414 | 0.00931 | 3 | 147,502,818 | GG/GA/AA | 14/45/30 | INTERGENIC |
| FLJ30375 | AGTR1 | rs6763183 | 0.01413 | 3 | 148,048,706 | CC/CA/AA | 20/41/28 | INTERGENIC |
| FLJ30375 | AGTR1 | rs1051322 | 0.04263 | 3 | 147,361,337 | CC/CA/AA | 2/29/58 | INTERGENIC |
| FLJ30375 | AGTR1 | rs6693640 | 0.04797 | 3 | 147,322,215 | GG/GA/AA | 3/27/59 | INTERGENIC |
| FLJ30375 | AGTR1 | rs67111729 | 0.04797 | 3 | 147,308,161 | GG/GA/AA | 3/27/59 | INTERGENIC |
| **ISR within 18 months** |
| FLJ30375 | AGTR1 | rs275685 | 0.00856 | 3 | 148,347,742 | GG/GA/AA | 0/8/36 | INTERGENIC |
| FLJ30375 | AGTR1 | rs7610743 | 0.00931 | 3 | 148,347,586 | AA/AT/TT | 0/11/33 | INTERGENIC |
| FLJ30375 | AGTR1 | rs4308618 | 0.02651 | 3 | 148,337,463 | GG/GA/AA | 8/19/17 | INTERGENIC |
| FLJ30375 | AGTR1 | rs16859648 | 0.03545 | 3 | 147,394,647 | CC/CA/AA | 7/21/16 | INTERGENIC |
| FLJ30375 | AGTR1 | rs4528908 | 0.04661 | 3 | 148,085,522 | GG/GA/AA | 8/20/15 | INTERGENIC |
| FLJ30375 | AGTR1 | rs6440544 | 0.04662 | 3 | 148,170,986 | GG/GA/AA | 8/22/14 | INTERGENIC |
| FLJ30375 | AGTR1 | rs9836223 | 0.04797 | 3 | 147,474,127 | GG/GA/AA | 2/17/25 | INTERGENIC |
| FLJ30375 | AGTR1 | rs2319323 | 0.04797 | 3 | 147,475,370 | GG/GA/AA | 2/17/25 | INTERGENIC |
| Gene Symbol | SNPs | P-value | CHR | BP | Gene type | Geno | Gene Location |
|------------|------|---------|-----|----|-----------|------|---------------|
| FLJ30375 | rs9873611 | 0.03093 | 3 | 147,270,026 | GG/GA/AA | 21/67/50 | INTERGENIC |
| FLJ30375 | rs200127120 | 0.03187 | 3 | 148,100,446 | CC/CA/AA | 8/54/76 | INTERGENIC |
| FLJ30375 | rs7610876 | 0.03187 | 3 | 148,103,369 | CC/CA/AA | 8/54/76 | INTERGENIC |
| NOS3 | rs7830 | 0.03477 | 7 | 150,709,571 | GG/GA/AA | 27/66/44 | INTERGENIC |
| FLJ30375 | rs7619579 | 0.03478 | 3 | 147,390,505 | GG/GA/AA | 4/22/68 | INTERGENIC |
| ISR within 30 months |
| FLJ30375 | rs202136024 | 0.000795 | 3 | 147,963,041 | GG/GA/AA | 29/40/27 | INTERGENIC |
| FLJ30375 | rs1874295 | 0.000879 | 3 | 147,955,974 | CC/CA/AA | 20/52/24 | INTERGENIC |
| FLJ30375 | rs4355248 | 0.002405 | 3 | 147,957,721 | CC/CA/AA | 6/43/47 | INTERGENIC |
| FLJ30375 | rs1685964 | 0.006205 | 3 | 147,394,647 | CC/CA/AA | 14/43/39 | INTERGENIC |
| FLJ30375 | rs10804724 | 0.009196 | 3 | 147,989,624 | GG/GA/AA | 5/35/56 | INTERGENIC |
| MMP1 | rs522616 | 0.009276 | 11 | 102,715,048 | GG/GA/AA | 14/56/68 | INTERGENIC |
| ISR within 42 months |
| FLJ30375 | rs202136024 | 0.01152 | 3 | 147,963,041 | GG/GA/AA | 9/23/67 | INTERGENIC |
| FLJ30375 | rs1874295 | 0.0168 | 3 | 147,955,974 | CC/CA/AA | 9/25/11 | INTERGENIC |
| FLJ30375 | rs4591472 | 0.01982 | 3 | 148,349,586 | AA/AT/TT | 2/28/66 | INTERGENIC |
| FLJ30375 | rs4269055 | 0.0236 | 3 | 148,347,742 | GG/GA/AA | 17/49/30 | INTERGENIC |
| FLJ30375 | rs6800835 | 0.04439 | 3 | 148,328,711 | GG/GA/AA | 6/20/19 | INTERGENIC |
Table 5: Results of individual gene set-base analysis of pathway genes associated with clinical outcome

| Gene Symbol | SNP     | Plink-P (within 18 months) | Plink-P (within 30 months) | Plink-P (within 42 months) | CHR | BP  | Gene type | GENO (within 18 months) | GENO (within 30 months) | GENO (within 42 months) | Gene Location |
|-------------|---------|----------------------------|---------------------------|----------------------------|-----|-----|-----------|------------------------|------------------------|------------------------|------------------|
| TCF4        | rs9966430 | 0.02164                    | 0.03221                   | 0.02041                    | 18  | 53,125,364 | AA/AC/CC   | 34/62/35               | 22/45/22               | 8/23/13                | INTRON          |
| PRKG1       | rs9943368 | 0.01008                    | 0.01812                   | 0.04452                    | 10  | 53,015,492 | GG/GA/AA   | 27/65/39               | 20/47/22               | 11/20/13               | INTRON          |
| VKORC1      | rs9934438 | 0.01287                    | 0.03725                   | 0.036                      | 16  | 31,104,878 | GG/GA/AA   | 1/19/111              | 1/12/76                | 1/4/39                 | INTRON          |
| VKORC1 | BCKDK | rs9923231 | 0.01287 | 0.03725 | 0.036 | 16 | 31,107,689 | GG/GA/AA | 1/19/111 | 1/12/76 | 1/4/39 | INTRON |
| TMEM132E | CCT6B | rs9916627 | 0.01855 | 0.01426 | 0.01529 | 17 | 33,010,157 | AA/AG/GG | 5/47/79 | 4/29/56 | 1/18/25 | INTRON |
| VKORC1 | BCKDK | rs9923231 | 0.01287 | 0.03725 | 0.036 | 16 | 31,107,689 | GG/GA/AA | 1/19/111 | 1/12/76 | 1/4/39 | INTRON |
| GRM7        | rs8853314 | 0.02919                    | 0.009172                  | 0.04203                    | 3   | 7,017,919  | AA/AG/GG   | 5/47/79               | 4/29/56                | 1/18/25               | INTRON          |
| TMEM132E | CCT6B | rs9943438 | 0.01287 | 0.03725 | 0.036 | 16 | 31,104,878 | GG/GA/AA | 1/19/111 | 1/12/76 | 1/4/39 | INTRON |
| TMEM132E | CCT6B | rs9883143 | 0.01741 | 0.02956 | 0.01888 | 17 | 33,042,432 | AA/AC/CC | 22/45/22 | 8/23/13 | 5/18/25 | INTRON |
| TMEM132E | CCT6B | rs945442 | 0.004404 | 0.01426 | 0.01529 | 17 | 33,010,157 | AA/AG/GG | 5/47/79 | 4/29/56 | 1/18/25 | INTRON |
| GPI         | rs191425 | 0.002074                   | 0.01426                   | 0.03953                    | 19  | 34,888,052 | GG/GA/AA   | 5/47/79               | 4/29/56                | 1/18/25               | INTRON          |
| GPI         | rs8075272 | 0.01741                    | 0.02956                   | 0.01888                    | 17  | 33,042,432 | AA/AC/CC | 22/45/22 | 8/23/13 | 5/18/25 | INTRON |
| ADAMTS11   | FAM154A | rs9923231 | 0.01287 | 0.03725 | 0.036 | 16 | 31,104,878 | GG/GA/AA | 1/19/111 | 1/12/76 | 1/4/39 | INTRON |
| GPI         | rs8853314 | 0.02919                    | 0.009172                  | 0.04203                    | 3   | 7,017,919  | AA/AG/GG   | 5/47/79               | 4/29/56                | 1/18/25               | INTRON          |
| GPI         | rs8853314 | 0.02919                    | 0.009172                  | 0.04203                    | 3   | 7,017,919  | AA/AG/GG   | 5/47/79               | 4/29/56                | 1/18/25               | INTRON          |
| GPI         | rs8853314 | 0.02919                    | 0.009172                  | 0.04203                    | 3   | 7,017,919  | AA/AG/GG   | 5/47/79               | 4/29/56                | 1/18/25               | INTRON          |
| GPI         | rs8853314 | 0.02919                    | 0.009172                  | 0.04203                    | 3   | 7,017,919  | AA/AG/GG   | 5/47/79               | 4/29/56                | 1/18/25               | INTRON          |
| GeneSymbol | SNP | Plink-P (within 18 months) | Plink-P (within 30 months) | Plink-P (within 42 months) | CHR | BP | Gene type | GENO (within 18 months) | GENO (within 30 months) | GENO (within 42 months) | GeneLocation |
|------------|-----|---------------------------|---------------------------|---------------------------|-----|----|-----------|-------------------|-------------------|-------------------|--------------|
| ADCY10     | rs203782 | 0.0009139 | 0.02799 | 0.02774 | 1 | 167,882,386 | 6/41/84 | 4/31/54 | 37,984 | INTRON |
| ADCY10     | rs203777 | 0.0009139 | 0.02799 | 0.02774 | 1 | 167,876,616 | 6/41/84 | 4/31/54 | 37,984 | INTRON |
| PRKG1      | rs1865645 | 0.02848 | 0.01812 | 0.04452 | 10 | 52,991,510 | 35/60/36 | 22/43/24 | 8/21/15 | INTRON |
| P2RY1 | LOC100287133 | 0.02622 | 0.02513 | 0.02448 | 3 | 152,831,259 | 0/19/112 | 0/13/76 | 0/638 | INTRON |
| TCF4       | rs1452788 | 0.028 | 0.04441 | 0.02954 | 18 | 53,117,304 | GG/GA/AA | 3/5/21 | 8/21/15 | INTRON |
| TCF4 | TXNL1 | rs12457258 | 0.0309 | 0.00162 | 0.01466 | 18 | 54,265,669 | GG/GA/AA | 6/33/50 | 3/14/27 | INTRON |
| MERTK | TMEM87B | rs11897014 | 0.03188 | 0.0448 | 0.03151 | 2 | 112,795,908 | GG/GA/AA | 0/12/77 | 0/8/36 | INTRON |
| PLA2G4A | FAM5C | rs11591211 | 0.03326 | 0.02318 | 0.046 | 17 | 64,725,031 | GG/GA/AA | 0/12/77 | 0/8/36 | INTRON |
| PRKCA      | rs11079667 | 0.02184 | 0.008135 | 0.03042 | 17 | 53,013,479 | AA/AC/CC | 23/58/13 | 8/21/15 | INTRON |
| ITPR2      | rs10842760 | 0.01335 | 0.04289 | 0.04301 | 12 | 26,722,311 | GG/GA/AA | 22/65/44 | 9/18/17 | INTRON |
| STIM1      | rs10835407 | 0.04901 | 0.02266 | 0.0188 | 11 | 3,993,231 | GG/GA/AA | 6/37/46 | 4/20/20 | INTRON |
| PRKG1      | rs10822496 | 0.01356 | 0.0245 | 0.0443 | 10 | 53,013,479 | AA/AC/CC | 23/68/40 | 10/21/13 | INTRON |
| ITPR2      | rs10743586 | 0.008208 | 0.01073 | 0.03645 | 12 | 26,642,577 | GG/GA/AA | 13/48/24 | 8/21/15 | INTRON |
| STIM1      | rs10742189 | 0.01766 | 0.03611 | 0.01824 | 11 | 3,928,752 | CC/CA/AA | 14/39/36 | 8/21/15 | INTRON |
| PRKG1      | rs1040978 | 0.03093 | 0.0245 | 0.0443 | 10 | 52,994,866 | GG/GA/AA | 26/65/40 | 10/21/13 | INTRON |
| GRLF1      | rs10425259 | 0.009649 | 0.01669 | 0.02654 | 19 | 47,492,475 | GG/GA/AA | 16/40/33 | 7/23/14 | INTRON |
| CXCL12     | rs1029153 | 0.007657 | 0.01525 | 0.02828 | 10 | 44,867,146 | GG/GA/AA | 5/24/60 | 38/654 | INTRON |
| ADCY2      | rs10066518 | 0.006643 | 0.01434 | 0.0378 | 5 | 7,518,855 | GG/GA/AA | 17/49/45 | 12/30/47 | INTRON |
| F2R | LOC100287744 | 0.02785 | 0.04026 | 0.01822 | 5 | 76,066,861 | GG/GA/AA | 15/50/61 | 4/38/41 | INTRON |
| PLA2G4A | FAM5C | kgp3945860 | 0.0268 | 0.02926 | 0.04043 | 3 | 152,815,451 | GG/GA/AA | 0/16/121 | 0/9/86 | INTRON |
| ISR        | P2RY1 | LOC100287133 | kgp30159942 | 0.03322 | 0.02581 | 0.04206 | 1 | 187,899,551 | GG/GA/AA | 16/48/74 | 4/15/25 | INTRON |
| ADAMTSL1   | rs10811043 | 0.03643 | 0.001305 | 0.04403 | 9 | 18,832,659 | GG/GA/AA | 7/49/40 | 3/25/17 | INTRON |
early ISR [30]. However, few studies have been conducted on rs62275874.

SNP rs7313458 is intron variant of ITPR2 (inositol 1,4,5-trisphosphate receptor type 2). The protein encoded by this gene belongs to the inositol 1,4,5-trisphosphate receptor family, whose members are second messenger intracellular Ca\^{2+} release channels. These proteins mediate a rise in cytoplasmic calcium in response to receptor-activated production of inositol triphosphate. In platelets, the elevation in the intracellular Ca\^{2+} concentration contributes to various steps of cellular activation, such as reorganization of the actin cytoskeleton necessary for shape change [31, 32] and degradation or inside-out activation of integrin \text{αIIbβ3} indispensable for platelet aggregation [33]. Polymorphism of ITPR2 in the change of platelet intracellular Ca\textsuperscript{2+} ion concentration offers a new explanation for platelet function of individual differences. 1,4,5-Inositol trisphosphate (IP3) opens Ca\textsuperscript{2+} channels in the platelet dense tubular system, raising intracellular Ca\textsuperscript{2+} levels. In addition, in our study, ITPR2 was associated with both platelet function and clinical outcomes, thus suggesting that ITPR2 gene polymorphism leads to adverse clinical outcomes by affecting platelet function. Another locus that affects both platelet function and clinical outcomes is the linkage rs471683 and rs7785386 of G protein subunit alpha i1 (GNAI1) and protein subunit alpha transducin 3 (GNAT3). Platelet G protein-coupled receptors (GPCRs) initiate and reinforce platelet activation and thrombus formation [34]. Activation of platelets by GPCRs mainly produces effects through the receptors P2Y12 and P2Y1 for ADP, TP receptors for TXA2, and PAR1 and PAR4 receptors for thrombin. Therefore, genetic polymorphisms of GNAI1 and GNAT3 have the potential to influence platelet function and clinical outcome.

The present study has some limitations. First, this is a retrospective study, for which all data were obtained from the previous medical records, and the majority of clinical outcome events were obtained by telephone follow-up. Second, the small sample size makes the identified associations between genes and revascularization just a possible indication.

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Data Availability Availability of data and material has been described in the manuscript. Data would be provided upon request from the authors and in accordance with local regulations.

Declarations

Ethics Approval All procedures involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Consent for Publication Written informed consent was obtained from all study participants.

Conflict of Interest The authors declare no competing interest.

Informed Consent All participants provided written informed consent before enrollment in this study.

References

1. Weintraub WS, Grau-Sepulveda MV, Weiss JM, et al. Prediction of long-term mortality after percutaneous coronary intervention in older adults: results from the National Cardiovascular Data Registry. Circulation. 2012;125:1501–10.

2. Xiu WJ, Yang HT, Zheng YY, Ma YT, Xie X. Drug-eluting balloons versus second-generation drug-eluting stents for treating in-stent restenosis in coronary heart disease after PCI: a meta-analysis. Cardiol Res Pract. 2018;2018:7658145.

3. Buccheri D, Piraino D, Andolina G, Cortese B. Understanding and managing in-stent restenosis: a review of clinical data, from pathogenesis to treatment. J Thorac Dis. 2016;8:E1150-e1162.

4. Omeh DJ, Shlofmitz E. Restenosis. StatPearls. Treasure Island (FL): StatPearls Publishing StatPearls Publishing LLC., 2020.

5. Siontis GC, Stefanini GG, Movridis D, et al. Percutaneous coronary interventional strategies for treatment of in-stent restenosis: a network meta-analysis. Lancet. 2015;386:655–64.

6. Dangas GD, Claessen BE, Caixeta A, Sanidas EA, Mintz GS, Mehran R. In-stent restenosis in the drug-eluting stent era. J Am Coll Cardiol. 2010;56:1897–907.

7. Lee MS, Banka G. In-stent restenosis. Interv. Cardiol Clin. 2016;5:211–20.

8. Gorog DA, Geisler T. Platelet inhibition in acute coronary syndrome and percutaneous coronary intervention: insights from the past and present. Thromb Haemost 2020.

9. Mangiacapra F, Patti G, Barbato E, et al. A therapeutic window for platelet reactivity for patients undergoing elective percutaneous coronary intervention: results of the ARMYDA-PROVE (Antiplalet therapy for Reduction of MYocardial Damage during Angioplasty-Platelet Reactivity for Outcome Validation Effort) study. JACC Cardiovasc Interv. 2012;5:281–9.

10. Li M, Wang H, Xuan L et al. Associations between P2RY12 gene polymorphisms and risks of clopidogrel resistance and adverse
cardiovascular events after PCI in patients with acute coronary syndrome. Medicine (Baltimore) 2017;96:e6553.
11. Bouman HJ, Schomig E, van Werkum JW, et al. Paraoxonase-1 is a major determinant of clopidogrel efficacy. Nat Med. 2011;17:110–6.
12. Park MW, Her SH, Kim CJ, et al. Evaluation of the incremental prognostic value of the combination of CYP2C19 poor metabolizer status and ABCB1 3435 TT polymorphism over conventional risk factors for cardiovascular events after drug-eluting stent implantation in East Asians. Genet Med. 2016;18:833–41.
13. Lewis JP, Horenstein RB, Ryan K, et al. The functional G143E variant of carboxylesterase 1 is associated with increased clopidogrel active metabolite levels and greater clopidogrel response. Pharmacogenet Genomics. 2013;23:1–8.
14. Pan Y, Chen W, Xu Y, et al. Genetic polymorphisms and clopidogrel efficacy for acute ischemic stroke or transient ischemic attack: a systematic review and meta-analysis. Circulation. 2017;135:21–33.
15. Zhou S, Mu G, Wei S, et al. Associations between polymorphisms of endothelial nitric oxide synthase, matrix metalloproteinase 3, angiotensinogen, and angiotensin II type 1 receptor and risk of restenosis after percutaneous coronary intervention: a meta-analysis. Clin Ther. 2020;42:458–74.
16. Ramanan VK, Shen L, Moore JH, Saykin AJ. Pathway analysis of genomic data: concepts, methods, and prospects for future development. Trends Genet. 2012;28:323–32.
17. Hu J, Tzeng JY. Integrative gene set analysis of multi-platform data with sample heterogeneity. Bioinformatics. 2014;30:1501–7.
18. Kunicki TJ, Williams SA, Nugent DJ. Genetic variants that affect platelet function. Curr Opin Hematol. 2012;19:371–9.
19. Geisler T, Schaeffeler E, Gawaz M, Schwab M. Genetic variation of platelet function and pharmacology: an update of current knowledge. Thromb Haemost. 2013;110:876–87.
20. Jiang XL, Samant S, Lesko LJ, Schmidt S. Clinical pharmacokinetics and pharmacodynamics of clopidogrel. Clin Pharmacokinet. 2015;54:147–66.
21. O I, M O, A AS, Hh CH, W SF, Rahman M. Evaluation of aspirin and clopidogrel resistance in patients with acute coronary syndrome by using adenosine diposphate test and aspirin test. Pakistan journal of medical sciences 2013;29:97–102.
22. Price MJ. Bedside evaluation of thienopyridine antiplatelet therapy. Circulation. 2009;119:2625–32.
23. Price MJ, Angiolillo DJ, Teirstein PS, et al. Platelet reactivity and cardiovascular outcomes after percutaneous coronary intervention: a time-dependent analysis of the Gauging Responsiveness with a VerifyNow P2Y12 assay: Impact on Thrombosis and Safety (GRAVITAS) trial. Circulation. 2011;124:1132–7.
24. Varenhorst C, James S, Erlinge D, et al. Assessment of P2Y(12) inhibition with the point-of-care device VerifyNow P2Y12 in patients treated with prasugrel or clopidogrel coadministered with aspirin. Am Heart J. 2009;157(562):e1-9.
25. Howie B, Fuchsberger C, Stephens M, Marchini J, Abecasis GR. Fast and accurate genotype imputation in genome-wide association studies through pre-phasing. Nat Genet. 2012;44:955–9.
26. Genomes Project C, Auton A, Brooks LD, et al. A global reference for human genetic variation. Nature. 2015;526:68–74.
27. Gresele P, Momi S, Guglielmini G. Nitric oxide-enhancing or -releasing agents as antithrombotic drugs. Biochem Pharmacol. 2019;166:300–12.
28. Moore C, Tymvios C, Emerson M. Functional regulation of vascular and platelet activity during thrombosis by nitric oxide and endothelial nitric oxide synthase. Thromb Res. 2010;124:342–9.
29. Cotta Filho CK, Oliveira-Paula GH, Rondon Pereira VC, Laccini R. Clinically relevant endothelial nitric oxide synthase polymorphisms and their impact on drug response. Expert Opin Drug Metab Toxicol. 2020;16:927–51.
30. Azova M, Timizheva K, Ait Aissa A, et al. Gene polymorphisms of the renin-angiotensin-aldosterone system as risk factors for the development of in-stent restenosis in patients with stable coronary artery disease. Biomolecules. 2021;11:763.
31. Varga-Szabo D, Braun A, Nieswandt B. Calcium signaling in platelets. J Thromb Haemost. 2009;7:1057–66.
32. Hathaway DR, Adelstein RS. Human platelet myosin light chain kinase requires the calcium-binding protein calmodulin for activity. Proc Natl Acad Sci U S A. 1979;76:1653–7.
33. Shattil SJ, Brass LF. Induction of the fibrinogen receptor on human platelets by intracellular mediators. J Biol Chem. 1987;262:992–1000.
34. Smyth SS, Woulfe DS, Weitz JI, et al. G-protein-coupled receptors as signaling targets for antiplatelet therapy. Arterioscler Thromb Vasc Biol. 2009;29:449–57.

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