Effect of platelet receptor gene polymorphisms on outcomes in ST-elevation myocardial infarction patients after percutaneous coronary intervention

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Abstract
Polymorphisms in platelet receptor genes may influence platelet function. This study aimed to assess the impact of five polymorphisms of genes encoding platelet receptors on the risk of ischemic and bleeding events in ST-elevation myocardial infarction (STEMI) patients after percutaneous coronary intervention (PCI). 503 consecutive Chinese patients with STEMI after an uneventful PCI and exposed to standard dual antiplatelet therapy for 12 months were enrolled. Polymorphisms of platelet receptors, GP1a (ITGA2, 807C>T, rs1126643), GPVI (GP6, 13254T>C, rs1613662), PAR-1 (F2R, IVS-14A>T, rs168753) and P2Y12 (P2RY12, 34C>T, rs6785930 and H1/H2 haplotype, 52G>T, rs6809699) were detected by the ligase detection reaction. The follow-up period was 12 months. Overall, 34 (6.8%) ischemic events occurred and 46 (9.1%) major bleedings occurred. Multivariate Cox regression analysis showed the carriage of F2R rs168753 minor allele was an independent predictor of major bleedings. The genetic testing of platelet receptors can be valuable in predicting adverse events in STEMI patients after PCI.

Keywords
Clinical outcomes, platelet receptor gene, polymorphism, STEMI

Introduction
In patients with ST-elevation myocardial infarction (STEMI) after percutaneous coronary intervention (PCI), aggressive antithrombotic and anticoagulant treatment regimens are routinely administered to provide protection against thrombotic complications. This protection, however, comes with an increased risk of bleedings during and after the procedure [1, 2]. Recent findings suggest that both thrombotic and bleeding complications have significant influence on patient prognosis [3, 4].

Platelet activation and aggregation plays a crucial role in the progression of the atherosclerotic vascular disease by triggering acute thrombotic complications such as myocardial infarction (MI) and stent thrombosis (ST). During standard dual antiplatelet therapy (DAPT), patients with high platelet reactivity are more likely to experience ischemic events, while low platelet reactivity may contribute to higher risk of bleedings [5]. A variety of specific membrane receptors have been involved in platelet activation [6]. Most of the genes encoding the platelet receptors have been sequenced, and polymorphisms have been described in coding and regulating regions. The consequences of these polymorphisms for platelet functions and their involvement in predispositions to excessive bleeding or thrombus formation begin to be determined [7–9]. However, there is less evidence about the relation of platelet receptor gene polymorphisms and adverse outcomes, especially bleeding events. The present study aimed to assess the impact of five polymorphisms of genes encoding platelet receptors on the risk of ischemic and bleeding events in STEMI patients undergoing PCI.

Methods
In our prospective, randomized and single center study, all patients enrolled were known to present with STEMI and have an uneventful PCI, between January 2011 and August 2012. The major exclusion criteria were active bleeding and bleeding diatheses, oral anticoagulation therapy, hemodynamic instability, use of intensified antiplatelet agents other than standard DAPT, contraindication to antiplatelet therapy, non-cardiac disease with a life expectancy of <1 year, or inability to follow the protocol. The Institutional Review Board approved the study protocol, and the patients provided written informed consent for participation and agreed to genotype determination. The study conformed to the principles outlined in the Declaration of Helsinki.
All patients were pre-treated with aspirin and a loading dose of 300 mg clopidogrel before PCI, followed by a maintenance dose of 100 mg/day aspirin for life and 75 mg/day clopidogrel for 1 year. Stents were deployed according to the current standard guidelines. The stent type was chosen by the operator, and tirosiban was administered if a glycoprotein IIb/IIIa receptor inhibitor (GPI) was required. Anticoagulation with low-molecular-weight heparin (enoxaparin) or unfractionated heparin was initiated before angiography in all patients.

Genomic DNA was extracted from peripheral whole blood samples using the salting-out protocol. Polymorphisms of platelet receptors, GPIa (ITGA2, 807C>T), rs1126643, GPVI (GP6, 13254T>C, rs1613662), PAR-1 (F2R, IVS-14A>T, rs168753) and P2Y12 (P2RY12, 34C>T, rs6785930 and H1/H2 haplotype, 52G>T, rs6809699) were selected with respect to the reported or supposed association with changes in platelet area [7–10]. All the five single nucleotide polymorphisms (SNPs) were detected by the ligase detection reaction and a commercially available detection system (ABI3130XL DNA Analyzer System; Applied Biosystems, Foster City, CA). Call rates of all the SNPs were over 99%. Repeat genotyping was performed on random duplicate samples (n = 25), and sequencing techniques were used to ensure quality control.

The primary clinical efficacy endpoint of this study was a composite of cardiovascular death, nonfatal MI, unplanned target vessel revascularization (TVR), and ST. MI was defined according to the universal definition [11]. Unplanned TVR was defined as any intervention required (surgical or percutaneous) to treat luminal stenosis (>75% on angiography) in the same coronary vessel that was treated at the index procedure, within and beyond the target lesion during the 12-month follow-up period [12]. ST was defined as definite ST according to the Academic Research Consortium [13]. The composite ischemic events indicated the composite of cardiovascular death, non-fatal MI, unplanned TVR, and ST. Two independent physicians blinded to the laboratory data adjudicated events after reviewing the source documents. The primary clinical safety endpoint of the present study was the incidence of major bleeding events. Major bleeding was quantified according to bleeding academic research consortium definition (BARC) criteria [14], including type 3 and 5 in the analysis.

Sample size calculation was based on our previous study (data not published), which included a cohort with the same selection criteria and treatment strategy. The previous study showed a significant association between the rs2168753 minor allele carrier and composite ischemic events (p = 0.01). Therefore, we assumed the minor T allele frequency was 0.44. The study was designed on the basis of the superiority principle to achieve 80% power to observe an incidence of composite ischemic events in the F2R rs168753 minor T carriers of 4% and 11.5% in wild-type homozygotes. Thus, a total of 463 patients were needed. To compensate for loss to follow-up, we recruited a population of about 500 (Statistical software: PASS 11, NCSS, LLC, Kaysville, UT).

Continuous variables were presented as the mean ± standard deviation and compared using the Student’s t test, Mann–Whitney U test, or one-way analysis of variance (ANOVA) test, as appropriate. Categorical variables were exposed as frequencies and percentages, which were compared with a chi-square test (χ²) or Fisher exact test. After significant differences among variables were demonstrated by the ANOVA test, post hoc comparisons between the groups were performed with the Student–Newman–Keuls test for multiple comparisons. Under three models (codominant, dominant and recessive), the relationships between all enrolled SNPs and the adverse events were analyzed. Clinical follow-up was censored on the day of the first cardiovascular event, which corresponded to the clinical endpoints. For subjects without a clinical event, clinical follow-up was censored either at the last clinic visit after 12 months of taking DAPT or on the day of DAPT discontinuation. A multivariate logistic regression model was used to test for an independent association of P2RY12 SNPs carriage with BARC ≥ 3 bleedings. Adjustment was made for the following risk factors: age, female gender, body mass index (BMI), renal function (serum creatinine), hypertension, hypercholesterolemia, diabetes mellitus, stent type, use of proton pump inhibitors and use of tirosiban. The odds ratio (OR) and the corresponding 95% CI were estimated for each variable included in the multivariate model. Multivariate Cox regression analysis was conducted to identify independent correlates of the composite ischemic events and to adjust for potential confounders as mentioned above. The measure of effect was the hazard ratio (HR). All statistical analyses were performed using SPSS ver. 18.0 (SPSS, Chicago, IL), and a two-tailed probability value of <0.05 was considered to be significant.

To validate the genetic associations in the study, we enrolled another cohort of 483 Chinese patients with STEMI in a second study from September 2012 to July 2013 in our hospital, based on the same inclusion and exclusion criteria. The second study protocol was basically the same as the first study. The logistic regression model and the Cox regression model were replicated in the second cohort.

Results

Genetic samples for analysis were available from 503 STEMI patients. All patients were from the Chinese Han population. PCIUs were all performed with drug-eluting stents (DES). For genotype distribution, no significant deviation from the Hardy–Weinberg equilibrium was observed, and all the allele frequencies were similar to the HapMap-HCB population (Table S1). At 1-year follow-up, 34 ischemic events occurred (6.8%): 13 (2.6%) cardiovascular deaths, 5 (1.0%) non-fatal MIs, 10 (2.0%) TVR and 6 (1.2%) ST. Forty-six BARC ≥ 3 bleeding events (9.1%) occurred, which included 11 (2.2%) cases of BARC 3b bleedings and 35 (6.9%) cases of BARC 3a bleedings. Baseline characteristics of the study population are presented in Table I.

Table S2 shows the clinical endpoints and their association with SNPs. According to F2R rs168753 genotype, the risk of composite ischemic events was lower in the minor T allele carriers. The SNP genotypes of P2RY12 rs6785930 and rs6809699 were significantly associated with BARC ≥ 3 bleedings. There were more bleedings for the minor allele carriers.

A multivariable Cox regression model demonstrated that carriage of F2R rs168753 minor allele was an independent predictor of composite ischemic events (HR 0.387, 95% CI 0.193–0.778, p = 0.008), after adjustment for traditional clinical risk factors (Table II). After controlling for potential clinical bleeding confounders, the association between P2RY12 rs6809699 minor allele and BARC ≥ 3 bleedings seen in the multivariate logistic regression analysis remained statistically significant (OR 2.71, 95% CI 1.298–5.659, p = 0.008, Table III). P2RY12 rs6785930 minor allele carriage was associated with a risk trend of bleedings (OR 1.831, 95% CI 0.944–3.552, p = 0.074, Table III).

We then carried out a second study (Tables S3 and S4), and similar results were obtained in the repeated study. In the second cohort, the association between F2R rs168753 and composite ischemic events was still significant (HR 0.308, 95% CI 0.148–0.641, p = 0.002), and P2RY12 rs6809699 minor allele carriage was an independent predictor of BARC ≥ 3 bleedings (OR 2.619, 95% CI 1.253–5.478, p = 0.011).

Discussion

To our knowledge, this is the first study to show the association between platelet receptor gene polymorphisms and clinical
adverse outcomes of STEMI patients after PCI. Several important findings of our study are that: (1) \( F2R \) rs168753 minor allele could predict composite ischemic events and (2) \( P2RY12 \) genetic locus harbors SNPs that could have influence on bleeding events in our study population.

There is growing evidence that genetic polymorphisms are important determinants of the large inter-individual variability in platelet reactivity, which could result in predispositions to adverse outcomes [15]. PAR-1, which \( F2R \) encodes, is the main thrombin receptor on platelets and plays a key role in platelet activation. The SNP rs168753 is one of the most important markers of \( F2R \) gene to be reported in many studies [16]. This polymorphism, an A\(\rightarrow\)T transversion, has been located in the intervening sequence (IVS), 14 nucleotides upstream of the exon 2 start site (IVSn-14 A/T). Although it is an intronic polymorphism, it may have an effect on the rate of mRNA processing, or augment transcription efficiency and the quantity of protein synthesized, which have been described for many genes [17–19]. Dupont et al. were able to prove that the minor T allele was associated with lower PAR-1 expression on the platelet surface and with a weaker aggregation and secretion response to PAR-1-activating peptide SFLLRN, and decreased procoagulant activity [16]. Smith et al. reported that the major A allele homozygotes have higher platelet reactivity both prior to and during clopidogrel treatment than patients with at least one copy of the minor T allele [9]. All the studies above focused on the relationship between rs168753 and platelet reactivity, but the association between this polymorphism and clinical outcomes remained unclear. In the current study, we found that STEMI patients undergoing PCI with at least one copy of the minor T allele had significantly lower risk of composite ischemic events than the major A homozygotes (HR 0.391, 95% CI 0.195–0.787, \( p = 0.009 \). Dupont’s and Smith’s studies could help to explain our results: the decreased expression of PAR-1 on the platelet surface may cause lower platelet reactivity, and then

| Table I. Baseline characteristics of the study population. |
|---------------------------------|
| Variables                     | Total \( N = 503 \) | Patients with CIE \( n = 34 \) | Patients with bleeding \( n = 46 \) |
| Age, years                    | 59 ± 12 | 58 ± 10 | 62 ± 12 |
| Female, \( n \) (%)            | 20.9   | 20.6   | 34.8* |
| BMI, kg/m²                     | 25.77 ± 3.59 | 25.19 ± 3.18 | 25.23 ± 3.92 |
| LVEF, g/dl                     | 52.65 ± 7.67 | 49.35 ± 10.21 | 49.35 ± 8.23 |
| Hemoglobin, g/dl               | 137.98 ± 19.31 | 138.44 ± 19.32 | 142.59 ± 26.67 |
| Platelet count, \( \times 10^9/\ell \) | 211.7 ± 71.7 | 226.11 ± 64.6 | 201 ± 60.3 |
| hs-CRP, mg/dl                  | 8.39 ± 5.28 | 8.93 ± 5.51 | 10.5 ± 5.36 |
| Risk factor, \( n \) (%)       | 30.6   | 35.3   | 37 |
| DM                             | 60.2   | 67.6   | 52.2 |
| Hypertension                   | 93.0   | 94.1   | 91.3 |
| Current smoking                | 65.8   | 67.6   | 56.5 |
| Alcohol drinking               | 26.6   | 23.5   | 19.6 |
| CHD family history             | 22.7   | 38.2   | 17.4 |
| History, \( n \) (%)           | 18.7   | 26.5   | 19.6 |
| Previous MI                    | 12.7   | 26.5   | 13.0 |
| Previous PCI                   | 0.8    | 5.9    | 2.2  |
| Infarct-related artery, \( n \) (%) | 50.9 | 38.2 | 52.2 |
| LAD                            | 20.1   | 23.5   | 19.6 |
| LCX                            | 42.9   | 41.2   | 52.2 |
| RCA                            | 3.8    | 8.8    | 13.0 |
| LM                             | 53.5   | 61.8   | 60.9 |
| Stent type, \( n \) (%)        | 46.5   | 38.2   | 39.1 |
| First-generation DES           | 98.6   | 94.1   | 100.0 |
| Second-generation DES          | 85.9   | 79.4   | 82.6 |
| Concomitant medications, \( n \) (%) | 73.8 | 55.9 | 56.5 |
| Statin                         | 9.9    | 12.1   | 4.3  |
| CCB                            | 71.1   | 85.3*  | 93.5* |

CIE, composite ischemic events; BMI, body mass index; LVEF, left ventricular ejection fraction; hs-CRP, high-sensitivity C-reactive protein; DM, diabetes mellitus; CHD, cardiac heart disease; MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; LAD, left anterior descending; LCX, left circumflex; RCA, right coronary artery; LM, left main; ACEI, angiotensin antagonist inhibitor; CCB, calcium channel blocker; PPI, proton pump inhibitor.

*Statistically significant, \( p < 0.05 \).

| Table II. Predictors of composite ischemic events by multivariate Cox regression analysis. |
|---------------------------------|
| Variable                        | Hazard ratio | 95% CI | \( p \) |
| \( F2R \) rs168753 minor allele carriage | 0.387 | 0.193–0.778 | 0.008 |
| Age                             | 0.973 | 0.942–1.006 | 0.109 |
| Female                          | 1.184 | 0.468–2.995 | 0.721 |
| Body mass index                 | 0.918 | 0.827–1.018 | 0.105 |
| Serum creatinine                | 1.019 | 1.008–1.030 | 0.001 |
| Hypertension                    | 1.804 | 0.844–3.857 | 0.128 |
| Hypercholesterolemia            | 1.732 | 0.400–7.498 | 0.462 |
| Diabetes mellitus               | 0.910 | 0.437–1.897 | 0.801 |
| Second-generation DES           | 0.631 | 0.311–1.280 | 0.202 |
| Use of proton pump inhibitors   | 0.402 | 0.152–1.061 | 0.066 |
| Use of tirofiban                | 2.460 | 1.185–5.105 | 0.016 |

Bold values indicate \( p < 0.05 \).
result in lower risk of ischemic events in minor T carriers. This result of our study had clinical implications that the SNP rs168753 may affect clinical benefit of antplatelet agent, for example, the orally active PAR-1 receptor antagonist vorapaxar, which has received its first global approval for the reduction of thrombotic cardiovascular events in patients with a history of MI or peripheral arterial disease in the USA.

The adenosine diphosphate purinergic subtype Y (P2Y) receptors are important signaling mediators for platelet activation and aggregation. The key role of the P2Y12 receptor is evidenced by the effectiveness of specific P2Y12 receptor antagonists (e.g., clopidogrel, prasugrel and ticagrelor) for the prevention of adverse cardiovascular events, which implies potential clinical relevance of P2Y12 function inter-individual variability. The P2RY12 gene is located on chromosome 3q24-q25 and the human P2Y12 receptor contains 342 amino acid residues [20]. Fontana et al. first reported that a minimum of four SNPs (rs2046934, rs10935838, rs5853517 and rs6809699) of the P2RY12 gene were in complete linkage disequilibrium, comprising the haplotype H1 and H2 [21]. H2 haplotype is one of the most important markers in P2RY12 gene to be studied in many researches, but evidence of the role of the H2 haplotype in relationship to platelet reactivity or clinical outcomes is incongruent. Most studies were unable to identify an association of H2 haplotype carriers with adverse cardiovascular events [7, 22–24]. For example, Rudez et al. [10] showed no correlation between the H2 haplotype and restenosis in PCI-treated patients, as was observed in our study. However, Zee et al. demonstrated that H2 haplotype was significantly associated with a lower risk of incident deep venous thromboembolism/pulmonary embolism (DVT/PE) as compared to the reference haplotype H1 (OR 0.50, 95% CI 0.27–0.93, p = 0.028) [25]. Furthermore, in a recent study by Oestreich et al. [26], H2/H2 carriers had greater platelet inhibition compared to both H1/H1 and H1/H2 subjects in the presence of cangrelor, a specific receptor antagonist for the P2Y12 receptor (p = 0.023). Galic et al. reported that stable coronary heart disease patients with H2/H2 haplotype displayed tendency toward greater than average response to clopidogrel and no resistance [27]. In the current study, we demonstrated that tag SNP rs6809699 of H2 haplotype was independently associated with BARC ≥3 bleedings. This study might be the first one to report association between P2RY12 polymorphisms and bleedings. The results of studies above helped to prove that H2 haplotype carriers may have higher platelet inhibition in response to P2Y12 receptor antagonist and then present with lower risk of thrombosis or higher risk of bleeding. Some studies showed relation between H2 haplotype carriers and maximal platelet aggregation [28], or thrombotic diseases [21]. Discrepancies between their studies and our study could attribute to differences in P2RY12 SNP frequencies among ethnic groups, differences in study population or phenotype/trait definition, or confounding by environmental factors.

The current study had some limitations to mention. First, this study was a monocentric rather than a multicenter investigation. Second, the sample size should be sufficient to draw a conclusion that may guide clinical practice but it was still not very large. The results gathered may be specific to the patient cohort and the way their cases were clinically managed. Further studies in diverse populations with multiple platelet function tests are required.

Conclusions

In STEMI patients after PCI, F2R rs168753 allele could significantly contribute to the risk of ischemic events, and P2RY12 rs6809699 alleles could predict bleedings. The genetic testing of platelet receptors can be valuable in predicting adverse events in STEMI patients after PCI.

Declaration of interest

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| Variable | Odds ratio | 95% CI | p     |
|----------|------------|--------|-------|
| P2RY12 rs6785930 minor allele carriage | 1.831 | 0.944–3.552 | 0.074 |
| P2RY12 rs6809699 minor allele carriage | 2.71 | 1.298–5.659 | 0.008 |
| Age | 1.004 | 0.971–1.037 | 0.822 |
| Female | 2.827 | 1.252–6.381 | 0.012 |
| Body mass index | 0.953 | 0.866–1.050 | 0.331 |
| Serum creatinine | 1.021 | 1.010–1.033 | 0.001 |
| Hypertension | 0.556 | 0.272–1.133 | 0.106 |
| Hypercholesterolemia | 0.560 | 0.163–1.925 | 0.357 |
| Diabetes mellitus | 1.303 | 0.638–2.664 | 0.467 |
| Second-generation DES | 0.743 | 0.373–1.483 | 0.4 |
| Use of proton pump inhibitors | 0.195 | 0.058–0.654 | 0.008 |
| Use of tirofiban | 4.217 | 2.006–8.861 | 0.001 |

Bold values indicate p < 0.05.
Platelet receptor gene SNPs predict outcomes

Supplementary material available online
Supplementary Table S1–S4