**Background:** High platelet reactivity (HPR) after clopidogrel treatment is linked to an increased risk of periprocedural myocardial infarction (PMI). The occurrence of PMI that could be associated with CYP2C19 genotype status was our hypothesis.

**Methods and Results:** A total of 233 patients with non-ST elevation acute coronary syndromes (NSTACS) undergoing uneventful elective percutaneous coronary intervention were included. Platelet reactivity was assessed by Thrombelastograph at 24 h after 300 mg clopidogrel loading. HPR was defined as ≥70% adenosine diphosphate-induced platelet aggregation. The CYP2C19*2 and *3 loss-of-function (LOF) alleles were determined using DNA microarray method. Patients with PMI had significantly higher on-clopidogrel platelet reactivity compared to those without PMI (60.0±24.4% vs. 43.0±24.0%, P<0.001). HPR was more frequently observed in patients with PMI and was the strongest risk factor of PMI in multivariate analysis (ORadj=4.348, 95% CI: 1.846–10.241, P=0.001). Furthermore, the incidence of HPR was significantly associated with the carriage of 2 CYP2C19 LOF alleles. Compared with non-carriers, patients carrying 2 CYP2C19 LOF alleles had a 3.000-fold increased risk (95% CI: 1.071–8.400, P=0.037) for PMI in multivariate analysis. However, inclusion of HPR as a covariate in the regression model changed the significant relationship between the carriage of 2 CYP2C19 LOF alleles and PMI.

**Conclusions:** Among Chinese patients with NSTACS, carriers with 2 CYP2C19 LOF alleles are more prone to HPR, which is associated with an increased risk for PMI. (*Circ J* 2012; 76: 2773–2778)

**Key Words:** Acute coronary syndrome; Antiplatelet; Clopidogrel; Genetics; Percutaneous coronary intervention
Methods

Study Population

From February 2011 to November 2011, a total of 447 Chinese patients with acute coronary syndrome (ACS) undergoing PCI were enrolled to investigate the relationship between genetic variants and clopidogrel response. Of the total cohort, 233 patients with non-ST elevation ACS undergoing uneventful elective PCI were included in this analysis due to available periprocedural cardiac necrosis markers and feasibility of determining the occurrence of PMI. In patients with increased baseline concentrations of creatine kinase-MB, the values should be stable or falling before the PCI procedure. Patients with adverse events, including side-branch occlusion, dissection, disruption of collateral flow, suspected thrombus, and coronary perforation during PCI, were not included. Exclusion criteria were: age less than 18 years, concomitant administration of cilostazol, allergies or contraindications to either aspirin or clopidogrel, upstream or bail-out use of glycoprotein IIb/IIIa inhibitors, malignancies, pregnancy, severe renal insufficiency (glomerular filtration rate, GFR, <30 ml·min⁻¹·1.73 m⁻²), severe hepatic insufficiency (total bilirubin >2× upper limit of normal, in association with alanine aminotransferase >3× upper limit of normal), total platelet count <100×10⁹/L, increased risk of bleeding, and hematologic disorder. The study protocol was approved by the hospital’s medical ethics committee, and informed consent was obtained from each patient.

All patients received drug-eluting stents and dual antiplatelet therapy. A loading dose of 300 mg clopidogrel was encouraged 16 h before the procedure and a daily regimen of 75 mg was given. Patients received a loading dose of 300 mg aspirin followed by 100 mg daily. Technicalities of the procedure, including use of anticoagulation drugs and type of stents, were left to the operator’s discretion. Sirolimus-eluting, Zotarolimus-eluting, Paclitaxel-eluting, and Everolimus-eluting stents were used in our study.

Table 1. Characteristics of the Study Population According to the Periprocedural Outcome

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Overall (n=233)</th>
<th>Without PMI (n=200)</th>
<th>With PMI (n=33)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline characteristic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>62±10</td>
<td>62±10</td>
<td>66±9</td>
<td>0.024</td>
</tr>
<tr>
<td>Male</td>
<td>181 (77.7%)</td>
<td>156 (78.0%)</td>
<td>25 (75.8%)</td>
<td>0.774</td>
</tr>
<tr>
<td>BMI</td>
<td>24.8±1.6</td>
<td>24.8±1.7</td>
<td>24.6±1.4</td>
<td>0.418</td>
</tr>
<tr>
<td>Hypertension</td>
<td>148 (63.5%)</td>
<td>127 (63.5%)</td>
<td>21 (63.6%)</td>
<td>0.988</td>
</tr>
<tr>
<td>DM</td>
<td>67 (28.8%)</td>
<td>52 (26.0%)</td>
<td>15 (45.5%)</td>
<td>0.022</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>48 (20.6%)</td>
<td>37 (18.5%)</td>
<td>11 (33.3%)</td>
<td>0.051</td>
</tr>
<tr>
<td>Smoking</td>
<td>131 (56.2%)</td>
<td>113 (56.5%)</td>
<td>18 (54.5%)</td>
<td>0.834</td>
</tr>
<tr>
<td>Stroke</td>
<td>13 (5.6%)</td>
<td>11 (5.5%)</td>
<td>2 (6.1%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Clinical presentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA</td>
<td>166 (71.2%)</td>
<td>141</td>
<td>25</td>
<td>0.679</td>
</tr>
<tr>
<td>NSTEMI</td>
<td>67 (28.8%)</td>
<td>59</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>233 (100%)</td>
<td>200 (100%)</td>
<td>33 (100%)</td>
<td>1.000</td>
</tr>
<tr>
<td>β-blockers</td>
<td>221 (94.8%)</td>
<td>190 (95.0%)</td>
<td>31 (93.9%)</td>
<td>0.618</td>
</tr>
<tr>
<td>ACEI or ARB</td>
<td>208 (89.3%)</td>
<td>178 (89.0%)</td>
<td>30 (90.9%)</td>
<td>1.000</td>
</tr>
<tr>
<td>PPI</td>
<td>52 (22.3%)</td>
<td>44 (22.0%)</td>
<td>8 (24.2%)</td>
<td>0.774</td>
</tr>
<tr>
<td>Coronary intervention procedure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Targeted coronary artery</td>
<td></td>
<td></td>
<td></td>
<td>0.824</td>
</tr>
<tr>
<td>LM</td>
<td>6 (2.6%)</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>LAD</td>
<td>124 (53.2%)</td>
<td>106</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>LCX</td>
<td>33 (14.2%)</td>
<td>27</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>RCA</td>
<td>70 (30.0%)</td>
<td>62</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>No. of stents</td>
<td>1.6±0.8</td>
<td>1.6±0.8</td>
<td>1.7±0.8</td>
<td>0.444</td>
</tr>
<tr>
<td>Length of stents (mm)</td>
<td>42.4±24.2</td>
<td>41.8±23.8</td>
<td>45.8±26.3</td>
<td>0.373</td>
</tr>
<tr>
<td>hs-CPR &gt;3.0 mg/L</td>
<td>126 (54.1%)</td>
<td>105 (52.5%)</td>
<td>21 (63.6%)</td>
<td>0.234</td>
</tr>
<tr>
<td>Clopidogrel response</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet reactivity rate (%)</td>
<td>45.4±24.8</td>
<td>43.0±24.0</td>
<td>60.0±24.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HPR</td>
<td>40 (17.2%)</td>
<td>26 (13.0%)</td>
<td>14 (42.4%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CYP2C19 LOF alleles</td>
<td></td>
<td></td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>No LOF carriers</td>
<td>84 (36.1%)</td>
<td>75</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>1 LOF carriers</td>
<td>98 (49.0%)</td>
<td>98</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>2 LOF carriers</td>
<td>37 (15.9%)</td>
<td>27</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed mean±SD or number of patients (percentage). ACEI, angiotensin-converting-enzyme inhibitors; ARB, angiotensin II receptor blockers; BMI, body mass index; DM, diabetes mellitus; HPR, high platelet reactivity; PMI, periprocedural myocardial infarction; LAD, left anterior descending artery; LCX, left circumflex artery; LM, left main artery; LOF, loss-of-function; NSTEMI, non-ST segment elevation myocardial infarction; PPI, proton pump inhibitors; RCA, right coronary artery; UA, unstable angina.


Platelet Function
At 24 h after clopidogrel loading, the magnitude of platelet reactivity was assessed by Thrombelastograph (TEG) Hemo-
stasis Analyzer (Haemoscope Corp, Niles, IL, USA). The Food
and Drug Administration-approved TEG relies on the measure-
ment of activator-induced clot strength to enable a quantitative
analysis of platelet function.20,21 This assay uses activator F, a
mixture of reptilase and factor XIII, to generate a cross-linked
fibrin clot to isolate the contributor of fibrin to clot strength.
Heparin is used as an anticoagulant to eliminate thrombin ac-
tivity in the sample. The dominant conventional parameter is
the maximum amplitude (MA), which is indicative of the
strength of the final clot. The contribution of P2Y12 receptor
pathway to the clot formation can be measured by the addition
of the agonist of adenosine diphosphate (ADP). Thrombin-
induced clot strength (MAthrombin) reflects the patient’s maxi-
mal potential platelet reactivity. MAfibrin reflects the contribu-
tion of fibrin alone to clot strength. MAADP represents the
contribution of platelets not inhibited by clopidogrel. Platelet
aggregation in response to ADP calculated with computerized
software on the basis of the formula:

\[
\text{%Aggregation} = \left( \frac{\text{MAADP} - \text{MAfibrin}}{\text{MAthrombin} - \text{MAfibrin}} \right) \times 100.
\]

Genotyping
Genomic DNA was extracted from whole-blood samples using
the Qiagen Blood Kit (Qiagen, Chatsworth, CA, USA), accord-
ing to the manufacturer’s instructions. DNA microarray (gene
chip) method was used to evaluate the genotypes of CYP2C19.
The CYP2C19*2 and CYP2C19*3 variant alleles were deter-
dined with BaO BE-2.0 Biochip diagnostic Analyzer (BaO
Technology Corp, Shanghai, China). The procedures of DNA
extraction, polymerase chain reaction amplification, hybrid-
ization, gene array detection, and analysis were strictly accord-
ing to the manuals of BaO genotype detecting gene array kit
equipment (BaO Technology Corp). These 2 polymor-
phisms were in Hardy-Weinberg equilibrium (P>0.05).

Participants were divided into 3 groups, based on the num-
ber of the CYP2C19 LOF allele: no LOF carriers (CYP2C19*1/*1),
1 LOF carriers (CYP2C19*1/*2 and CYP2C19*1/*3), and 2 LOF
carriers (CYP2C19*2/*2, CYP2C19*2/*3, and CYP2C19*3/*3).
No carrier of CYP2C19*3/*3 was found in the study popula-
tion.

Main Outcomes
The main outcomes of the study included high platelet reac-
tivity (HPR) and PMI. The cut-off points for HPR were defined
as ≥70% ADP-induced aggregation with 2-μmol/L ADP as
measured by TEG.20 All patients below these cut-off points were
defined as exhibiting normal on-treatment platelet reac-
tivity. PMI was defined as a post-procedural increase in tropo-
nin T ≥3 times (±0.09 ng/ml) the 99th percentile the upper
reference limit for patients with baseline negative myocardial
necrosis markers.22 In patients with increased baseline concen-
trations of creatine kinase-MB, if the values are stable or fall-
ing, a subsequent increase ≥50% the baseline value fulfilled the
criteria for periprocedural MI.23,24 Blood samples were drawn
from all patients before PCI and at 24 h after intervention for
measurement of creatine kinase-MB and troponin T concen-
trations.

Statistical Analyses
Continuous variables were expressed as mean±SD. Categori-
ical variables were expressed as frequencies and percentage.
For analysis of relationship between categorical variables we
used the chi-square test or Fisher’s exact test when appropri-
ate. Kolmogorov-Smirnov test was used to check for normal
distribution of continuous data. T-test or Wilcoxon rank sum
test for unpaired samples was used to compare any continuous
variable with a normal or non-normal distribution, respectively.
Univariate and multivariate logistic regression analyses was
performed to determine the significant factors indicating the
occurrence of PMI. All tests were 2-sided with a significance
level of P<0.05. All statistical analyses were performed with
SPSS software package, version 17 (SPSS Inc, Chicago, IL,
USA).

Results
Demographic and baseline clinical characteristics in these co-
horts are shown in Table 1, as well as the procedural variables.
PMI was documented in 33 of 233 patients (14.2%). Compared
with patients without PMI, those with PMI were significantly
older and exhibited a considerably higher prevalence of dia-
betes mellitus (Table 1). No significant differences in gender,
body mass index, or baseline medications existed between the
2 groups. Likewise, the proportion of patients with hyperten-
sion, hypercholesterolemia, current smoking, or history of stroke
did not differ significantly. No significant differences between the
2 groups were observed with respect to targeted coronary
artery and the number and length of implanted stents.

Mean platelet aggregation was significantly higher in patients
with PMI than in those without PMI as measured by TEG
(60.0±24.4% vs. 43.0±24.0%, P<0.001; Table 1). Compared
with subjects without PMI, patients with PMI had a signifi-
cantly higher prevalence of HPR (42.4% vs. 13.0%, P<0.001; 
Table 1). Moreover, the distribution of CYP2C19 LOF alleles
differed significantly between the 2 groups in Table 1. As dem-
onstrated in Figure 1, 2 LOF carriers had an increased inci-
dence of PMI compared to no LOF carriers (27.0% vs. 10.7%, 
P=0.023). An increased incidence of PMI was also observed
in 2 LOF carriers compared with 1 LOF carrier (27.0% vs.
12.5%, P=0.037; Figure 1). However, there was no significant
difference in the prevalence of PMI between no LOF carriers
and 1 LOF carrier (10.7% vs. 12.5%, P=0.701; Figure 1).

Univariate and multivariate logistic regression analyses were
Table 2. Predictors for PMI by Univariate and Multivariate Logistic Regression Models

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th>Multivariate (exclusion of HPR)</th>
<th>Multivariate (inclusion of HPR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P value</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Age</td>
<td>1.046</td>
<td>0.026</td>
<td>1.042</td>
</tr>
<tr>
<td></td>
<td>(1.005–1.087)</td>
<td></td>
<td>(1.001–1.085)</td>
</tr>
<tr>
<td>DM</td>
<td>2.372</td>
<td>0.012</td>
<td>2.275</td>
</tr>
<tr>
<td></td>
<td>(1.115–5.044)</td>
<td></td>
<td>(1.048–4.939)</td>
</tr>
<tr>
<td>HPR</td>
<td>4.931</td>
<td>0.0001</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>(2.207–11.018)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C19 genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 LOF allele carriage</td>
<td>1.190</td>
<td>0.701</td>
<td>1.291</td>
</tr>
<tr>
<td></td>
<td>(0.489–2.898)</td>
<td></td>
<td>(0.522–3.196)</td>
</tr>
<tr>
<td>2 LOF alleles carriage</td>
<td>3.086</td>
<td>0.028</td>
<td>3.000</td>
</tr>
<tr>
<td></td>
<td>(1.133–8.409)</td>
<td></td>
<td>(1.071–8.400)</td>
</tr>
</tbody>
</table>

OR, odds ratio. Other abbreviations as in Table 1.

Discussion

The present study was conducted for the first time to investigate the influence of CYP2C19 LOF alleles on periprocedural outcomes in patients receiving clopidogrel pretreatment and undergoing PCI. Even though the study population underwent successful and uneventful PCI, PMI still occurred in 14.2% of cases. In line with previous evidence, our study demonstrated that HPR was associated with an increased risk for periprocedural myocardial injury. Furthermore, our study demonstrated that patients carrying 2 CYP2C19 LOF alleles had a more than 3-fold increased risk for PMI in the univariate and multivariate logistic regression analysis. However, inclusion of HPR as a covariate in the multivariate regression model changed the significant relationship between the CYP2C19 LOF allele carriage and PMI. When we analyzed the incidence of HPR with respect to the CYP2C19 LOF allele status, we found that only the carriage of 2 CYP2C19 LOF alleles was significantly associated with the prevalence of HPR (OR=2.322, 95% CI: 0.978–5.515, P=0.056).

There is controversy surrounding the issue as to whether PMI is associated with poor clinical outcome. In a recent meta-analysis applying the universal definition of periprocedural MI (type 4a), patients with type 4a MI had an increased risk of major adverse cardiac events compared with those without troponin elevation at an average follow-up of about 17.7 months (OR=2.25, 95% CI: 1.26–4.00, P=0.006). Troponin elevation following PCI could be attributed to extrinsic and intrinsic mechanisms. Visible procedural-related adverse events, such as side-branch occlusion, dissection, disruption of collateral flow, and distal embolization, have been identified as extrinsic factors leading to PMI. Recently, accumulative evidences performed to determine the significant factor indicating the occurrence of PMI (Table 2). Univariate analysis showed that age, diabetes mellitus, HPR, and the carriage of 2 CYP2C19 LOF alleles were positively associated with PMI. The summary odds ratio (OR) for age, diabetes mellitus, HPR, and the carriage of 2 CYP2C19 LOF alleles were 1.046 (95% CI: 1.005–1.087, P=0.026), 2.372 (95% CI: 1.115–5.044, P=0.012), 4.931 (95% CI: 2.207–11.018, P=0.001), and 3.086 (95% CI: 1.133–8.409, P=0.037; Table 2). However, when HPR was included as a covariate in the multivariate logistic regression model, HPR and diabetes mellitus were independent risk factors of PMI, whereas there was no significant relationship between the carriage of 2 CYP2C19 LOF alleles and PMI.

When we analyzed the incidence of HPR with respect to the CYP2C19 LOF allele status, we found that 27.0% (10/37) of 2 LOF carriers were classified as HPR, and 19.6% (22/112) of 1 LOF carrier were categorized as HPR, whereas only 9.5% (8/84) of no LOF carriers were identified as HPR (P=0.039; Figure 2). Additionally, compared with no LOF carriers, 2 LOF carriers had a 3.519-fold increased risk (95% CI: 1.258–9.838, P=0.016) for HPR. However, the carriage of 1 LOF allele was not significantly associated with the prevalence of HPR (OR=2.322, 95% CI: 0.978–5.515, P=0.056).
showed that intrinsic platelet activation played a pivotal role in the pathophysiological development of PMI\textsuperscript{11,12} and that pretreatment with clopidogrel has significantly decreased the risk of PMI.\textsuperscript{6} Accordingly, current common clinical practice is pretreatment with a 300-mg loading dose of clopidogrel at least 6 h before the procedure in patients undergoing elective intervention. Wide response variability has been observed in patients treated with clopidogrel. Moreover, poor response to clopidogrel is associated with an increased risk for periprocedural myocardial necrosis.\textsuperscript{13–15} Our results support these findings in Chinese patients and show that HPR is the strongest risk factor for PMI. Given the risks associated with decreased clopidogrel responsiveness, alternative treatment strategies, either higher clopidogrel dosing regimens, or upstream use of glycoprotein IIb/IIIa inhibitors, or selection of a novel platelet ADP P2Y12 receptor antagonist, might be considered in patients with HPR. However, it is embarrassing that the anti-platelet effect of clopidogrel could be assessed only after clopidogrel intake, even after PCI.

CYP2C19 genotype has been repeatedly reported to be associated with the HPR phenotype. CYP2C19*2 (containing a splicing defect) and CYP2C19*3 (containing a premature stop codon), are 2 major LOF alleles of CYP2C19. A recent Korean study\textsuperscript{13} suggested that CYP2C19 LOF alleles had a gene-dose effect on post-clopidogrel platelet reactivity and that the effect of the CYP2C19*2 vs. *3 LOF allele carriage on platelet reactivity did not differ in East Asians. Additionally, there is no difference in the prevalence of HPR between CYP2C19*2/*2 and CYP2C19*2/*3 carriers.\textsuperscript{14} The fact that the magnitude of platelet reactivity increased by the presence of CYP2C19 LOF alleles encourages the search for the additional effect of CYP2C19 genotype on periprocedural outcome during PCI.

We demonstrated that the status of CYP2C19 LOF allele was associated with the occurrence of PMI after uneventful PCI in Chinese ACS patients and that the carriage of 2 CYP2C19 LOF alleles was a significant factor indicating the occurrence of PMI. Thus, our current data seems to support the strategy of CYP2C19 genotyping as a way to identify patients who might be at risk for periprocedural myocardial necrosis. Although the effect of HPR on PCI surpassed the genotype of CYP2C19 and the effect of CYP2C19 genotype on PMI might be mediated through platelet reactivity, genotyping has potential advantages for the optimization of antiplatelet therapy before PCI. Independent of clopidogrel intake, CYP2C19 genotyping might give an estimate of the probability of a low response to clopidogrel and provide an opportunity for physicians to individualize antiplatelet therapy before PCI.

In this observational study, we cannot completely exclude possible bias by various risk factors and patient characteristics although the multivariable adjustment models confirmed the primary analyses. The present study has limitations that merit mention. First, this study was a single-center investigation, which might be limited in making definite conclusion. Second, sample size calculation was not performed in our study. Third, we are aware that using only 1 method to test platelet function might not be sufficient to fully diagnose the response to antiplatelet therapy. Finally, we did not assay platelet reactivity before clopidogrel treatment. Additionally, this study concerns Chinese patients, a population characterized by a high prevalence of the CYP2C19 LOF allele, hence, the results cannot be generalized to the western populations.

Conclusions

In Chinese patients with non-ST elevation ACS, carriers with 2 CYP2C19 LOF alleles are more prone to HPR, which is associated with an increased risk for PMI.

Disclosures

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No conflict of interest exists. No relationship with industry exists.

References


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