Effect of CYP2C19*2 and *3 Loss-of-Function Alleles on Platelet Reactivity and Adverse Clinical Events in East Asian Acute Myocardial Infarction Survivors Treated With Clopidogrel and Aspirin

Young-Hoon Jeong, MD, PhD; Udaya S. Tantry, PhD; In-Suk Kim, MD, PhD; Jin-Sin Koh, MD; Tae Jung Kwon, MD; Yongwhi Park, MD, PhD; Seok-Jae Hwang, MD, PhD; Kevin P. Bliden, MBA; Choong Hwan Kwak, MD, PhD; Jin-Yong Hwang, MD, PhD; Paul A. Gurbel, MD

Background—As compared with whites, East Asians more often carry the cytochrome P450 (CYP) 2C19 loss-of-function (LOF) allele with the CYP2C19*3 variant. The influence of the CYP2C19 LOF alleles (*2 and *3) on clopidogrel response and clinical outcomes in East Asians with acute myocardial infarction (AMI) has not been reported. We sought to evaluate the effect of the CYP2C19 variants on clopidogrel pharmacodynamics and long-term prognosis in these patients.

Methods and Results—Patients who survived an AMI (n=266) were enrolled in a single-center registry. Predischarge platelet reactivity was assessed with light transmittance aggregometry and the VerifyNow P2Y12 assay; the CYP2C19*2, *3, *17 and ABCB1 3435C>T variants were determined. The primary clinical end point was the composite of cardiovascular death, nonfatal MI, and ischemic stroke. The median exposure to clopidogrel was 21 months (interquartile range, 13–29). The ABCB1 3435C>T was not related to clopidogrel response or cardiovascular events. Carriage of the CYP2C19 LOF variant allele was relatively high (60.9%, n=162; *2/*17=2, *3/*17=1, *1/*2=96, *1/*3=29, *2/*2=20, and *2/*3=14). Platelet reactivity increased proportionally according to the number of the CYP2C19 LOF alleles. In a multivariate regression analysis, the risk of high on-treatment platelet reactivity (HPR) increased depending on the number of CYP2C19 LOF allele [1 LOF allele; odds ratio (OR), 1.8; 95% confidence interval (CI), 0.8 to 4.2, P=0.152; and 2 LOF alleles; OR, 2.8; 95% CI, 1.2 to 6.5; P=0.016]; platelet reactivity and the rate of HPR did not differ between the CYP2C19*2 versus *3 allele carriage. In addition, cardiovascular event occurrence increased according to the number of the CYP2C19 LOF allele; compared with noncarriers, carriers of 1 [hazard ratio (HR), 3.1; 95% CI, 0.8 to 11.6; P=0.089] and 2 CYP2C19 LOF allele(s) (HR, 10.1; 95% CI, 1.8–58.8; P=0.008) were associated with clinical end point. The clinical impact of the CYP2C19*2 versus *3 allele carriage also did not differ.

Conclusions—Among East Asian patients who survived an AMI, the CYP2C19 LOF allele carriage appears to affect clopidogrel pharmacodynamics and cardiovascular events according to the number of the CYP2C19 LOF allele; the influence of the CYP2C19*2 and *3 alleles on clopidogrel response and long-term outcomes does not differ. (Circ Cardiovasc Interv. 2011;4:585-594.)

Key Words: platelet ■ acute myocardial infarction ■ clopidogrel ■ CYP2C19 polymorphism ■ East Asian population

Dual antiplatelet therapy with aspirin and clopidogrel is a mainstay treatment in patients with acute coronary syndrome (ACS) or undergoing percutaneous coronary intervention (PCI).1,2 However, the antiplatelet effect of clopidogrel is variable and high on-treatment platelet reactivity (HPR) is accepted as an established risk factor for post-PCI ischemic event.3 Because clopidogrel is a prodrug that requires the hepatic cytochrome P450 (CYP) mediated conversion to an active

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From the Division of Cardiology, Department of Internal Medicine, Gyeongsang National University Hospital, Jinju, Korea (Y.-H.J., T.I.K., Y.P., S.-J.H., C.H.K., J.-Y.H.); Sinai Center for Thrombosis Research, Baltimore, MD (Y.-H.J., U.S.T., K.P.B., P.A.G.), the Department of Laboratory Medicine, Gyeongsang National University Hospital, Jinju, Korea (I.-S.K.); and the Department of Internal Medicine and Cardiovascular Center, Seoul National University Hospital, Seoul, Korea (J.-S.K.).

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WHAT IS KNOWN
- CYP2C19*2 allele carriage has shown significant association with the antiplatelet response and ischemic event occurrence in patients who suffered acute coronary syndrome or who were treated with percutaneous coronary intervention during dual antiplatelet therapy with aspirin and clopidogrel.
- Asian population has a high prevalence of the CYP2C19 loss-of-function (LOF) genotype compared with white population (~70% versus ~35%), with the CYP2C19*3 LOF allele and considerable portion of poor metabolizers (10~15%).

WHAT THE STUDY ADDS
- In Asian survivors of acute myocardial infarction, platelet reactivity increases proportionally according to the number of the CYP2C19 LOF allele (*2 or *3), which is related to a high prevalence of the consensus-defined high on-treatment platelet reactivity (more than 50%).
- The CYP2C19 LOF allele (*2 or *3) carriage is an important predictor of ischemic events, but long-term clinical outcome seems similar or lower compared with whites.
- The influence of the CYP2C19*3 allele on clopidogrel response and clinical outcome is as strong as the CYP2C19*2 allele.

metabolite, its pharmacokinetic and pharmacodynamic effects can be influenced by multiple factors that affect intestinal absorption and the CYP isoenzyme activity. In addition to the single-nucleotide polymorphisms (SNPs) of the gene encoding the ABCB1 transporter, SNPs of the gene encoding the CYP2C19 isoenzyme have been consistently linked to clopidogrel response and ischemic events in ACS patients or PCI-treated subjects.

Intriguingly, there are considerable ethnic differences in the distribution and type of the CYP2C19 loss-of-function (LOF) alleles. The carriage prevalence of the CYP2C19 LOF variant is 35% to 45% and 25% to 35% among blacks and whites, respectively, whereas it is 55% to 70% among Asians. The prevalence of CYP2C19 poor metabolizers (subjects carrying 2 LOF alleles) is ~5% among blacks and whites, whereas it is 10% to 20% among Asians. In addition to the CYP2C19*2 allele, 10% to 20% of Asians also carry another defective allele, CYP2C19*3.

We and other groups reported that the CYP2C19*3 allele as well as the CYP2C19*2 allele could affect the magnitude of clopidogrel responsiveness in patients undergoing elective PCI. However, contrary to the consistent link between the CYP2C19*2 allele carriage and worse clinical outcomes among white patients, there are no substantial clinical data linking the CYP2C19*3 alleles to increased ischemic event occurrence. In addition, several clinical data from East Asians raise the concern regarding the role of the CYP2C19 LOF alleles in Asians. Despite a high prevalence of the CYP2C19 LOF allele, the observations have suggested similar or relatively low ischemic event occurrence followed by ACS or PCI in East Asians compared with Western population.

Therefore, the present study was performed to evaluate the effect of the CYP2C19 variants, including the CYP2C19*3 allele, on clopidogrel pharmacodynamics and long-term clinical outcome in high-risk East Asian survivors from acute myocardial infarction (AMI).

Figure 1. Flow diagram of the study population. CYP indicates cytochrome P450; LOF, loss of function. "Intensified antiplatelet therapy was mostly related with the ACCEL-AMI (Adjunctive Clopidogrel versus High Maintenance-Dose Clopidogrel in Acute Myocardial Infarction Patients According to CYP2C19 Polymorphism) study."

Study Population
Korean patients who survived an AMI were selected at the Department of Cardiology of the Gyeongsang National University Hospital between September 2007 and August 2009 (Figure 1). Of the total cohort, 266 patients were included in this analysis due to available genetic analyses, platelet function measurements and long-term prognosis. Patients were eligible for enrollment if they were ≥18 years of age, underwent coronary angiography or had an uneventful PCI, and could be followed up over 1 year after coronary angiography. AMI was defined as clinical symptoms compatible with acute myocardial ischemia within 12 hours before admission with a subsequently documented increase in cardiac markers. ST-segment elevation–MI (STEMI) was prespecified as ST-segment elevation ≥1 mm in at least 2 contiguous leads in the admission ECG or left bundle-branch block, and STEMI patients were recommended to undergo primary PCI within 12 hours of pain onset. Of the 140 STEMI patients, 131 patients (93.6%) were treated with primary PCI. The remaining patients constituted the non–ST-segment elevation–MI (NSTEMI) cohort, and all NSTEMI patients were treated with PCI within 48 hours after admission. Major exclusion criteria were hemodynamic instability, active bleeding and bleeding diatheses, oral anticoagulation therapy, use of intensified antiplatelet agents other than standard dual antiplatelet therapy, contraindication to antiplatelet therapy, noncardiac disease with a life expectancy <1 year, or inability to follow the protocol. The study complied with the Declaration of Helsinki and was approved by the Institutional Review Board of Gyeongsang National University Hospital, and the patients provided written informed consent for the procedure, clinical follow-up and agreement for genetic analyses.

Study Design
Immediately after hospital arrival, all patients received aspirin (300 mg loading, followed by 200 mg per day for 1 month and 100~200 mg per day indefinitely) and clopidogrel (600 mg loading and then...
was drawn into a Greiner Bio-One 3.2% citrate Vacutube tube (Greiner Bio-One, Kremsmünster, Austria). The assay channel contains fibrinogen-coated polystyrene beads, 20 μmol/L ADP, and 22 nmol/L PGE$_1$. The instrument measures an optical signal, reported as P2Y12 reaction units (PRU).

**Data Collection and Follow-Up**

The cardiovascular risk factor data were obtained during the index hospitalization or from the patient chart. Clinical follow-up after PCI was performed at 1 month, 6 months, and 1 year and annually thereafter. Follow-up angiography was performed at the attending doctor’s decision. For the validation of follow-up medication and clinical end point data, additional information was obtained by interview or questionnaire, telephone contacts or from outpatient medical records, as necessary.

**End Points and Definitions**

The primary pharmacodynamic end point was 20 and 5 μmol/L ADP-induced Agg$_\text{max}$, according to the metabolizer status. The secondary pharmacodynamic end points were PRU and the frequency and predictors of HPR according to the metabolizer status. Of the definitions suggested by the consensus document,2,3 we adapted 20 μmol/L ADP-induced Agg$_\text{max}$ >59% as the criteria of HPR, considering that a high concentration of ADP may more represent the high thrombogenic conditions such as AMI.24 The primary clinical end point was the composite of cardiovascular death, nonfatal MI, and ischemic stroke, according to the metabolizer status. The secondary clinical end point was the composite of major or minor bleeding according to the metabolizer status. MI was defined as ischemic symptoms with ECG abnormalities and upper normal limits of troponin.1,2 Ischemic stroke was defined as focal loss of neurological function caused by an ischemic event, with residual symptoms lasting at least 24 hours or leading to death.2 Stent thrombosis was defined as definite stent thrombosis according to the Academic Research Consortium.25 Bleeding was quantified according to Thrombolysis in Myocardial Infarction (TIMI) criteria.2 Two independent physicians blinded to the laboratory data adjudicated events after reviewing the source documents.

**Sample Size Calculation and Statistical Analysis**

In terms with clopidogrel pharmacodynamics, we assumed a 20% relative difference in 20 μmol/L ADP-induced Agg$_\text{max}$ between carriers versus noncarriers of the CYP2C19 LOF variant (PS program version 3.0.14).6 Because the prevalence of the CYP2C19 LOF allele carriage in the East Asian is approximately 60%,11,13,14 it was estimated that a total of 203 patients (122 carriers and 81 noncarriers of the CYP2C19 LOF allele) would be required to provide a power of 95% to detect a statistically significant difference with a 2-sided α-level of 0.05. With regard to clinical outcome, we assumed a 15% incidence of ischemic events in the CYP2C19 LOF carriers as compared with 5% incidence in noncarriers during follow-up period.7 At least 249 patients (150 carriers and 99 noncarriers of the CYP2C19 LOF allele) were needed to detect a statistically significant difference at the level of a power of 80% with a 2-sided α-level of 0.05 and SD of 0.25.

Categorical variables were presented as numbers or percentages, and compared using χ² test or Fisher exact test. Continuous variables were presented as mean±SD and compared using the Student t test, Mann-Whitney U test, or 1-way ANOVA test, as appropriate. After demonstrating significant differences among variables by the ANOVA test, post hoc comparisons between the groups were performed with the Student-Newman-Keuls test for multiple comparisons. To evaluate the impact of covariates on HPR, a logistic regression analysis was performed including known clinical variables to show a significant difference between the HPR and non-HPR group.26 In addition to the CYP2C19 phenotype and ticagrelor use: sex, age, body mass index (BMI), smoking status, hypertension, diabetes status, chronic kidney disease (CKD), calcium channel blocker (CCB), and proton pump inhibitor.

**Platelet Function Measurement**

The considerable clinical thrombotic and bleeding events occur early after the procedure, and platelet reactivity during this period may be associated with adverse clinical events. However, the magnitude of platelet activation can be variably changed by concomitant administration of antiplatelet agents.22 Platelet-rich plasma was obtained after the procedure, and platelet reactivity during this period may be assessed by a whole-blood point-of-care system that assesses responsiveness to P2Y$_{12}$ antagonists.22
Table 1. Baseline Characteristics According to the CYP2C19 Genotype

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total (n=266)</th>
<th>*1/*1 (n=104)</th>
<th>*1/*2 (n=98)</th>
<th>*1/*3 (n=30)</th>
<th>*2/*2 (n=20)</th>
<th>*2/*3 (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>63.0±11.9</td>
<td>63.0±12.4</td>
<td>61.6±12.1</td>
<td>64.3±12.3</td>
<td>68.2±8.7</td>
<td>62.1±10.1</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>195 (73.3)</td>
<td>72 (69.2)</td>
<td>82 (71.8)</td>
<td>17 (56.7)</td>
<td>14 (70.0)</td>
<td>10 (71.4)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.2±3.1</td>
<td>24.2±2.8</td>
<td>24.5±3.0</td>
<td>23.8±2.5</td>
<td>24.1±2.5</td>
<td>25.3±3.3</td>
</tr>
<tr>
<td>Hemoglobin A1C, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFR, MDRD, mL/min/1.73 m²</td>
<td>79</td>
<td>101</td>
<td>95</td>
<td>93</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>189±51</td>
<td>190±49</td>
<td>189±51</td>
<td>181±61</td>
<td>191±40</td>
<td>209±52</td>
</tr>
<tr>
<td>Infarct-related artery, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left anterior descending</td>
<td>128 (48.1)</td>
<td>54 (51.9)</td>
<td>52 (40.6)</td>
<td>10 (33.3)</td>
<td>9 (45.0)</td>
<td>3 (21.4)</td>
</tr>
<tr>
<td>Left circumflex</td>
<td>53 (19.9)</td>
<td>22 (21.2)</td>
<td>16 (30.2)</td>
<td>8 (26.7)</td>
<td>4 (20.0)</td>
<td>3 (21.4)</td>
</tr>
<tr>
<td>Right coronary</td>
<td>84 (31.6)</td>
<td>28 (26.9)</td>
<td>29 (34.5)</td>
<td>12 (40.0)</td>
<td>7 (35.0)</td>
<td>8 (57.1)</td>
</tr>
<tr>
<td>Left main</td>
<td>1 (0.4)</td>
<td>0 (0)</td>
<td>1 (1.0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Thrombectomy, n (%)</td>
<td>44 (16.5)</td>
<td>14 (13.5)</td>
<td>17 (17.3)</td>
<td>5 (16.7)</td>
<td>4 (20.0)</td>
<td>4 (28.6)</td>
</tr>
<tr>
<td>Administration of GPI, n (%)</td>
<td>16 (6.0)</td>
<td>6 (5.8)</td>
<td>4 (4.1)</td>
<td>2 (6.7)</td>
<td>2 (10.0)</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>IVUS guidance, n (%)</td>
<td>156 (58.6)</td>
<td>64 (61.5)</td>
<td>56 (62.5)</td>
<td>15 (57.1)</td>
<td>13 (65.0)</td>
<td>8 (57.1)</td>
</tr>
<tr>
<td>Use of IABP, n (%)</td>
<td>2 (0.8)</td>
<td>1 (1.0)</td>
<td>1 (1.0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Multivessel intervention, n (%)</td>
<td>68 (25.6)</td>
<td>23 (22.1)</td>
<td>25 (25.5)</td>
<td>10 (33.3)</td>
<td>5 (25.0)</td>
<td>5 (35.7)</td>
</tr>
<tr>
<td>Intervention method, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug-eluting stent</td>
<td>239 (89.8)</td>
<td>94 (90.4)</td>
<td>89 (90.8)</td>
<td>26 (86.7)</td>
<td>19 (95.0)</td>
<td>11 (78.6)</td>
</tr>
<tr>
<td>Bare metal stent</td>
<td>2 (0.8)</td>
<td>1 (1.0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (7.1)</td>
</tr>
<tr>
<td>Ballooning only</td>
<td>9 (3.4)</td>
<td>3 (2.9)</td>
<td>2 (2.0)</td>
<td>2 (6.7)</td>
<td>1 (5.0)</td>
<td>1 (7.1)</td>
</tr>
<tr>
<td>Stent diameter, mm</td>
<td>3.1±0.4</td>
<td>3.0±0.4</td>
<td>3.2±0.4</td>
<td>3.1±0.4</td>
<td>3.1±0.4</td>
<td>3.1±0.3</td>
</tr>
<tr>
<td>Stents per patient</td>
<td>1.7±0.9</td>
<td>1.5±0.8</td>
<td>1.8±0.9</td>
<td>1.5±0.9</td>
<td>2.0±1.2</td>
<td>2.1±1.2</td>
</tr>
<tr>
<td>Total stent length, mm</td>
<td>37±26</td>
<td>35±24</td>
<td>38±26</td>
<td>30±20</td>
<td>46±28</td>
<td>42±24</td>
</tr>
<tr>
<td>Post-PCI slow flow, n (%)</td>
<td>14 (5.3)</td>
<td>3 (2.9)</td>
<td>6 (6.1)</td>
<td>3 (10.0)</td>
<td>1 (5.0)</td>
<td>1 (7.1)</td>
</tr>
</tbody>
</table>

CYP indicates cytochrome P450; NSTEMI, non–ST-segment elevation–myocardial infarction; STEMI, ST-segment elevation–myocardial infarction; MI, myocardial infarction; CABG, coronary artery bypass grafting; PCI, percutaneous coronary intervention; LV, left ventricular; WBC, white blood cell; hs-CRP, high-sensitivity C-reactive protein; Hb A1c, hemoglobin A1c; GFR, glomerular filtration rate; GPI, glycoprotein IIb/IIIa inhibitor; NUS, intravascular ultrasound; and IABP, intra-aortic balloon pump.

*P<0.05.

*1/*1, *1/*2, and *1/*3 groups include 3 cases of *1/*17, 2 cases of *2/*17, and 1 case of *3/*17.
Clinical follow-up was censored at the day of the first cardiovascular event corresponding to the clinical end point. For subjects without a clinical event, clinical follow-up was censored either at the last clinic visit while taking clopidogrel or at the day of clopidogrel discontinuation. To assess cumulative event-free survival for primary clinical end point, a Kaplan-Meier analysis was performed. Data were stratified according to the metabolizer status or genotype and were compared with log-rank test. We conducted multivariate Cox regression analysis to identify independent correlates of the primary end point and to adjust for potential confounders27: age, sex, BMI, smoking status, hypertension, diabetes status, CKD, left ventricular EF, use of tirofiban/CCB/proton pump inhibitor, left anterior descending artery infarction, post-PCI slow flow (TIMI flow 0–2), drug-eluting stent implantation, and stent length. All univariate variables with a probability value <0.10 also were included in multivariate analysis. All statistical analyses were performed using the SPSS version 13.0 (SPSS Inc, Chicago, IL) and a 2-tailed probability value <0.05 was considered significant.

Results

Patient Characteristics and Clinical Follow-Up

The average age was 63.0 (SD, 11.9) years, and about three-fourths of patients were men (Table 1). More than half of the patients presented with STEMI. PCI with drug-eluting stents was mostly performed (89.8%). All SNPs were in Hardy-Weinberg equilibrium (89.8%).

Table 2. Allelic, Genotypic, and Phenotypic Distributions of the Study Population

<table>
<thead>
<tr>
<th>Gene</th>
<th>Allele Frequency, %</th>
<th>Genotype Distribution, n (%)</th>
<th>Predicted Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19</td>
<td>*1 62.0</td>
<td>*17/*17 0 (0)</td>
<td>Ultrarapid</td>
</tr>
<tr>
<td></td>
<td>*2 28.6</td>
<td>*1/*17 3 (1.1)</td>
<td>Rapid heterozygote</td>
</tr>
<tr>
<td></td>
<td>*3 8.3</td>
<td>*1/*1 101 (38.0)</td>
<td>Extensive</td>
</tr>
<tr>
<td></td>
<td>*17 1.1</td>
<td>*2/*17 2 (0.8)</td>
<td>Poor or rapid heterozygote</td>
</tr>
<tr>
<td></td>
<td>*3/*17 1 (0.4)</td>
<td>*1/*2 96 (36.1)</td>
<td>Intermediate</td>
</tr>
<tr>
<td></td>
<td>*1/*3 29 (10.9)</td>
<td>*2/*2 20 (7.5)</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>*2/*3 14 (5.2)</td>
<td>*2/*3 0 (0)</td>
<td>Poor</td>
</tr>
<tr>
<td>ABCB1</td>
<td>3435C&gt;T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>66.9</td>
<td>CC 124 (46.6)</td>
<td>High expression</td>
</tr>
<tr>
<td>T</td>
<td>33.1</td>
<td>CT 108 (40.6)</td>
<td>Intermediate expression</td>
</tr>
<tr>
<td>TT</td>
<td>34 (12.8)</td>
<td></td>
<td>Low expression</td>
</tr>
</tbody>
</table>

ABCB1 indicates P-glycoprotein gene; CYP, cytochrome P450.

The average age was 63.0 (SD, 11.9) years, and about three-fourths of patients were men (Table 1). More than half of the patients presented with STEMI. PCI with drug-eluting stents was mostly performed (89.8%). All SNPs were in Hardy-Weinberg equilibrium (P>0.05). The frequencies of CYP2C19 LOF allele, platelet measures and the prevalence of HPR were similar across the ABCB1 genotype groups (data not shown). Thirteen patients (4.9%) experienced the composite end point of cardiovascular death, nonfatal MI, and ischemic stroke while on clopidogrel [12 nonfatal MI events (6 STEMI and 6 NSTEMI) and 1 ischemic stroke], and median time to event was 7 months (IQR, 3–8). There were 2 noncardiovascular deaths (sepsis and pneumonia). Seven patients had a definite stent thrombosis, all presented with nonfatal MI (4 STEMI and 3 NSTEMI). Stent thrombosis occurred in 6 patients with drug-eluting stent and 1 with bare metal stent. Two of these stent thrombosis were subacute (<30 days), 2 were late (30 days to 1 year), and 3 were very late (>1 year). In addition, 8 patients (3.0%) had the composite of bleeding events, and 5 had TIMI major bleeding and three had TIMI minor bleeding. Three patients had a bleeding event within 30 days after discharge, and the remaining 5 patients experienced bleeding between 30 days and the end of follow-up.

Pharmacodynamic End Points

The median levels of 20 and 5 μmol/L ADP-induced Aggmax and PRU were 59.3% (IQR, 46.4–68.2%), 44.4% (IQR, 34.5–55.7%), and 259 (IQR, 196–304), respectively. Platelet measures did not differ according to the presence of tirofiban administration (data not shown). Predischarge platelet function measurements and the prevalence of HPR were similar across the ABCB1 genotype groups (Table 3). The CYP2C19*17 allele frequency was rare and platelet function measurements were assessed according to the CYP2C19 LOF carrier or genotype only.

Platelet reactivity and the prevalence of HPR increased proportionally according to the number of the CYP2C19 LOF allele (data not shown). Regardless of the carriage of CYP2C19*2 or *3 LOF allele, platelet measures and the prevalence of HPR increased depending on the number of the LOF allele (P<0.031 and P<0.009 for trend, respectively) (Table 3). The influence of the CYP2C19*2 and *3 carriage
on platelet measures appeared to be additive, and the influence of the CYP2C19*2 versus *3 allele carriage on platelet reactivity appeared to be similar (Table 3). In multivariate regression analysis to determine predictors of HPR, carriage of two CYP2C19 LOF alleles could predict the risk of HPR [odds ratio (OR) 2.8, 95% confidence interval (CI) 1.2 to 6.5, \(P=0.016\)] (Table 4); compared with noncarriers, the risk of HPR was numerically greater in patients with one CYP2C19 LOF allele, but did not reach statistical significance (OR, 1.8; 95% CI, 0.8–4.2; \(P=0.152\))

**Clinical End Points**

The rate of the composite of cardiovascular death, nonfatal MI, and ischemic stroke did not differ according to the ABCB1 genotype groups [hazard ratios (HR), 0.8; 95% CI, 0.3 to 1.9; \(P=0.603\) by Kaplan-Meier estimate]. The primary end point during the follow-up period occurred in 2 noncarriers (1.9%), 6 patients carrying 1 CYP2C19 LOF allele (4.7%: 4.1% in *1/*2 and 6.7% in *1/*3), and 5 patients carrying 2 CYP2C19 LOF alleles (14.7%: 15.0% in *2/*2 and 14.3% in *2/*3). If primary end point was divided depending on the presence of NSTEMI and STEMI, the results were consistent (see the Appendix in the online-only Data Supplemental Table). The status of the CYP2C19 metabolizer was significantly associated with an increase in the rate of cardiovascular events (\(P=0.013\)) (Figure 2A). In Kaplan-Meier analysis with CYP2C19 genotype, the risk of cumulative cardiovascular events showed an increased trend by the number of the CYP2C19 LOF allele (\(P=0.057\)) (Figure 2B). The influence of the CYP2C19*2 versus *3 allele carriage on clinical events did not differ, and appeared to be additive.

In multivariable stepwise Cox regression analysis, the CYP2C19 LOF allele carriage (\(P=0.029\)) was a significant contributor linked to the occurrence of cardiovascular events, in addition to age (per 1-year increment; HR, 1.1; 95% CI, 1.0–1.1; \(P=0.049\)). Compared with noncarriers, carriers of 1 (HR, 3.1; 95% CI, 0.8–11.6; \(P=0.089\)) and 2 CYP2C19 LOF allele(s) (HR, 10.1; 95% CI, 1.8–58.8; \(P=0.008\)) were associated with clinical end point. Inclusion of platelet reactivity or HPR as a covariate in the regression model did not significantly change the relation between the CYP2C19 LOF allele carriage and cardiovascular events (2 CYP2C19 LOF alleles: all \(P<0.05\), suggesting that the effect of the genotype on clinical outcomes may not be mediated solely through platelet reactivity.

Bleeding risk did not differ according to the CYP2C19 or ABCB1 phenotype (Table 3). If bleeding risk was divided, depending on the presence of NSTEMI and STEMI, its prevalence did not differ according to genotyping (see the Appendix in the online-only Data Supplemental Table). Between patients with versus without bleeding events, there were no differences in platelet function measurements (data not shown).

**Discussion**

To the best of our knowledge, this is the first study to demonstrate the influence of the CYP2C19*2 and *3 LOF alleles on both the antiplatelet effect of clopidogrel and
clinical outcome in AMI patients. The important findings of the present study are (1) East Asian patients had a high prevalence of the CYP2C19 LOF genotype (∼60% including 12.7% 2 LOF alleles carriers) and HPR; over half of the patients met the criteria of HPR; (2) the prevalence of HPR increased according to the number of the CYP2C19 LOF allele; (3) the influence of the CYP2C19*3 LOF allele on clopidogrel response and the prevalence of HPR was as strong as the CYP2C19*2 allele; (4) despite the high prevalence of HPR, long-term rate of the composite clinical events appeared to be low in East Asian patients; (5) the CYP2C19 LOF allele carriage was significantly associated with long-term ischemic events; and (6) the ABCB1 C3435C>T variant did not have any influence on the antiplatelet effect of clopidogrel or clinical outcomes.

Because the P2Y12 receptor plays a crucial role in the growth and stabilization of a thrombus, it has been strongly targeted for inhibition by multiple agents.28,29 Optimal inhibition of the P2Y12 receptor to reach efficacy while avoiding let effect of clopidogrel or clinical outcomes.

Asian population has almost twice the prevalence of the CYP2C19 LOF genotype as compared with white population (for PMs, over 3–5 times).11–14 Therefore, it is important to determine the influence of the CYP2C19 LOF allele on clopidogrel response and the long-term clinical outcomes among Asians. Several factors (eg, old age, BMI, diabetes, CKD, congestive heart failure, drug-drug interaction, the CYP2C19 LOF/GOF allele, and the ABCB1 C3435T variant) have been suggested to determine clopidogrel response and HPR.9,10,30 There are prominent differences in BMI and the CYP2C19 genotype between white and East Asian patients. Although clopidogrel response may be enhanced due to low BMI in East Asians (∼24 kg/m² in this study), the influence of the CYP2C19 LOF allele on clopidogrel response seems to surpass the extent of BMI.13,14

including the present study identified an uncommon carriage of the CYP2C19*17 allele among East Asians (∼4%), validating the linkage disequilibrium between the CYP2C19 LOF and GOF variants.6 Because the CYP2C19*2 and *3 account for 99% of the nonfunctional alleles in Asians,31 the additive impact of the CYP2C19*2 and *3 LOF alleles may increase the level of platelet reactivity and the prevalence of HPR.13,14

However, whether the link between the level of platelet reactivity and clinical outcome is proportional across the races is a matter of debate. Multiple evidences from registries and prospective studies have also demonstrated that the risk of stent thrombosis in East Asians (annually 0.2–0.6%) was not higher compared with whites.15–18 In addition, despite a high prevalence of the CYP2C19 LOF carriage in East Asians, a similar rate of clinical events was observed between this Korean and French AMI cohorts (∼5% during 2 years).7 Although South and East Asian populations have similarly high prevalence of the CYP2C19 LOF variant (10–15% PMs),32 the benefit of intensified platelet inhibition was quiet different. In the CURRENT-OASIS 7 (Clopidogrel and Aspirin Optimal Dose Usage to Reduce Recurrent Events—Seventh Organization to Assess Strategies in Ischemic Symptoms) trial,33 compared with standard-dose clopidogrel, 1-week double-dose clopidogrel in PCI-treated patients was associated with 19% decrease in East Asian (n=2363), but 40% increase in South Asians (n=1570) in terms with ischemic events occurrence. These observations might indicate that the clinical impact of the CYP2C19 LOF carriage and platelet reactivity can be different depending on the ethnicity.

Although there are no conclusive evidences to explain relatively low ischemic events in East Asians, some potential explanations can be extrapolated based on the recent studies. Hypercoagulability is a complex phenomenon incorporating platelet, procoagulant and fibrinolytic systems, and dysfunctional
Thrombin-induced platelet-fibrin clot strength (MAthrombin) measured by thrombelastography, an indicator of hypercoagulability, has been shown to be associated with post-PCI ischemic events occurrence. Interestingly, Korean patients with coronary artery disease appeared to have relatively low levels of MAthrombin compared with Western population (mean, 61.5 versus 68 mm), suggesting that thrombin-mediated hemostasis plays a little role in atherothrombosis among East Asians. Low level of prothrombotic potency in East Asian may suggest an underlying mechanism to explain more bleeding tendency in this ethnicity. Besides, inflammation has been closely associated with platelet activation, atherogenesis, and post-PCI ischemic events occurrence including stent thrombosis. Asian patients have shown to have low high-sensitivity C-reactive protein levels compared with other races, indicating less effect of inflammation on post-PCI ischemic events among Asians. Taken together, these findings may suggest that the relation between ischemic event rate and platelet reactivity in East Asians can be different compared with Western population. In support of the latter hypothesis, some recent studies including Koreans have identified higher cutoffs of HPR to predict ischemic event occurrence (=275 PRU) compared with Western studies (235–240 PRU).

In the Cox regression analysis that included predischarge platelet reactivity or the criteria of HPR, platelet function measurements were not associated with clinical prognosis in the current study suggesting CYP2C19 LOF allele may independently influence adverse cardiovascular events. The latter observation may be due to chance or due to independent influence of the CYP2C19 LOF allele on adverse cardiovascular events. In the CHARISMA (Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance) study, the CYP2C19 LOF PMs in the placebo-treated group showed a higher risk than noncarriers (HR, 1.8; 95% CI, 0.7–4.7). The French AMI cohort also exhibited an unexpected greater risk of primary ischemic events occurrence in the carriers versus noncarriers of the CYP2C19 LOF allele (adjusted HR, 5.38). One of the postulated mechanisms is the CYP epoxygenase system including the CYP2C19 enzyme in various tissue and cell types. Recent studies have shown an important role of this system in controlling oxidative stress, inflammation, vascular tone, hemostasis, and ischemia-reperfusion injury. SNPs of this system may decrease the enzymatic activity, and consecutively cause the in vivo proatherothrombotic milieu.

**Limitations**

First, this study was a single-center experience, which may be limited in making definite conclusion. The potential selection bias on patients’ ischemic event rates can be derived from the fact that only patients who had platelet function and genotyping were included. Therefore, our findings should be considered primarily hypothesis generating. Second, this study included only East Asians. Because the strength of the CYP2C19 LOF allele on clinical events can be different, depending on gene variation or environment, the mechanistic globalization from the result of the present study may be inappropriate. Third, the curve of clinical outcome according to the CYP2C19 phenotype was more divergent as time passed. The present study stressed the clinical impact of the CYP2C19 LOF allele itself rather than platelet reactivity, and multiple studies also have shown a similar trend by the CYP2C19 phenotype. Finally, this study was performed using candidate gene analysis, and other unknown genetic variants may be relevant in risk stratification and clopidogrel response.

**Conclusions**

Among East Asian patients who survived an AMI, the CYP2C19 LOF allele carriage appears to affect clopidogrel pharmacodynamics and cardiovascular events according to the number of the CYP2C19 LOF allele; the influence of the CYP2C19*2 and *3 alleles on clopidogrel response and long-term outcomes does not differ.
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Disclosures

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References


Effect of CYP2C19*2 and *3 Loss-of-Function Alleles on Platelet Reactivity and Adverse Clinical Events in East Asian Acute Myocardial Infarction Survivors Treated With Clopidogrel and Aspirin

Young-Hoon Jeong, Udaya S. Tantry, In-Suk Kim, Jin-Sin Koh, Tae Jung Kwon, Yongwhi Park, Seok-Jae Hwang, Kevin P. Bliden, Choong Hwan Kwak, Jin-Yong Hwang and Paul A. Gurbel

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Supplemental Table. Ischemic and bleeding clinical outcome according to index clinical diagnosis

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<tr>
<th></th>
<th>CYP2C19</th>
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<th></th>
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<td>1/*1</td>
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<td>1/*3</td>
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<td><strong>Non-ST-elevation MI, n (%)</strong></td>
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<td>3 (6.4)</td>
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<td>TIMI major/minor bleeding</td>
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<td>0 (0)</td>
<td>3 (17.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.468</td>
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<td>2 (4.3)</td>
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<tr>
<td>CV death/MI/stroke</td>
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<td>3 (5.5)</td>
<td>1 (7.7)</td>
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<td>1 (7.7)</td>
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<td>3 (4.8)</td>
<td>1 (1.6)</td>
<td>0 (0)</td>
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*1/*1, *1/*2, and *1/*3 groups include three cases of *1/*17, two cases of *2/*17, and one case of *3/*17, respectively.

ABCB1, P-glycoprotein gene; CYP, cytochrome P450; CV, cardiovascular; MI, myocardial infarction; TIMI, Thrombolysis In Myocardial Infarction.