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

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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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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,[4] its pharmacokinetic and pharmacodynamic effects can be influenced by multiple factors that affect intestinal absorption and the *CYP* isoenzyme activity.[3] In addition to the single-nucleotide polymorphisms (SNPs) of the gene encoding the *ABCB1* transporter,[5] 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.[6–10]

Intriguingly, there are considerable ethnic differences in the distribution and type of the *CYP2C19* loss-of-function (LOF) alleles.[11–14] 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*.[11–14]

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.[13,14] However, contrary to the consistent link between the *CYP2C19**2 allele carriage and worse clinical outcomes among white patients,[5–10] 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.[15–18]

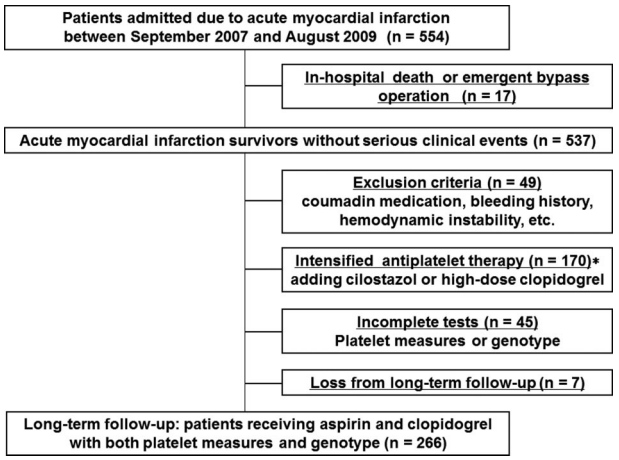

Figure 1. Flow diagram of the study population. *CYP* indicates cytochrome P450; LOF, loss of function. *Intensified antiplatelet therapy was mostly related with the ACCEL-AMI (Adjunctive Cilostazol versus High Maintenance-Dose Clopidogrel in Acute Myocardial Infarction Patients According to *CYP2C19* Polymorphism) study.[19]

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).

Methods

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.[3] 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 Gyeongsnag 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

75 mg per day for at least 1 year). PCI was decided after coronary angiography, and all interventions were conducted according to the current standard guidelines.[1,2] Stent type was chosen by the operator, and tirofiban with a short half-life was administered if a glycoprotein IIb/IIIa inhibitor was required. Anticoagulation with low-molecular-weight heparin (enoxaparin) or unfractionated heparin was initiated before angiography in all patients.

Genetic Analysis

Genotyping [the *CYP2C19*2* or **3* LOF allele, the *CYP2C19*17* gain-of-function (GOF) allele, and the *ABCB1 3435C>T]* was performed using a commercially available kit (QIAamp DNA Blood Mini Kit, Qiagen, Hilden, Germany) after extracting genomic DNA from whole-blood leukocytes.[20] The numbering and allele definitions are based on the nomenclature of the Human *CYP* Allele Nomenclature Committee. Since the frequencies of the *CYP2C19*4, *5, *6, *7,* and **8* LOF alleles are extremely rare in East Asians,[11] only the *CYP2C19*2* (rs4244285, c.681G>A) and **3* (rs4986893, c.636G>A) alleles were genotyped using the ABI SNaPshot (Applied Biosystems, Foster City, CA) reaction. Polymerase chain reaction (PCR) product was processed according to the ABI SNaPshot protocol, using primers designed for fluorescent dideoxy nucleotide termination. SNP analysis was carried out on the ABI 3100 genetic analyzer (Applied Biosystems). The *CYP2C19*17* (rs12248560, g.-808C>T) and *ABCB1 3435C>T* (rs1045642) were identified using the TaqMan method and a commercially available detection system (ABI PRISM 7900HT Sequence Detection System, Applied Biosystems). The PCR amplification protocol for the TaqMan assays included denaturation at 95°C for 10 minutes, followed by 40 cycles at 92°C for 15 seconds, 60°C for 1 minute, and 72°C for 45 seconds, followed by elongation at 72°C for 5 minutes. The TaqMan assays were then read on a 7900HT Fast Real-Time PCR System and alleles were called using SDS software (www.appliedbiosystems.com).

We classified every *CYP2C19* phenotype based on the established nomenclature and its reported effect on enzyme function according to the published reports.[5–10] In addition, participants were also divided into 3 groups, based on the number of the *CYP2C19* LOF allele: extensive (no LOF carriers), intermediate (1 LOF carriers), and poor metabolizers (2 LOF carriers). For *ABCB1 3435C>T*, patients were classified as homozygous for the C allele (CC: high expression), heterozygous (CT: intermediate expression), and homozygous for the T allele (TT: low expression).

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 antithrombotic agents and the status of patients. Because there may be no significant changes of platelet reactivity from days 3–5 in AMI patients undergoing PCI,[21] we measured the patients' platelet reactivity at these periods: ≥3 days after PCI in those individuals not treated with tirofiban or ≥5 days after among patients treated with tirofiban. Blood samples were drawn from the antecubital vein into Vacutainer tubes containing 3.2% sodium citrate (Becton-Dickinson, San Jose, CA) at 2–6 hours after clopidogrel administration. The first 2–4 mL free flowing blood was discarded to avoid spontaneous platelet activation.

Light transmittance aggregometry was performed according to a standard protocol.[22] Platelet-rich plasma was obtained after centrifugation of the blood at 120 g for 10 minutes. The remaining blood was further centrifuged at 1200*g* for 10 minutes to collect platelet-poor plasma. Platelet-rich plasma was adjusted to platelet counts of 250 000/mm^3 by adding platelet-poor plasma whenever required. The samples were processed within 2 hours. Twenty and 5 μmol/L ADP-induced platelet aggregations at the maximal magnitude (Agg_{max}) were assessed at 37°C using an AggRAM aggregometer (Helena Laboratories Corp, Beaumont, TX).

The VerifyNow P2Y12 Assay is a whole-blood, point-of-care system that assesses responsiveness to $P2Y_{12}$ antagonists.[22] Blood was drawn into a Greiner Bio-One 3.2% citrate Vacuette 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_{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,[3,23] we adapted 20 μmol/L ADP-induced Agg_{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_{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 χ^2 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 tirofiban use: sex, age, body mass index (BMI), smoking status, hypertension, diabetes status, chronic kidney disease (CKD), calcium channel blocker (CCB), and proton pump inhibitor.

Table 1. Baseline Characteristics According to the *CYP2C19* Genotype

Variables	Total (n=266)	*1/*1 (n=104)	*1/*2 (n=98)	*1/*3 (n=30)	*2/*2 (n=20)	*2/*3 (n=14)
Age, y	63.0±11.9	63.0±12.4	61.6±12.1	64.3±12.3	68.2±8.7	62.1±10.1
Male, n (%)	195 (73.3)	72 (69.2)	82 (71.8)	17 (56.7)	14 (70.0)	10 (71.4)
Body mass index, kg/m^2	24.2±3.1	24.2±2.8	24.5±3.0	23.8±2.5	24.1±2.5	25.3±3.3
Index clinical presentation, n (%)						
NSTEMI	126 (47.4)	49 (47.1)	43 (43.9)	17 (56.7)	9 (45.0)	8 (57.1)
STEMI	140 (52.6)	55 (52.9)	55 (56.1)	13 (43.3)	11 (55.0)	6 (42.9)
Risk factor, n (%)						
Diabetes mellitus	70 (26.3)	26 (25.0)	26 (26.5)	6 (20.0)	7 (35.0)	5 (35.7)
Hypertension	125 (47.0)	48 (46.2)	43 (43.9)	17 (56.7)	11 (55.0)	6 (42.9)
Hypercholesterolemia	71 (26.7)	21 (20.2)	31 (31.6)	10 (33.3)	4 (20.0)	5 (35.7)
Current smoking	141 (53.0)	54 (51.9)	60 (51.9)	13 (61.2)	8 (40.0)	6 (42.9)
Chronic kidney disease	70 (26.3)	28 (26.9)	22 (22.4)	10 (33.3)	7 (35.0)	3 (21.4)
History, n (%)						
Previous MI	15 (5.6)	4 (3.8)	9 (9.2)	0 (0)	1 (5.0)	1 (7.1)
Previous CABG	2 (0.8)	0 (0)	1 (1.0)	0 (0)	1 (5.0)	0 (0)
Previous PCI	20 (7.5)	7 (6.7)	10 (10.2)	0 (0)	2 (10.0)	1 (7.1)
Previous stroke	7 (2.6)	3 (2.9)	1 (1.0)	2 (6.7)	1 (5.0)	0 (0)
Concomitant medications, n (%)						
Statin	257 (96.6)	101 (97.1)	95 (94.7)	28 (93.3)	19 (95.0)	14 (100.0)
CYP3A4 metabolized	172 (64.7)	63 (60.6)	67 (68.4)	18 (60.0)	16 (80.0)	8 (57.1)
β-blocker	223 (83.8)	87 (83.7)	78 (79.6)	26 (86.7)	19 (95.0)	13 (92.9)
Angiotensin antagonist	239 (89.8)	96 (92.3)	87 (88.8)	27 (90.0)	19 (95.0)	10 (71.4)
Calcium channel blocker	30 (11.3)	10 (9.6)	12 (12.2)	4 (13.3)	2 (10.0)	2 (14.3)
Proton pump inhibitor	4 (1.5)	1 (1.0)	2 (2.0)	1 (3.3)	0 (0)	0 (0)
LV ejection fraction ≤45%, n (%)	56 (21.1)	26 (25.0)	20 (20.4)	6 (20.0)	1 (5.0)	3 (21.4)
WBC count, ×10^3/mm^3	10.8±3.6	11.3±3.5	10.9±3.7	10.1±4.1	9.9±3.0	9.4±2.9
Hemoglobin, g/dL	13.8±1.8	13.8±1.8	13.9±1.6	13.5±2.0	13.4±2.0	14.0±2.3
Platelet count, ×10^3/mm^3	272±71	280±81	267±81	277±69	259±56	261±66
hs-CRP, mg/dL	5.0±7.5	5.6±8.4	5.0±7.5	3.7±4.7	3.7±4.0	4.9±9.1
Hb A1$_C$, %	6.5±1.4	6.6±1.4	6.5±1.6	6.1±1.2	6.5±1.0	6.5±1.2
GFR, MDRD, mL/min/1.73 m^2	79±31	79±32	83±30	73±32	75±26	82±29
Total cholesterol, mg/dL	189±51	190±49	189±51	181±61	191±40	209±52
Infarct-related artery, n (%)*						
Left anterior descending	128 (48.1)	54 (51.9)	52 (40.6)	10 (33.3)	9 (45.0)	3 (21.4)
Left circumflex	53 (19.9)	22 (21.2)	16 (30.2)	8 (26.7)	4 (20.0)	3 (21.4)
Right coronary	84 (31.6)	28 (26.9)	29 (34.5)	12 (40.0)	7 (35.0)	8 (57.1)
Left main	1 (0.4)	0 (0)	1 (1.0)	0 (0)	0 (0)	0 (0)
Aspiration thrombectomy, n (%)	44 (16.5)	14 (13.5)	17 (17.3)	5 (16.7)	4 (20.0)	4 (28.6)
Administration of GPI, n (%)	16 (6.0)	6 (5.8)	4 (4.1)	2 (6.7)	2 (10.0)	2 (14.3)
IVUS guidance, n (%)	156 (58.6)	64 (61.5)	56 (62.5)	15 (57.1)	13 (65.0)	8 (57.1)
Use of IABP, n (%)	2 (0.8)	1 (1.0)	1 (1.0)	0 (0)	0 (0)	0 (0)
Multivessel intervention, n (%)	68 (25.6)	23 (22.1)	25 (25.5)	10 (33.3)	5 (25.0)	5 (35.7)
Intervention method, n (%)						
Drug-eluting stent	239 (89.8)	94 (90.4)	89 (90.8)	26 (86.7)	19 (95.0)	11 (78.6)
Bare metal stent	2 (0.8)	1 (1.0)	0 (0)	0 (0)	0 (0)	1 (7.1)
Ballooning only	9 (3.4)	3 (2.9)	2 (2.0)	2 (6.7)	1 (5.0)	1 (7.1)
Stent diameter, mm	3.1±0.4	3.0±0.4	3.2±0.4	3.1±0.4	3.1±0.4	3.1±0.3
Stents per patient	1.7±0.9	1.5±0.8	1.8±0.9	1.5±0.9	2.0±1.2	2.1±1.2
Total stent length, mm	37±26	35±24	38±26	30±20	46±28	42±42
Post-PCI slow flow, n (%)	14 (5.3)	3 (2.9)	6 (6.1)	3 (10.0)	1 (5.0)	1 (7.1)

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 A1$_C$, hemoglobin A1$_C$; GFR, glomerular filtration rate; GPI, glycoprotein IIb/IIIa inhibitor; IVUS, 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.

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

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

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

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 confounders[27]: age, sex, BMI, smoking status, hypertension, diabetes status, CKD, left ventricular EF ≤45%, 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 ($P>0.05$). The frequencies of the *CYP2C19* LOF allele and the predicted phenotype were representative of the East Asian population (Table 2). We observed a high prevalence of the *CYP2C19* LOF genotype (60.9%) and a low frequency of the *CYP2C19*17* allele (1.1%). Moreover, there were 54 carriers of the *CYP2C19*3* LOF allele (16.5%). Baseline demographics, clinical presentation, and treatment were mostly well balanced between the *CYP2C19* LOF carrier groups, except for more stent use in 2 LOF carriers, and there were no significant differences across the *ABCB1* genotype groups (data not shown). Even though we classified the patients by *CYP2C19* genotype, baseline characteristics did not differ across the groups, except of distribution of infarct-related artery (Table 1).

During the follow-up, 96% of the patients (n=255) received clopidogrel for more than 1 year. Clopidogrel discontinuation was mostly decided by the attending physician, except for 5 cases (1.9%). The median clopidogrel exposure time was 21 months (interquartile range [IQR], 13–29) and did not differ between the *CYP2C19* LOF carrier 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 Agg_{max} 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 ($P\leq0.031$ and $P=0.009$ for trend, respectively) (Table 3). The influence of the *CYP2C19*2* and **3* carriage

Table 3. Platelet Function Measurement and the Prevalence of HPR and Cardiovascular Events According to Metabolizer Status

	CYP2C19						*ABCB1 3435C>T*			
	1/*1 (n=104)	*1/*2 (n=98)	*1/*3 (n=30)	*2/*2 (n=20)	*2/*3 (n=14)	*P*	CC (n=124)	CT (n=108)	TT (n=34)	*P*
LTA, %										
5 μmol/L ADP-Agg_{max}	41.9±15.7	45.4±16.1	45.9±15.8	50.4±15.1	53.9±15.1	0.031	44.7±16.2	44.9±16.1	43.4±17.5	0.887
20 μmol/L ADP-Agg_{max}	53.8±15.7	58.1±14.7	59.0±15.1	64.1±12.1	67.2±11.3	0.003	57.0±15.2	57.5±15.7	56.2±16.5	0.906
VerifyNow P2Y12 assay										
P2Y12 reaction unit	231±88	245±80	261±76	276±71	291±46	0.020	245±78	245±83	244±97	0.996
HPR rate, n (%)	45 (43.3)	50 (51.0)	16 (53.3)	13 (65.0)	10 (71.4)	0.009	64 (51.6)	54 (50.0)	16 (47.1)	0.638
MACE,* n (%)	2 (1.9)	4 (4.1)	2 (6.7)	3 (15.0)	2 (14.3)	0.004	7 (5.6)	5 (4.6)	1 (2.9)	0.512
Bleeding,† n (%)	3 (2.9)	1 (1.0)	4 (13.3)	0 (0)	0 (0)	0.810	4 (3.2)	3 (2.8)	1 (2.9)	0.879

HPR indicates high on-treatment platelet reactivity (20 μmol/L ADP-induced maximal platelet reactivity>59%); *ABCB1*, P-glycoprotein gene; ADP, adenosine diphosphate; Agg_{max}, maximal platelet aggregation; and *CYP*, cytochrome P450.

*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.

*MACE indicates major adverse cardiovascular events, including cardiovascular death, nonfatal myocardial infarction, and ischemic stroke.

†Bleeding indicates Thrombolysis in Myocardial Infarction major or minor bleeding.

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 C*YP2C19* 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* LOF 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

Table 4. Predictors for HPR by Univariate and Multivariate Logistic Regression Models

	Univariate Analysis		Multivariate Analysis	
Variables	OR (95% CI)	*P*	OR (95% CI)	*P*
CYP2C19 LOF allele carriage				
One LOF allele carriage	1.96 (0.88–4.37)	0.097	1.83 (0.80–4.17)	0.152
Two LOF alleles carriage	2.74 (1.21–6.21)	0.015	2.81 (1.21–6.54)	0.016
Female	2.06 (1.18–3.61)	0.011	1.92 (1.00–3.70)	0.050
Age, per 1-y increment	1.02 (1.00–1.05)	0.024	1.01 (0.99–1.04)	0.321
Body mass index, per 1 increment	1.01 (0.93–1.10)	0.787	1.01 (0.92–1.10)	0.901
Current smoking	0.69 (0.43–1.13)	0.139	0.86 (0.48–1.51)	0.590
Hypertension	1.13 (0.70–1.82)	0.628	1.47 (0.85–2.51)	0.165
Diabetes mellitus	1.02 (0.59–1.76)	0.942	1.10 (0.61–2.00)	0.755
Chronic kidney disease	1.45 (0.83–2.51)	0.188	1.32 (0.61–2.27)	0.636
Usage of tirofiban	0.98 (0.36–2.70)	0.975	0.93 (0.31–2.75)	0.897
Calcium channel blocker	1.18 (0.55–2.53)	0.666	1.19 (0.53–2.68)	0.672
Proton pump inhibitor	1.02 (0.14–7.32)	0.988	1.12 (0.15–8.61)	0.911

HPR indicates high on-treatment platelet reactivity (20 μmol/L ADP-induced maximal platelet reactivity >59%); ADP, adenosine diphosphate; CI, confidence interval; *CYP,* cytochrome P450; and OR, odds ratio.

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* LOF 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 3435C>T* variant did not have any influence on the antiplatelet effect of clopidogrel or clinical outcomes.

Because the $P2Y_{12}$ 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 $P2Y_{12}$ receptor to reach efficacy while avoiding bleeding has been a major area of clinical interest.

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 C3435*T 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/m^2 in this study), the influence of the *CYP2C19* LOF allele on clopidogrel response seems to surpass the extent of BMI.[13,14] In addition, recent studies[31] 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 dysfunc-

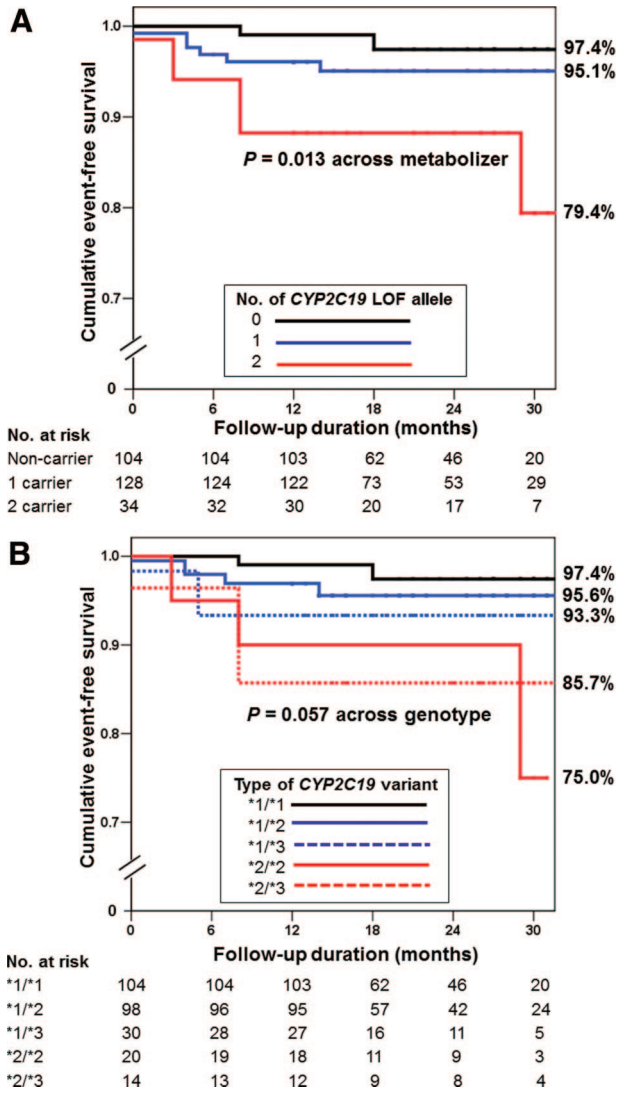

Figure 2. Cumulative risk of cardiovascular death, myocardial infarction, and ischemic stroke according to *CYP2C19* phenotype metabolizer (**A**) and genotype (**B**) (Kaplan-Meier estimates). *CYP* indicates cytochrome P450.

tional endothelium, which are finely tuned by gene variation and environment.[34] Thrombin-induced platelet-fibrin clot strength ($MA_{thrombin}$) measured by thrombelastography, an indicator of hypercoagulability, has been shown to be associated with post-PCI ischemic events occurrence.[35] Interestingly, Korean patients with coronary artery disease appeared to have relatively low levels of $MA_{thrombin}$ compared with Western population (mean, 61.5 versus ≈68 mm),[35,36] suggesting that thrombin-mediated hemostasis plays a little role in atherothrombosis among East Asians.[37] Low level of prothrombotic potency in East Asian may suggest a underlying mechanism to explain more bleeding tendency in this ethnicity.[37,38] Besides, inflammation has been closely associated with platelet activation, atherogenesis, and post-PCI ischemic events occurrence including stent thrombosis.[39,40] Asian patients have shown to have low high-sensitivity C-reactive protein levels compared with other races,[41] 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)[16,31,42] compared with Western studies (235–240 PRU).[3]

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 play of the 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,[43] 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 unexpected greater risk of primary ischemic events occurrence in the carriers versus noncarriers of the *CYP2C19* LOF allele (adjusted HR, 5.38).[7] 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.[44] 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,[6] 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.[5–10] 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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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

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SUPPLEMENTAL MATERIAL

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

	CYP2C19						***ABCB1* C3435T**			
	1/*1	***1/*2**	***1/*3**	***2/*2**	***2/*3**		**CC**	**CT**	**TT**	
	(n = 104)	**(n = 98)**	**(n = 30)**	**(n = 20)**	**(n = 14)**	***P***	**(n = 124)**	**(n = 108)**	**(n = 34)**	***P***
Non-ST-elevation MI, n (%)	**(n = 49)**	**(n = 43)**	**(n = 17)**	**(n = 9)**	**(n = 8)**		**(n = 61)**	**(n = 47)**	**(n = 18)**	
CV death/MI/stroke	1 (2.0)	1 (2.3)	1 (5.9)	1 (11.1)	1 (12.5)	0.074	2 (3.3)	3 (6.4)	0 (0)	0.852
TIMI major/minor bleeding	1 (2.0)	0 (0)	3 (17.6)	0 (0)	0 (0)	0.468	1 (1.6)	2 (4.3)	1 (5.6)	0.334
ST-elevation MI, n (%)	**(n = 55)**	**(n = 55)**	**(n = 13)**	**(n = 11)**	**(n = 6)**		**(n = 63)**	**(n = 61)**	**(n = 16)**	
CV death/MI/stroke	1 (1.8)	3 (5.5)	1 (7.7)	2 (18.2)	1 (16.7)	0.018	5 (7.9)	2 (3.3)	1 (6.3)	0.478
TIMI major/minor bleeding	2 (3.6)	1 (1.8)	1 (7.7)	0 (0)	0 (0)	0.662	3 (4.8)	1 (1.6)	0 (0)	0.213

*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.