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CVN Interview

Carriage of Cytochrome *2C19* Polymorphism Is Associated With Risk of High Post-Treatment Platelet Reactivity on High Maintenance-Dose Clopidogrel of 150 mg/day

Results of the ACCEL-DOUBLE (Accelerated Platelet Inhibition by a Double Dose of Clopidogrel According to Gene Polymorphism) Study

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Objectives This study sought to determine the impact of gene polymorphisms on platelet reactivity (PR) after clopidogrel 150 mg/day in patients treated with percutaneous coronary intervention (PCI).

Background Although high maintenance-dose (MD) clopidogrel reduces PR, it is unknown whether gene polymorphisms are related with the risk of high post-treatment PR (HPPR) after high-MD clopidogrel.

Methods We included mostly patients receiving high-MD clopidogrel after PCI from previously registered Gyeongsang National University Hospital data. A total of 126 PCI-treated patients receiving high-MD clopidogrel were enrolled. Platelet reactivity was assessed with conventional aggregometry and VerifyNow (Accumetrics Inc., San Diego, California) after receiving clopidogrel 150 mg/day for at least 1 month. *CYP3A5*, *CYP2C19,* and *ABCB1* genotyping was performed. We defined HPPR as 5 μmol/l adenosine diphosphate (ADP)–induced maximal PR (PR_{max}) >50%.

Results *CYP3A5* and *ABCB1* polymorphisms did not influence PR. Carriers of *CYP2C19* variant (**2* or **3*) (n = 80) had significantly higher 5 and 20 μmol/l ADP-induced PR_{max} than did noncarriers (n = 46) (40.7 ± 16.8% vs. 30.3 ± 12.6%, $p < 0.001$; 54.2 ± 16.2% vs. 40.5 ± 15.8%, $p < 0.001$, respectively). Late PR and VerifyNow results indicated consistently greater measures in carriers versus noncarriers of *CYP2C19* variant. All platelet measures proportionally increased according to the number of *CYP2C19* variant alleles. Twenty-seven (21.4%) patients met the criteria for HPPR. Prevalence of HPPR was 8.7%, 21.7%, and 50.0% in carriers of 0, 1, and 2 *CYP2C19* variant alleles, respectively ($p < 0.001$). By multivariate analysis, carriage of *CYP2C19* variant was a significant predictor of HPPR (odds ratio: 5.525, 95% confidence interval: 1.333 to 23.256, $p = 0.018$).

Conclusions Among PCI-treated patients receiving high-MD clopidogrel, carriage of *CYP2C19* variant relates to increased PR and predicts risk of HPPR. (Adjunctive Cilostazol Versus High Maintenance-dose ClopidogrEL in Acute Myocardial Infarction [AMI] Patients According to CYP2C19 Polymorphism [ACCELAMI2C19]; NCT00915733; and Comparison of Platelet Inhibition With Adjunctive Cilostazol Versus High Maintenance-Dose Clopidogrel According to Hepatic Cytochrome 2C19 Allele (CYP2C19) Polymorphism [ACCEL2C19]; NCT00891670). (J Am Coll Cardiol Intv 2010;3:731–41) © 2010 by the American College of Cardiology Foundation

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Because platelets play a critical role in atherothrombosis after acute coronary syndrome (ACS) or percutaneous coronary intervention (PCI) (1,2), adequate platelet inhibition is important to minimize the risk of recurrent ischemic cardiovascular events. Although a combination of aspirin and clopidogrel is the current standard antiplatelet therapy following ACS and PCI (3–5), antiplatelet response to clopidogrel is highly variable (6,7), and a higher level of on-treatment platelet reactivity (PR) has been associated with an increased risk of cardiovascular events (8–10). Therefore, intensified antiplatelet regimens have been introduced to achieve consistent and potent inhibition of adenosine diphosphate (ADP)–induced platelet aggregation (11–13).

Abbreviations and Acronyms

ACS = acute coronary syndrome
ADP = adenosine diphosphate
BMI = body mass index
CKD = chronic kidney disease
CYP = the hepatic cytochrome P450
DNA = deoxyribonucleic acid
HPPR = high post-treatment platelet reactivity
MD = maintenance-dose
PCI = percutaneous coronary intervention
PCR = polymerase chain reaction
PR = platelet reactivity
PR_{late} = late platelet aggregation at 5 min
PR_{max} = maximal platelet aggregation
PRU = $P2Y_{12}$ reaction unit
RM = reduced metabolizer

A higher dose of clopidogrel also can obtain a more rapid and greater degree of platelet inhibition (14,15), and the CURRENT/OASIS 7 (Clopidogrel Optimal Loading Dose Usage to Reduce Recurrent Events/Optimal Antiplatelet Strategy for Interventions) study (13) showed that a double dose of clopidogrel for 7 days in PCI-treated patients with ACS can reduce the risk of major adverse cardiovascular events by 15%. Although various factors including patient characteristics and drug-drug interactions can affect antiplatelet response to clopidogrel, gene polymorphisms underlie enhanced PR and higher rates of ischemic events to a great extent (16,17). Because clopidogrel, regardless of dose, is converted to its active metabolite through the same metabolic pathway, it is uncertain whether gene polymorphisms are associated with the risk of high post-treatment PR (HPPR) in patients on a higher dose of clopidogrel (18).

The purpose of this study, therefore, was to determine the impact of gene polymorphisms on residual PR with a high dose of clopidogrel. We assessed the risk of HPPR and the association of gene polymorphisms with HPPR after a high maintenance-dose (MD) clopidogrel of 150 mg/day in PCI-treated patients.

Methods

Patient population and study design. A total of 126 PCI-treated patients with available deoxyribonucleic acid (DNA) genotyping could be enrolled to the ACCEL-DOUBLE (Accelerated Platelet Inhibition by a Double Dose of Clopidogrel According to Gene Polymorphism) study (Fig. 1). Patients were recruited at the Department of Cardiology of the Gyeongsang National University Hospital between January 2008 and June 2009. One-hundred and six patients were collected from a registry of the ACCEL (Adjunctive Cilostazol Versus High-MD Clopidogrel) studies (19,20), which were performed to compare the degree of platelet inhibition by adjunctive cilostazol versus high-MD clopidogrel in patients within a specific subset: HPPR, diabetes, drug-eluting stent implantation for complex lesions, and acute myocardial infarction. The high-MD group received a high-MD clopidogrel of 150 mg/day for 1 month. A minority (n = 20, 15.9%) of patients received high-MD clopidogrel for over 1 month after PCI at the attending physician's discretion. Patient compliance to antiplatelet therapy was assessed by interview and tablet counting at the follow-up visit. Because previous definitions of clopidogrel response using the difference between pre- and post-treatment PR overestimate ischemic risk (7), we assessed the post-treatment threshold of PR. Because pharmacokinetic and pharmacodynamic responses may vary profoundly during the initial days or weeks after antiplatelet therapy (15), PR was assessed during the maintenance phase of high-MD clopidogrel in addition to aspirin 200 mg/day after PCI (≥1 month).

Patients were eligible for enrollment if they were ≥18 years of age and had been treated with PCI for symptomatic coronary artery disease. Exclusion criteria were known allergies to antiplatelet therapy, left ventricular ejection fraction <30%, active bleeding and bleeding diatheses, oral

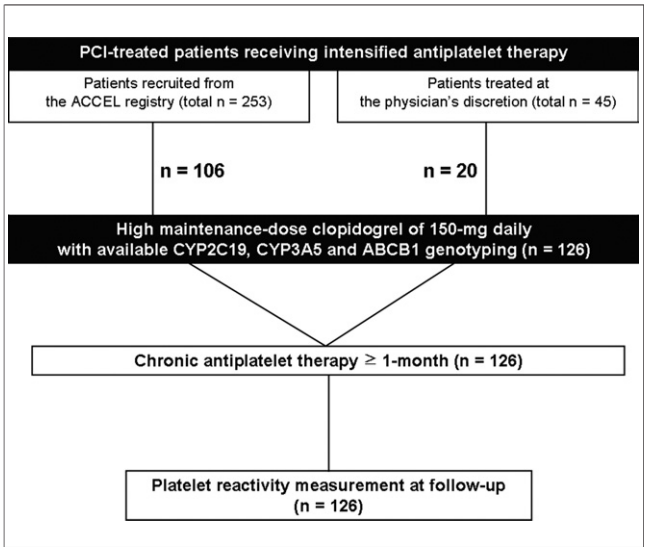

Figure 1. Flow Diagram of the ACCEL-DOUBLE Study

Flow diagram of the ACCEL-DOUBLE (Accelerated Platelet Inhibition by a Double Dose of Clopidogrel According to Gene Polymorphism) study. *ABCB1* = P-glycoprotein gene; ACCEL = Adjunctive Cilostazol Versus High Maintenance-Dose Clopidogrel; *CYP* = the hepatic cytochrome P450; PCI = percutaneous coronary intervention.

anticoagulation therapy with Coumadin, a leukocyte count $<3{,}000/mm^3$ and/or a platelet count $<100{,}000/mm^3$, an aspartate aminotransferase level or an alanine aminotransferase level ≥3 times the upper normal limit, serum creatinine level ≥2.5 mg/dl, stroke within 3 months, noncardiac disease with a life expectancy <1 year and inability to receive the regimen. The study protocol was approved by the Institutional Ethics Committee of Gyeongsang National University Hospital, Korea, and the patients provided written informed consent to participate in this study.

Platelet function assays. Within 2 to 4 h after the last intake of regimen, blood samples were collected using the double-syringe technique, and the first 2 to 4 ml of blood was discarded to avoid the bias of spontaneous platelet activation. Platelet reactivity was simultaneously measured <1 h after venipuncture by light transmittance aggregometry and the VerifyNow $P2Y_{12}$ assay (Ultegra rapid platelet function assay, Accumetrics Inc., San Diego, California). Correlation between 2 assays at our laboratory has been reported (21).

Platelet aggregation was assessed by light transmittance aggregometry according to standard protocol (15,19,20). Blood samples were drawn into Vacutainer tubes containing 0.5 ml of 3.2% sodium citrate (Becton-Dickinson, San Jose, California) and processed within 60 min. Platelet-rich plasma was obtained as a supernatant fluid after centrifuging the blood at 120 g for 10 min. The remaining blood was further centrifuged at 1,200 g for 10 min to prepare platelet-poor plasma. Examined sample was adjusted to platelet counts of $250{,}000/mm^3$. Platelet aggregation was assessed at 37°C using an AggRAM aggregometer (Helena Laboratories Corporation, Beaumont, Texas). Platelet measures were performed after the addition of 5 and 20 μmol/l ADP, and the curves were recorded for 10 min. Platelet reactivity was measured at the peak (maximal PR [PR_{max}]) and at 5 min (late PR [PR_{late}]) by laboratory personnel blinded to the study protocol.

The VerifyNow $P2Y_{12}$ assay is a whole-blood, point-of-care system that has been developed to assess 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 device consists of 2 whole-blood assay channels. One contains fibrinogen-coated polystyrene beads, 20 μmol/l ADP, and 22 nmol/l prostaglandin E1. The other separate channel contains fibrinogen-coated polystyrene beads and isothrombin receptor activating protein. Platelet aggregation by the isothrombin receptor activating protein (BASE) can occur independently of $P2Y_{12}$ receptors. The results are reported in $P2Y_{12}$ reaction unit (PRU), BASE and % inhibition. The % inhibition is calculated as: ([BASE-PRU]/BASE) × 100.

Genotyping and metabolic phenotype. We performed genotyping of the *CYP3A5*3*, *CYP2C19*2* and **3*, and *ABCB1* variants according to previous studies (16,17,23–25). The base numbering and allele definitions follow the nomenclature of the Human *CYP* Allele Nomenclature Committee. Genomic DNA was extracted from leukocytes of whole-blood specimens with a commercially available kit (QIAamp DNA Blood Mini Kit, Qiagen, Hilden, Germany).

Because the allelic frequencies of the *CYP2C19*4* to **6* are extremely rare in East Asians (26,27), genotyping for *CYP2C19*2* (rs4244285, c.681G>A) and *CYP2C19*3* (rs4986893, c.636G>A) were investigated using the ABI SNaPshot (Applied Biosystems, Foster City, California) reaction (Fig. 2). Polymerase chain reaction (PCR) was carried out by using the same primers as previously described (24). The PCR product was processed as per the ABI SNaPshot protocol, using primers designed for fluorescent dideoxy nucleotide termination. Single nucleotide polymorphisms analysis was carried out on the ABI 3100 genetic analyzer (Applied Biosystems, Foster City, California). Genotyping for the *CYP3A5*3* (rs776746, g.6986A>G) and *ABCB1* (rs1045642, c.3435C>T) was performed with the use of an allelic discrimination assay based on the TaqMan method (Applied Biosystems) and ABI PRISM 7900HT Sequence Detection System (SDS) (Applied Biosystems). The PCR amplification protocol for the TaqMan assays included denaturation at 95°C for 10 min, followed by 40 cycles at 92°C for 15 s, 60°C for 1 min, and 72°C for 45 sec, followed by elongation at 72°C for 5 min. The TaqMan assays were then read on a 7900HT Fast Real-Time PCR System and alleles were called using SDS software.

In the case of the *CYP2C19* and *ABCB1*, carriers of any loss-of-function allele were categorized as reduced metabolizers (RMs). In terms of the *CYP3A5,* carriers of 2 mutant alleles were defined as RMs (25).

End points and definition. Primary end point was PR_{max} according to DNA genotypes. Secondary end points were: 1) PR_{late}; 2) PRU; 3) percentage of inhibition; and 4) the rate and predictors of HPPR according to DNA genotypes. On chronic clopidogrel therapy, patients with 5 μmol/l ADP-induced PR_{max} >50%, or diabetic subjects with 20 μmol/l ADP-induced PR_{max} >62% have shown a higher risk of long-term adverse cardiovascular events (28,29). Because we demonstrated that these cutoffs were similar (21), HPPR was defined as 5 μmol/l ADP-induced PR_{max} >50%.

Sample size calculation and statistical analysis. We assumed a 25% difference in 5 μmol/l ADP-induced PR_{max} between carriers versus noncarriers of the *CYP2C19* variant on a high-MD clopidogrel. Based on our previous study (24), prevalence of the *CYP2C19* variant carriage was considered as approximately 60% in the East Asian population. Thus, it was estimated that a total of 95 patients (57 carriers and 38 noncarriers of the *CYP2C19* variant allele) would be required to provide a power of 90% to detect a statistically significant difference with a 2-sided alpha-level of 0.05.

Continuous variables, presented as mean ± SD, were compared using the Student unpaired *t* test or Mann-Whitney *U* test. Categorical variables, presented as numbers or percentages, were compared using chi-square tests or Fisher exact tests

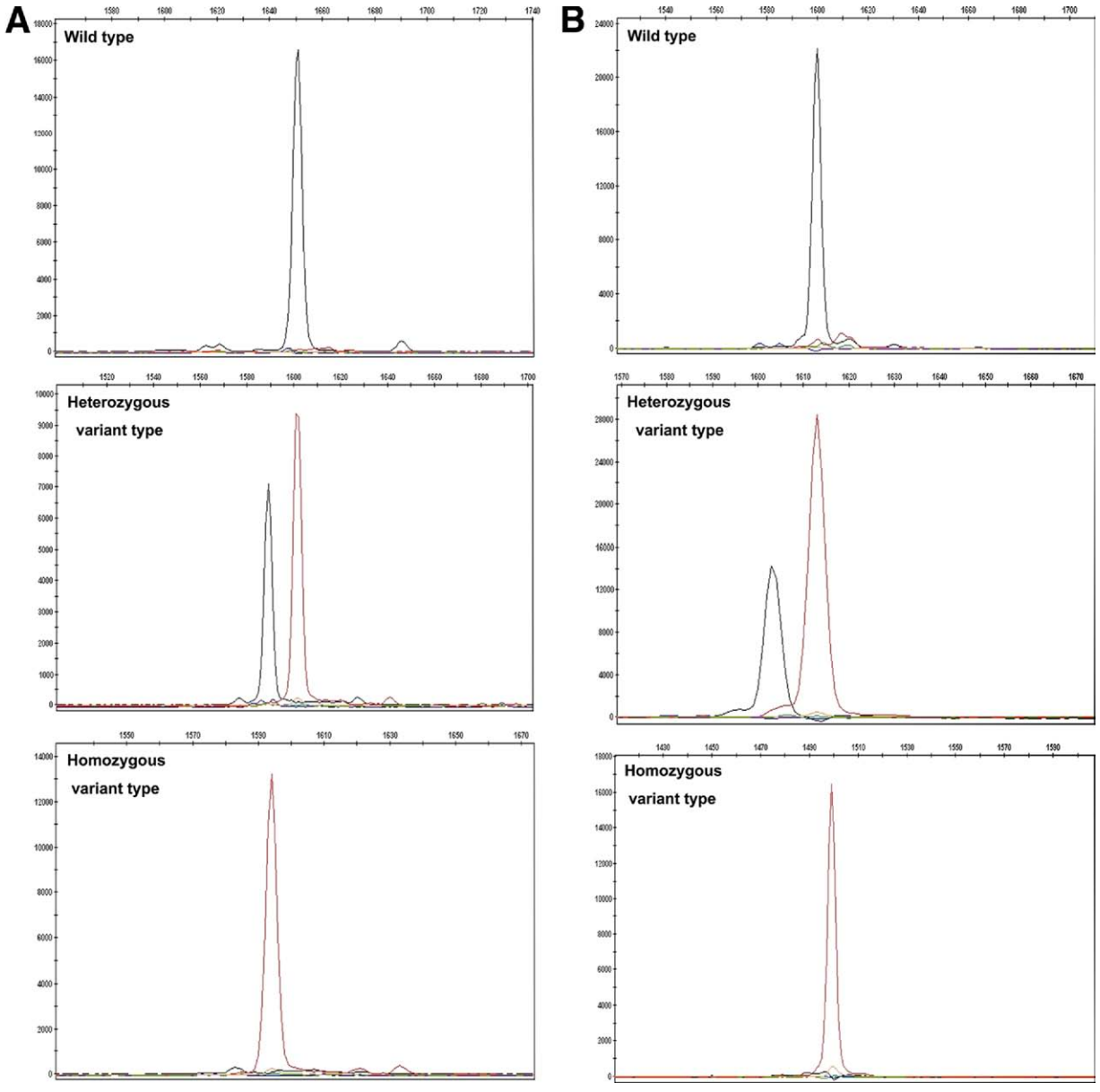

Figure 2. Genotyping Using ABI SNaPshot Method

*CYP2C19*2* (rs4244285, c.681G>A) **(A)** and *CYP2C19*3* (rs4986893, c.636G>A) **(B)** genotyping using ABI SNaPshot method (Applied Biosystems, Foster City, California).

as appropriate. Platelet measures and baseline characteristics according to DNA genotyping were analyzed using a 1-way analysis of variance test. After demonstration of significant differences among variables by analysis of variance, post hoc comparisons among the groups were made with the Student-Newman-Keuls procedure for multiple comparisons. The genotypic frequencies were compared by chi-square analyses, and departure from Hardy-Weinberg equilibrium was tested. To determine predictors of HPPR, a logistic regression analysis was performed using known variables, and odds ratio (OR) and 95% confidence interval (CI) were also calculated. A value of $p < 0.05$ was considered to indicate a significant difference. All the statistical analyses were performed using SPSS version 13.0 (SPSS Inc., Chicago, Illinois).

Results

Patient characteristics and DNA genotypes. All patients showed complete compliance with a high-MD clopidogrel for at least 30 days. No major cardiovascular and bleeding

events were observed in any patient during the follow-up. One patient in the noncarrier group suffered from TIMI (Thrombolysis In Myocardial Infarction) minor bleeding during the follow-up period, which was related with an entry-site hematoma. Baseline characteristics of the patients are demonstrated in Table 1. Patients comprised relatively high-risk cohorts. Sixty-four patients (50.6%) were admitted with acute myocardial infarction; 96.8% of the cohorts were treated with drug-eluting stents, and 59 patients (46.8%) had multivessel disease.

The genetic distributions for evaluated polymorphisms were similar to the published East Asian frequencies (30)

Table 1. Baseline Characteristics According to Carriage of the *CYP2C19* Variant Allele (*2 or *3)

Variables	Total (n = 126)	Noncarrier (n = 46)	1-Variant Carrier (n = 60)	2-Variant Carrier (n = 20)	p Value
Age, yrs	61.6 ± 11.0	61.8 ± 9.4	60.4 ± 12.5	64.6 ± 9.0	0.327
Male sex	85 (67.5)	29 (63.0)	40 (66.7)	16 (80.0)	0.215
Body mass index, kg/m^2	24.8 ± 2.9	24.8 ± 2.7	24.8 ± 3.1	25.2 ± 2.7	0.817
Index clinical presentation					0.134
Stable angina	28 (22.2)	6 (13.0)	14 (23.3)	8 (40.0)	
Unstable angina	34 (27.0)	15 (32.6)	17 (28.3)	2 (10.0)	
NSTEMI	28 (22.2)	9 (19.6)	14 (23.3)	5 (25.0)	
STEMI	36 (28.6)	16 (34.8)	15 (25.0)	5 (25.0)	
Risk factor					
Diabetes mellitus	31 (24.6)	12 (26.1)	15 (25.0)	4 (20.0)	0.634
Hypertension	67 (53.2)	28 (60.9)	30 (50.0)	9 (45.0)	0.185
Hypercholesterolemia	46 (36.5)	15 (32.6)	25 (41.7)	6 (30.0)	0.896
Current smoking	35 (27.8)	11 (23.9)	18 (30.0)	6 (30.0)	0.526
Chronic kidney disease	4 (3.2)	0 (0)	3 (5.0)	1 (5.0)	0.183
History					
Previous MI	70 (55.6)	28 (60.9)	32 (53.3)	10 (50.0)	0.360
Previous CABG	1 (0.8)	0 (0)	1 (1.7)	0 (0)	0.766
Previous stroke	6 (4.8)	3 (6.5)	3 (5.0)	0 (0)	0.290
Concomitant medications					
Statin	112 (88.9)	40 (87.0)	53 (88.3)	19 (95.0)	0.390
CYP3A4 metabolized	89 (70.6)	28 (60.9)	45 (75.0)	16 (80.0)	0.106
Beta-blocker	102 (81.0)	35 (76.1)	50 (83.3)	17 (85.0)	0.321
Angiotensin antagonist	114 (90.5)	44 (95.7)	53 (88.3)	17 (85.0)	0.130
Nitrate	91 (72.2)	31 (67.4)	44 (73.3)	16 (80.0)	0.281
Calcium-channel blocker	30 (23.8)	9 (19.6)	17 (28.3)	4 (20.0)	0.721
Proton pump inhibitor	1 (0.8)	1 (2.2)	0 (0)	0 (0)	0.253
LV ejection fraction <45%	18 (14.3)	7 (15.2)	7 (11.7)	4 (20.0)	0.794
Hemoglobin, g/dl	13.7 ± 2.6	13.3 ± 1.3	13.9 ± 3.5	14.1 ± 1.5	0.413
Platelet count, ×10^3/mm^3	265 ± 68	272 ± 75	261 ± 67	261 ± 53	0.673
HbA1C, %	6.4 ± 1.2	6.3 ± 1.3	6.4 ± 1.4	6.2 ± 0.8	0.814
GFR (MDRD, ml/min/1.73 m^2)	91.8 ± 19.5	93.5 ± 19.4	90.1 ± 20.3	91.9 ± 17.9	0.753
Total cholesterol, mg/dl	141.8 ± 36.9	141.5 ± 36.9	141.0 ± 31.4	145.1 ± 51.4	0.913
Multivessel disease	59 (46.8)	16 (34.8)	33 (55.0)	10 (50.0)	0.113
Intervention method					0.005
Drug-eluting stent	122 (96.8)	46 (100.0)	59 (95.0)	17 (85.0)	
Bare-metal stent	0 (0)	0 (0)	0 (0)	0 (0)	
Ballooning only	4 (3.2)	0 (0)	1 (1.7)	3 (15.0)	
Multivessel intervention	35 (27.8)	9 (19.6)	19 (31.7)	7 (35.0)	0.136
Stent diameter, mm	3.1 ± 0.4	3.0 ± 0.3	3.1 ± 0.4	3.1 ± 0.5	0.061
Stents per patient	1.6 ± 0.8	1.6 ± 0.9	1.6 ± 0.7	1.5 ± 0.7	0.873
Total stent length, mm	38.1 ± 21.7	37.2 ± 25.7	39.0 ± 19.2	37.8 ± 18.4	0.916

Values are mean ± SD or n (%) unless otherwise indicated.

CABG = coronary artery bypass grafting; *CYP* = the hepatic cytochrome P450; GFR = glomerular filtration rate; HbA1C = hemoglobin A1C; LV = left ventricular; MDRD = maximum deflection ratio detector; MI = myocardial infarction; NSTEMI = non–ST-segment elevation myocardial infarction; STEMI = ST-segment elevation myocardial infarction.

Table 2. Distributions of Gene Polymorphisms

Gene	Genotypes	Predicted Function	Distribution
CYP2C19	*1/*1	Normal	46 (36.5)
	*1/*2	Decreased	45 (35.7)
	*1/*3	Decreased	15 (11.9)
	*2/*2	Decreased or absent	13 (10.3)
	*2/*3	Decreased or absent	7 (5.6)
	*3/*3	Decreased or absent	0 (0)
CYP3A5	*1/*1	Normal	8 (6.3)
	*1/*3	Normal	35 (27.8)
	*3/*3	Decreased or absent	83 (65.9)
ABCB1	CC	Normal	56 (44.4)
	CT	Decreased	55 (43.7)
	TT	Decreased or absent	15 (11.9)

Values are n (%).
ABCB1 = P-glycoprotein gene; other abbreviation as in Table 1.

and did not significantly deviate from Hardy-Weinberg equilibrium (Table 2). As expected (24), carriage of the *CYP2C19* variant allele (**2* or **3*) was relatively high, 63.5% of total (n = 80): 60 carriers with 1 variant allele (47.6%) and 20 carriers with 2 variant alleles (15.9%). There were no differences in platelet measures between carriers of the *CYP2C19*2* versus **3* variant allele (data not shown). The frequency of RM was sufficient to perform the analyses for the *CYP3A5*3* and *ABCB1* (Table 2).

In terms of the *CYP2C19* mutant allele, baseline characteristics were well matched between the groups except for a higher prevalence of ballooning only in 2-variant carriers (Table 1). Baseline characteristics did not differ between normal metabolizers versus RMs of the *CYP3A5*3* and *ABCB1* c. 3435C>T polymorphisms (data not shown).

ADP-induced PR. According to predicted function determined by gene polymorphisms, ADP-induced PR on a high-MD clopidogrel is demonstrated in Table 3. Reduced metabolizers of the *CYP3A5*3* and *ABCB1* c. 3435C>T genotypes did not show increased ADP-induced PR.

The PR_{max} values in carriers of the *CYP2C19* mutant allele were significantly higher than the values in noncarriers (Table 3). PR_{max} with 5 μmol/l ADP stimulus was consistently increased to 30.3 ± 12.6%, 38.4 ± 14.3%, and 47.8 ± 21.9% in patients with 0, 1, and 2 *CYP2C19* variant allele(s) ($p < 0.001$), whereas it was 40.5 ± 15.8%, 51.3 ± 15.3%, and 62.8 ± 16.3% with 20 μmol/l ADP stimulus, respectively ($p < 0.001$) (Fig. 3).

The PR_{late} values were also significantly greater in carriers of the *CYP2C19* mutant allele than in noncarriers (Table 3). PR_{late} after the addition of 5 μmol/l ADP was 17.5 ± 12.6% in noncarriers, 26.1 ± 16.0% in carriers of 1 *CYP2C19* mutant allele, and 37.2 ± 26.6% in carriers of 2 *CYP2C19* mutant alleles ($p < 0.001$), and 24.3 ± 17.7%, 38.8 ± 20.0%, and 51.8 ± 25.0% after the addition of 20 μmol/l ADP, respectively ($p < 0.001$) (Fig. 4).

The VerifyNow $P2Y_{12}$ assay. Compared with normal metabolizers on a high-MD clopidogrel, RMs of the *CYP3A5*3* and *ABCB1* genotypes did not differ in the results of the VerifyNow $P2Y_{12}$ assay. Subjects without the *CYP2C19* mutant allele presented lower PRU and

Table 3. Platelet Reactivity and HPPR According to Gene Polymorphisms

	CYP2C19		*CYP3A5*		*ABCB1*	
	***1/*1** (n = 46)	**RMs*** (n = 80)	***1/*1+ *1/*3** (n = 43)	***3/*3** (n = 83)	**CC** (n = 56)	**CT+TT** (n = 70)
Light transmittance aggregometry						
5 μmol/l ADP—PR_{max} (%)	30.3 ± 12.6	40.7 ± 16.8	35.3 ± 15.1	37.8 ± 16.8	38.4 ± 16.0	35.8 ± 16.4
p value	<0.001		0.424		0.382	
5 μmol/l ADP—PR_{late} (%)	17.5 ± 12.6	28.8 ± 19.6	21.7 ± 16.5	26.3 ± 18.9	25.7 ± 17.9	23.9 ± 18.5
p value	0.001		0.183		0.597	
20 μmol/l ADP—PR_{max} (%)	40.5 ± 15.8	54.2 ± 16.2	48.2 ± 17.5	49.6 ± 17.3	51.3 ± 17.8	47.4 ± 16.9
p value	<0.001		0.670		0.211	
20 μmol/l ADP—PR_{late} (%)	24.3 ± 17.7	42.0 ± 21.7	33.1 ± 21.8	36.8 ± 22.1	37.8 ± 22.2	33.8 ± 21.9
p value	<0.001		0.370		0.313	
VerifyNow $P2Y_{12}$ assay						
$P2Y_{12}$ reaction unit	149.3 ± 74.5	197.7 ± 78.1	184.0 ± 80.0	178.0 ± 80.3	187.2 ± 87.2	174.3 ± 73.9
p value	0.001		0.693		0.370	
% inhibition	55.7 ± 20.4	37.6 ± 22.4	45.2 ± 21.4	43.7 ± 24.4	42.1 ± 23.7	45.9 ± 23.1
p value	<0.001		0.734		0.364	
HPPR,† n (%)	4 (8.7)	23 (28.8)	10 (23.3)	17 (20.5)	13 (23.2)	14 (20.0)
p value	0.012		0.819		0.669	

*RMs indicate reduced metabolizers (*CYP2C19 1/2, 1/3, 2/2, 2/3* and *3/3*). †HPPR indicates high post-treatment platelet reactivity (5 μmol/l ADP-induced maximal platelet reactivity > 50%).
ADP = adenosine diphosphate; PR_{late} = late platelet reactivity at 5 min; PR_{max} = maximal platelet reactivity; other abbreviations as in Tables 1 and 2.

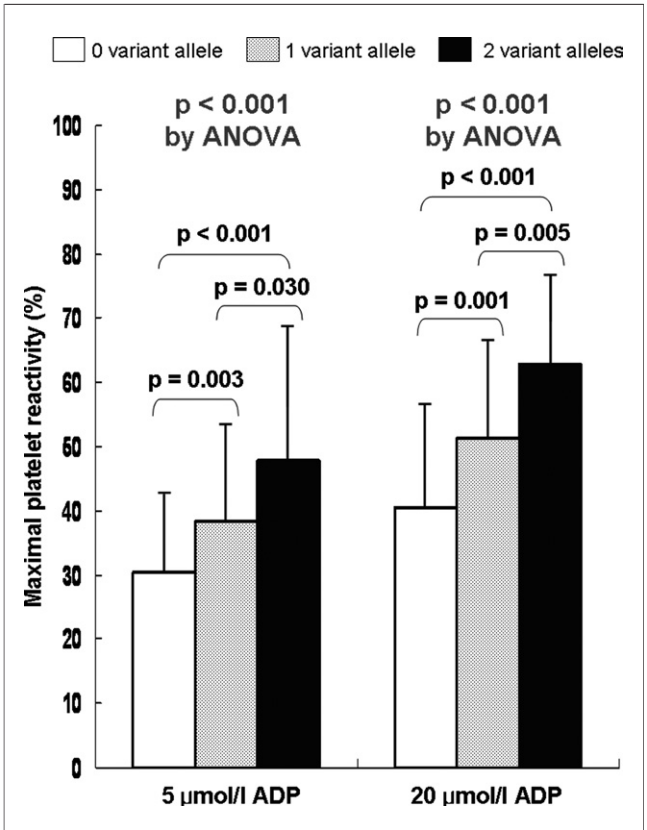

Figure 3. Maximal Platelet Reactivity After High Maintenance-Dose Clopidogrel

Maximal platelet reactivity after high maintenance-dose clopidogrel. Numbers indicate numbers of the *CYP2C19* variant alleles present. **Bars** indicate standard deviations. ADP = adenosine diphosphate; ANOVA = analysis of variance.

higher % inhibition as compared with RMs (Table 3). PRU consecutively increased according to the number of the *CYP2C19* variant alleles (149.3 ± 74.5 in no-variant carriage vs. 182.5 ± 72.9 in 1-variant carriage vs. 243.4 ± 76.9 in 2-variant carriage, $p < 0.001$) (Fig. 5A). The % inhibition was reduced 55.7 ± 20.4%, 42.7 ± 20.3%, and 22.0 ± 21.8% in subjects with 0, 1, and 2 *CYP2C19* mutant allele(s) ($p < 0.001$) (Fig. 5B).

Rate and predictors of HPPR. Twenty-seven (21.4%) patients among all cohorts receiving a high-MD clopidogrel met the criteria of HPPR. The *CYP3A5*3* and *ABCB1* c.3435C>T polymorphisms were not correlated with the increased risk of HPPR (Table 3). Carriers of the *CYP2C19* mutant allele showed a greater risk of HPPR than did noncarriers (28.8% vs. 8.7%, $p = 0.012$). According to carriage of the *CYP2C19* variant, prevalence of HPPR was 8.7% in noncarriers, 21.7% in carriers of 1 variant, and 50.0% in carriers of 2 variants ($p < 0.001$) (Fig. 6).

To determine predictors of HPPR, a logistic regression analysis was performed to evaluate the impact of sex, age, body mass index (BMI), gene polymorphisms, acute coronary syndrome at index PCI, current smoking, hypertension, diabetes mellitus, chronic kidney disease (CKD), left ventricular ejection fraction <45%, and use of the *CYP3A4*-metabolized statin, beta-blocker, angiotensin antagonists, nitrate, calcium channel blocker, and proton pump inhibitor (Table 4). Carriage of the *CYP2C19* variant allele (OR: 5.525, 95% CI: 1.333 to 23.256, $p = 0.018$) and diabetes mellitus (OR: 4.539, 95% CI: 1.030 to 20.012, $p = 0.046$) were significant predictors of HPPR. In addition, carriers of 2 *CYP2C19* variant alleles showed a more significant association with the risk of HPPR than did noncarriers (OR: 6.428, 95% CI: 2.271 to 18.190, $p < 0.001$).

Discussion

This ACCEL-DOUBLE study demonstrates that despite use of a high-MD clopidogrel in high-risk patients treated with PCI, carriers of the *CYP2C19* variant allele show higher platelet measures and an increased risk of HPPR than do noncarriers. Furthermore, this study identifies that the number of the *CYP2C19* variant allele carriage proportionally increases the risk of HPPR. These

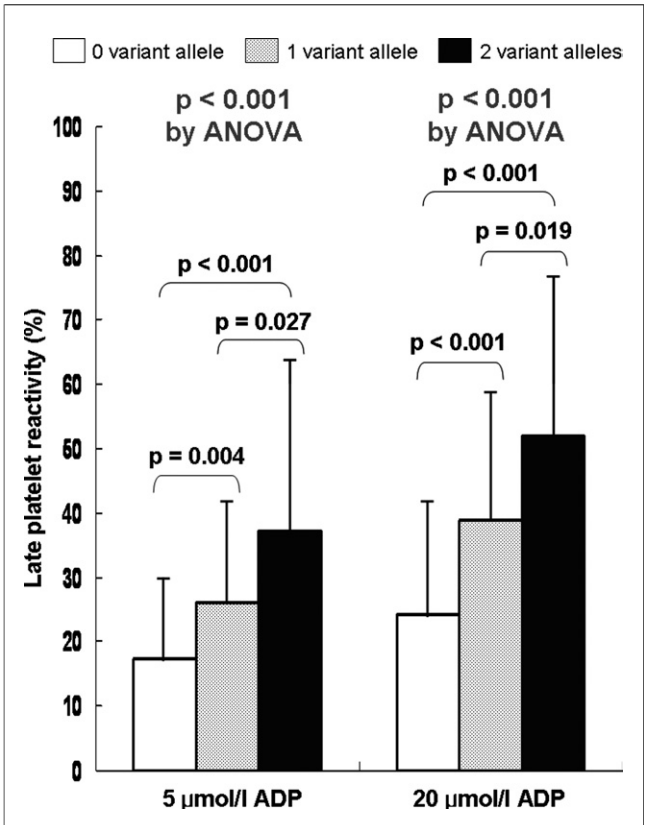

Figure 4. Late Platelet Reactivity After High Maintenance-Dose Clopidogrel

Late platelet reactivity after high maintenance-dose clopidogrel. Numbers indicate numbers of the *CYP2C19* variant alleles present. **Bars** indicate standard deviations. Abbreviations as in Figure 3.

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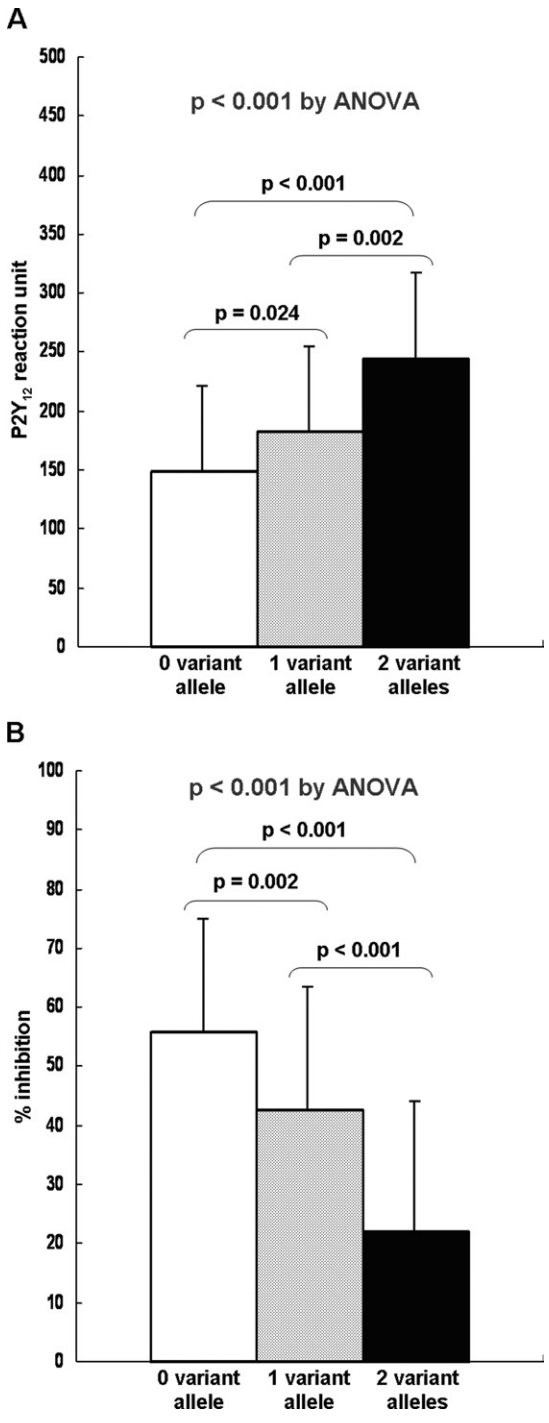

Figure 5. $P2Y_{12}$ Reaction Unit and % Inhibition After High Maintenance-Dose Clopidogrel

$P2Y_{12}$ reaction unit **(A)** and percentage of inhibition **(B)** after high maintenance-dose clopidogrel. Numbers indicate numbers of the *CYP2C19* variant alleles present. **Bars** indicate standard deviations. Abbreviation as in Figure 3.

results suggest that carriage of the *CYP2C19* polymorphism is associated with the risk of HPPR in PCI-treated subjects on a high-MD clopidogrel.

After the prodrug clopidogrel is orally administered, conversion to the active metabolites requires absorption, distribution, and metabolism. Response to clopidogrel is related with intestinal absorption, the *CYP* enzymatic activity (due to interacting drugs and genetic variants), $P2Y_{12}$ receptor density, and other factors, which account for the highly variable prevalence of inadequate response to clopidogrel (31). Because recent large trials have demonstrated that the *CYP2C19* loss-of-function allele is an exceedingly important risk factor to predict enhanced on-treatment PR and adverse clinical outcomes (16,17,32), effective antiplatelet therapy to override the impact of the *CYP2C19* mutant allele must be evaluated.

Although a high-MD clopidogrel achieves a greater platelet inhibition than a standard-MD clopidogrel of 75 mg/day (15,33), the efficacy of a high-MD clopidogrel in high-risk patients remains questionable. In the OPTIMUS (Optimizing Antiplatelet Therapy in Diabetes Mellitus) study, high-MD clopidogrel, when compared with standard-MD clopidogrel, enhanced antiplatelet effects in

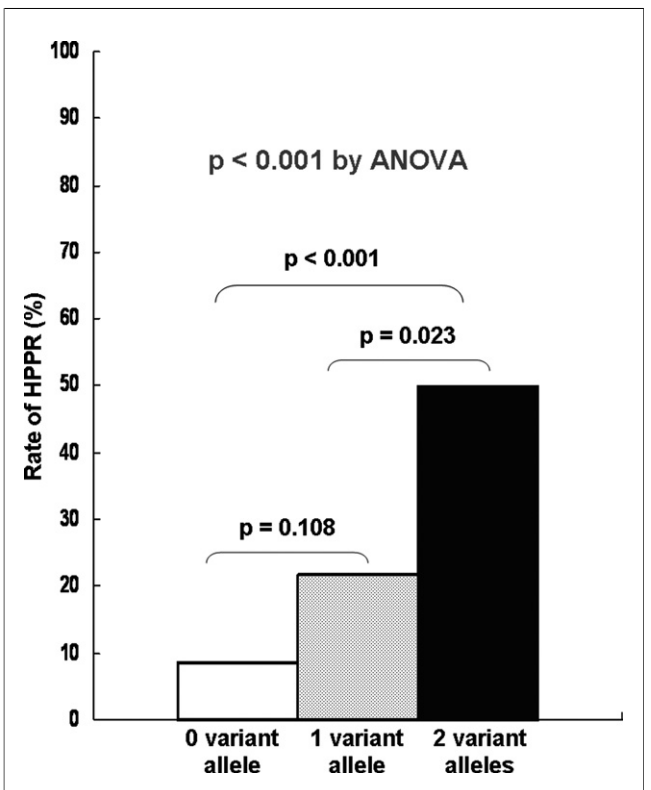

Figure 6. Rate of HPPR After High Maintenance-Dose Clopidogrel

Rate of HPPR after high maintenance-dose clopidogrel. Numbers indicate numbers of the *CYP2C19* variant alleles present. HPPR = high post-treatment platelet reactivity (5 μmol/l ADP-induced maximal platelet reactivity >50%); other abbreviation as in Figure 3.

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

	Univariate Analysis		Multivariate Analysis	
Variables	OR (95% CI)	p Value	OR (95% CI)	p Value
Carriage of *CYP2C19* variant	4.237 (1.362–13.158)	0.013	5.525 (1.333–23.256)	0.018
*CYP3A5**3/*3* carriers	0.850 (0.351–2.061)	0.719	0.609 (0.575–4.914)	0.369
Carriage of *ABCB1* variant	0.827 (0.352–1.941)	0.662	0.691 (0.243–1.961)	0.487
Female sex	1.931 (0.806–4.627)	0.140	2.370 (0.707–7.937)	0.162
Age (per 10-yr increment)	1.267 (0.847–1.901)	0.248	1.070 (0.599–1.912)	0.819
Body mass index (per 1 increment)	1.014 (0.874–1.176)	0.853	1.075 (0.887–1.304)	0.457
Acute coronary syndrome at index PCI	2.040 (0.850–4.896)	0.111	2.828 (0.589–13.579)	0.194
Current smoking	0.523 (0.181–1.511)	0.231	0.691 (0.113–4.237)	0.690
Hypertension	1.070 (0.456–2.508)	0.877	1.073 (0.346–3.333)	0.902
Diabetes mellitus	3.155 (0.879–11.318)	0.078	4.539 (1.030–20.012)	0.046
Chronic kidney disease	1.230 (0.123–12.346)	0.860	1.410 (0.102–19.498)	0.798
LV ejection fraction <45%	2.410 (0.518–11.200)	0.262	2.598 (0.5442–15.275)	0.291
CYP3A4-metabolized statin	1.268 (0.509–3.156)	0.610	1.624 (0.505–5.223)	0.416
Beta-blocker	3.571 (0.784–16.393)	0.100	4.608 (0.780–27.027)	0.092
Angiotensin blocker	0.800 (0.201–3.185)	0.752	0.807 (0.145–4.498)	0.806
Calcium-channel blocker	1.156 (0.434–3.077)	0.771	1.785 (0.456–6.996)	0.405
Nitrate	0.690 (0.252–1.885)	0.469	0.528 (0.166–1.676)	0.279
Proton pump inhibitor	—	1.000	—	1.000

OR = odds ratio; PCI = percutaneous coronary intervention; other abbreviations as in Tables 1 to 3.

type 2 diabetic patients, but a suboptimal response to clopidogrel (20 μmol/l ADP-induced PR_{max} >50%) persisted in 60% of patients on the 150-mg regimen. Because a high-MD clopidogrel also may inhibit ADP-stimulated platelet aggregation depending on the *CYP2C19* genotype, there was a need to determine if carriers of the *CYP2C19* variant may benefit from increased dose of clopidogrel (32). It is noteworthy that in a small-size study (n = 6), increasing the dose of clopidogrel could not override the effect of the *CYP2C19* variant in patients with stent thrombosis (18). Furthermore, in a recent study including Korean patients, Park et al. (34) demonstrated that antiplatelet response to clopidogrel decreased more in CKD patients than in controls, and a double dose of clopidogrel in CKD patients could not sufficiently inhibit increased PR. Although this result may reflect platelet dysfunction in CKD patients, it also may imply the limited benefit of high-MD clopidogrel in patients with high prevalence of the *CYP2C19* variant (about 60% in East Asians) (24). In studies of the PREDICT (Residual Platelet Aggregation After Deployment of Intracoronary Stent) score (29) and Bonello-Palot et al. (35), prediction of HPPR after a 600-mg loading dose of clopidogrel was significantly influenced by the *CYP2C19* polymorphism and clinical risk factors (high BMI, ACS, age >65 years, diabetes mellitus, CKD, and reduced left ventricular function). Thus, CKD patients with the *CYP2C19* variant may not achieve adequate platelet inhibition despite use of high-MD clopidogrel. Moreover, the present study provides laboratory evidence that high-risk carriers of the *CYP2C19* variant still have a risk for suboptimal platelet inhibition despite use of a high-MD clopidogrel (21.7% in heterozygotes and 50.0% in homozygotes). These results provide a rationale for further studies to assess whether high-MD clopidogrel, as compared with noncarriers of the *CYP2C19* variant, provides long-term clinical benefits in carriers.

Subjects with the *CYP2C19* polymorphism may benefit more from antiplatelet regimens that do not include clopidogrel, such as use of third-generation thienopyridines (14,15,32), or the addition of a third antiplatelet agent, such as cilostazol (19,20). Third-generation thienopyridines predominantly do not use the *CYP2C19* pathway for activation, and therefore can inhibit platelet aggregation sufficiently regardless of *CYP2C19* polymorphism. Cilostazol is mainly converted into active metabolites by the *CYP3A* system (36), which implies less of an impact of the *CYP2C19* mutant allele on the additive platelet inhibition by cilostazol.

Because the *CYP2C19* genotype may be useful to determine antiplatelet regimen and dose (32), this concept may be more important among East Asians with a higher prevalence of the *CYP2C19* variant than in Caucasians. However, results of the CHARISMA (Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance) substudy (37) undermined this reasoning. Compared with other ethnic groups, Asian cohorts showed the lowest incidence of cardiovascular mortality and a higher risk of moderate bleeding (37). The risk associated the *CYP2C19* variant can differ according to

genetic background or environmental exposures (32). Because East Asians show a lower response to clopidogrel with lesser rates of clinical events than Caucasians do, the cutoff points of HPPR suggested by platelet function assays can differ from those based on the Caucasian population. Therefore, the cutoff of HPPR and the effect of intensified antiplatelet regimens according to ethnicity must be evaluated to balance the efficacy and safety of intensified antiplatelet therapy. In addition, we should not neglect the complex interaction between specific environmental factors and gene expression in the context of the thrombotic process. Although some medications and environmental factors cause decreased response to clopidogrel in terms of gene expression, these factors also can differentially affect gene expression related to atherothrombosis. Consequently, intricate interactions of environmental factors and gene expression may make it difficult to identify direct cause-effect networks.

Study limitations. First, the present study is an observational study including a heterogeneous set of subjects with a small sample size. Sample size and frequency of HPPR are insufficient to yield solid predictors in multivariate analysis, as indicated by the wide CIs. Second, the present study showed laboratory data only without clinical outcomes. Third, the impact of the *CYP2C19* genotype on clopidogrel response can change with time. The present study may not validate the optimal duration of intensified antiplatelet therapy in high-risk patients. Fourth, proton pump inhibitors can affect the enzymatic activity of the *CYP2C19* pathway, especially in carriers of the *CYP2C19* variant. However, because few patients were on proton pump inhibitors due to a physician's preference in the present study, we could not ascertain this hypothesis appropriately. Fifth, Bonello-Palot et al. (35) showed that high BMI could be related with HPPR after a 600-mg loading dose of clopidogrel. Because our patients' mean BMI is relatively lower than that in Bonello-Palot et al. (35) (24.8 ± 2.7 kg/m^2 vs. 27.1 ± 4.1 kg/m^2), the impact of BMI on HPPR can be underestimated in the present study. Finally, we performed DNA genotyping for some chosen gene polymorphisms, and we could not absolutely exclude the impact of other gene polymorphisms.

Conclusions

Among PCI-treated patients receiving a high-MD clopidogrel of 150 mg/day, carriage of the *CYP2C19* variant allele is related to increased PR and predicts risk of HPPR. It remains to be evaluated whether carriers of the *CYP2C19* variant allele treated with a high-MD clopidogrel may show poorer cardiovascular outcomes than noncarriers would.

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Key Words: platelet ■ high post-treatment platelet reactivity ■ *CYP2C19* polymorphism ■ high maintenance-dose clopidogrel.