Cytochrome 2C19 Polymorphism and Response to Adjunctive Cilostazol Versus High Maintenance-Dose Clopidogrel in Patients Undergoing Percutaneous Coronary Intervention

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Background—Among patients treated with clopidogrel, carriers of the cytochrome P450 (*CYP*) *2C19* loss-of-function allele have shown increased platelet reactivity and higher rates of ischemic events. Although adjunctive cilostazol to dual antiplatelet therapy (or "triple antiplatelet therapy") intensifies platelet inhibition, it remains unknown whether triple antiplatelet therapy after percutaneous coronary intervention can achieve adequate platelet inhibition in patients with the *CYP2C19* mutant allele.

Methods and Results—*CYP2C19* genotyping for **1*, **2*, and **3* was performed in 134 high-risk patients undergoing elective percutaneous coronary intervention. After measurement of preprocedural platelet reactivity, patients were randomly assigned to receive either adjunctive cilostazol 100 mg twice daily (triple group; n=69) or high maintenance-dose (MD) clopidogrel of 150 mg daily (high-MD group; n=65). Using light transmittance aggregometry and the VerifyNow $P2Y_{12}$ assay, platelet reactivity was assessed before the index procedure and at 30-day follow-up. The primary end point was absolute change in maximal platelet aggregation (ΔAgg_{max}) according to *CYP2C19* genotyping. High posttreatment platelet reactivity was defined as 5 μmol/L ADP–induced maximal platelet aggregation >50%. In noncarriers of the *CYP2C19*2/*3* mutant allele, ΔAgg_{max} values after 5 and 20 μmol/L ADP stimuli did not differ significantly between the triple (n=22) versus the high-MD group (n=22) (23.6±21.6% versus 16.6±15.4%, P=0.224 and 26.4±22.2% versus 18.6±14.9%, P=0.174, respectively). Absolute changes in late platelet aggregation and $P2Y_{12}$ reaction unit were not different between the groups. The rate of high posttreatment platelet reactivity at 30-day follow-up also was comparable between the triple versus the high-MD group (4.5% versus 13.6%, P=0.607). In carriers of at least 1 CYP2C19**2/*3* mutant allele, the triple group (n=47) showed greater values of ΔAgg_{max} after addition of 5 μmol/L (25.8±16.8% versus 11.1±19.8%, P<0.001) and 20 μmol/L ADP (26.3±16.0% versus 11.5±16.3%, P<0.001) compared with the high-MD group (n=43). Likewise, absolute changes in late platelet aggregation and $P2Y_{12}$ reaction unit were consistently greater in the triple versus the high-MD group. Fewer patients in the triple group met the criteria of high posttreatment platelet reactivity at 30-day follow-up compared with the high-MD group (6.4% versus 37.2%, P<0.001).

Conclusions—Among high-risk patients undergoing elective percutaneous coronary intervention, adjunctive cilostazol can achieve consistently intensified platelet inhibition and reduce the risk of high posttreatment platelet reactivity irrespective of *CYP2C19* genotyping.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01012193.

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Key Words: *CYP2C19* protein ■ platelet aggregation ■ cilostazol ■ dose clopidogrel ■ polymorphism

In patients undergoing percutaneous coronary intervention (PCI), antiplatelet treatment with aspirin and clopidogrel has reduced the risk of adverse cardiovascular events with a favorable safety profile, which has made this combination the treatment of choice in clinical practice.[1–3] However, recent data have shown that there is a wide interindividual variabil-

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ity in antiplatelet response to clopidogrel,[4–6] and increased platelet reactivity despite clopidogrel is associated with a higher risk of ischemic events including stent thrombosis.[6–9] Furthermore, patients with suboptimal response to clopidogrel also have a higher rate of aspirin resistance, which may increase the risk of thrombotic complications.[10,11] Although various factors can affect response to clopidogrel, polymorphisms of alleles related with absorption and metabolism can decrease the thiol metabolite level of clopidogrel, which may lead to low inhibition of ADP-induced platelet reactivity.[12,13] Of those, the hepatic cytochrome P450 (*CYP*) mutant alleles, especially the dominant *CYP2C19* isoenzyme, have shown an association with increased platelet reactivity and consequent risk of major adverse cardiovascular events.[14–17]

Clinical Perspective on p 459

The Adjunctive Cilostazol versus High Maintenance-dose (MD) Clopidogrel (ACCEL) trials have demonstrated that adjunctive cilostazol can intensify platelet inhibition and reduce the rate of high posttreatment platelet reactivity (HPPR) in high-risk patients compared with high-MD clopidogrel of 150 mg daily.[18,19] Cilostazol is a unique antiplatelet agent that acts by selective dual inhibition of phosphodiesterase type 3 (PDE3) and adenosine uptake in various cardiovascular systems.[20] Unlike other antiplatelet agents, cilostazol not only inhibits platelet aggregation[20] but also has favorable pleiotropic effects on neointimal hyperplasia after PCI[21] and the diverse processes of atherosclerosis.[22–24] Furthermore, cilostazol is mainly converted into the active metabolites by the *CYP3A* system,[25] which might imply less impact of the *CYP2C19* mutant allele for additive platelet inhibition with cilostazol.

Although there are numerous studies providing evidences for the beneficial role of cilostazol, it remains unknown whether adjunctive cilostazol to dual antiplatelet therapy (or "triple antiplatelet therapy") in PCI-treated patients can achieve adequate platelet inhibition irrespective of the *CYP2C19* loss-of-function allele carriage. If adjunctive cilostazol shows potency to surmount the risk of clopidogrel resistance in carriers of the *CYP2C19* variant, triple antiplatelet therapy could be an optimal antithrombotic regimen. We thus performed the present study to assess the degree of intensified platelet inhibition by adjunctive cilostazol according to *CYP2C19* genotyping in high-risk patients treated with PCI.

Methods

Patient Population

Between January 2008 and June 2009, patients were recruited at the Department of Cardiology of the Gyeongsang National University Hospital (Jinju, Korea). In the setting of elective PCI, 150 patients were prospectively enrolled in the ACCEL trials,[18] which were performed to compare the degree of platelet inhibition by adjunctive cilostazol with dual antiplatelet therapy versus high-MD clopidogrel in high-risk patients such as HPPR, diabetes, and drug-eluting stent implantation for complex lesions.[18] For the prespecified analysis, 134 patients (89.3%) of the cohort with available deoxyribonucleic acid (DNA) genotyping were recruited, which constituted the study subjects. Compared with patients enrolled in this study, 16 patients who could not undergo DNA genotyping (10.7%) did not show any difference in terms of baseline characteristics ($P>0.05$).

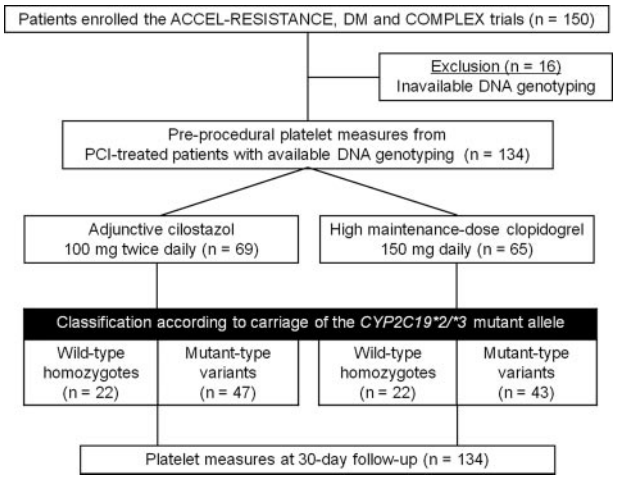

Figure 1. Flow diagram of the ACCEL-POLYMORPHISM study. PCI indicates percutaneous coronary intervention; and CYP, the hepatic cytochrome P450.

Patients were eligible for enrollment if they were ≥18 years of age and identified to have the enrolled criteria (HPPR, diabetes, or drug-eluting stent for complex lesions). Exclusion criteria were (1) acute myocardial infarction or hemodynamic instability; (2) active bleeding and bleeding diatheses; (3) warfarin therapy; (4) use of periprocedural glycoprotein IIb/IIIa inhibitors; (5) contraindication to antiplatelet therapy; (6) left ventricular ejection fraction <30%; (7) leukocyte count <3000/mm^3 and/or platelet count <100 000/mm^3; (8) aspartate aminotransferase or alanine aminotransferase level ≥3 times the upper normal limit; (9) serum creatinine level ≥2.5 mg/dL; (10) stroke within 3 months; (11) noncardiac disease with a life expectancy <1 year; and (12) inability to follow the protocol. The institutional review board approved the study protocol, and the patients provided written informed consent for participation. The present study protocol complies with the Declaration of Helsinki and was approved by the Institutional Ethics Committee of the Gyeongsang National University Hospital. All of the patients provided written informed consent for the intervention, platelet function assays, and DNA genotyping.

Study Design

This ACCEL-POLYMORPHISM (Adjunctive Cilostazol versus High-MD Clopidogrel According to the *CYP2C19* Polymorphism) study is an analysis of the subjects drawn from the prospective, randomized, parallel-group platelet function trials. The flow diagram of the study is illustrated in Figure 1. All patients received a 300-mg loading dose of clopidogrel at least 12 hours before PCI (n=98) or were receiving chronic clopidogrel therapy (75 mg daily for ≥7 days, n=36). All patients received a 300-mg loading dose of aspirin, followed by 200 mg daily for 1 month. Whole blood for preprocedural platelet measures was obtained immediately after insertion of the arterial sheath in the catheterization room. Diagnostic and interventional procedures were performed according to standard techniques. In patients with multiple lesions, the first treated lesion was considered as the target lesion.

If subjects met the inclusion criteria, they were randomly assigned to adjunctive cilostazol (triple group) or high-MD clopidogrel (high-MD group) using a computer-generated randomization table. The triple group (n=69) received adjunctive cilostazol 100 mg twice daily to clopidogrel 75 mg daily for 30 days. The high-MD group (n=65) received clopidogrel 150 mg daily for 30 days. At the 30-day follow-up visit, patient compliance to antiplatelet therapy was assessed by interview and pill counting. Blood samples also were obtained 2 to 4 hours after the last drug ingestion. Peripheral venous

blood samples were drawn through a venous catheter inserted into a forearm vein.

CYP2C19 Genotyping

Base numbering and allele definitions followed the nomenclature of the Human *CYP* Allele Nomenclature Committee.[26] Genomic DNA was extracted from leukocytes of whole-blood specimens with an extraction 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,[27,28] genotyping for *CYP2C19*2* (rs4244285, c.681G>A) and *CYP2C19*3* (rs4986893, c.636G>A) were conducted using single base primer extension assay using SNaPshot assay kit (Applied Biosystems, Foster City, Calif).[29] Briefly, the genomic DNA region containing 1 of the 2 single nucleotide polymorphism (SNP) was amplified with polymerase chain reaction (PCR) separately. PCR was carried out by using the same primers as previously described. The PCR product was processed as per the ABI SNaPshot protocol, using primers designed for fluorescent dideoxy nucleotide termination. SNP analysis was carried out on the ABI 3100 genetic analyzer.

Platelet Function Assays

Blood samples were collected using the double-syringe technique, in which the first 2 to 4 mL of blood was discarded to avoid spontaneous platelet activation. Platelet reactivity was simultaneously measured by light transmittance aggregometry and the VerifyNow $P2Y_{12}$ assay (Accumetrics Inc, San Diego, Calif). Correlation between 2 methods at our laboratory has been previously reported.[30]

Light transmittance aggregometry was performed according to standard protocol, as described in detail.[18,19] Blood samplings were drawn into Vacutainer tubes containing 0.5 mL of 3.2% sodium citrate (Becton-Dickinson, San Jose, Calif), and the samples were processed within 60 minutes. Platelet-rich plasma (PRP) was obtained as a supernatant fluid after centrifuging the blood at 120*g* for 10 minutes. The remaining blood was further centrifuged at 1200*g* for 10 minutes to prepare platelet-poor plasma (PPP). PRP was adjusted to platelet counts of 250 000/mm^3 by adding PPP as needed. Platelet aggregation was assessed at 37°C using an AggRAM aggregometer (Helena Laboratories Corp, Beaumont, Tex). Tests were performed after the addition of 5 and 20 μmol/L ADP, and the curves were recorded for 10 minutes. Platelet reactivity was measured at the peak (maximal platelet aggregation; Agg_{max}) and at 5 minutes (late platelet aggregation; Agg_{late}) by laboratory personnel blinded to the study protocol. Absolute changes in platelet aggregation (ΔAgg_{max} and ΔAgg_{late}) were defined as changes of values between preprocedural and 30-day follow-up time points: ΔAgg=(preprocedural platelet aggregation−platelet aggregation at 30-day follow-up).

The VerifyNow $P2Y_{12}$ assay is a whole-blood, point-of-care system.[8] Blood was drawn into a Greiner Bio-One 3.2% citrate Vacuette tube (Greiner Bio-One, Kremsmünster, Austria). The assay consists of 2 whole-blood channels containing fibrinogen-coated polystyrene beads. One contains 20 μmol/L ADP and 22 nmol/L PGE_1 to reduce the nonspecific contribution of other pathways. The other channel contains iso-thrombin receptor activating protein (iso-TRAP), and a baseline value (BASE) is obtained. The results are reported in $P2Y_{12}$ reaction unit (PRU), BASE and % inhibition. The percent inhibition is calculated as: ([BASE-PRU]/BASE)×100. Absolute changes in PRU (ΔPRU) were defined as changes of PRU values between preprocedural and 30-day follow-up time points. ΔPRU=(preprocedural PRU−PRU at 30-day follow-up).

End Points and Definitions

The primary end point was ΔAgg_{max} according to *CYP2C19* genotyping. Secondary end points were (1) ΔAgg_{late}; (2) ΔPRU; and (3) the rate of HPPR at 30-day follow-up according to *CYP2C19* genotyping. In addition, we assessed the composite of death, acute myocardial infarction, urgent target vessel revascularization, or stent thrombosis during 30-day follow-up. Bleeding was defined according to the criteria used in the Thrombolysis in Myocardial Infarction trials. HPPR was defined as 5 μmol/L ADP–induced Agg_{max} >50% of light transmission.[18,19,31] Because a 5 μmol/L ADP–induced Agg_{max} >50% was similar to a PRU value ≥235 based on previously reported data,[30] this threshold of HPPR might be an acceptable level of suboptimal response. Patients without the *CYP2C19*2/*3* mutant allele were categorized as wild-type homozygotes (wt/wt). Mutant-type variants consisted of carriers with 1 (wt/*2 or wt/*3) and 2 *CYP2C19* mutant allele (*2/*2, *3/*3 or *2/*3).[29]

Sample Size Calculation and Statistical Analysis

The sample size calculation was based on the observed 5 μmol/L ADP–induced ΔAgg_{max} (31.8) by adjunctive cilostazol and on the observed 5-μmol/L ADP-induced ΔAgg_{max} (17.2) by high-MD clopidogrel.[18] Based on previous studies,[29] prevalence of wild-type homozygotes was considered as approximately 40% in the East Asian population. To detect such a 45.9% relative difference in 5 μmol/L ADP–induced ΔAgg_{max} with a power of 95% and a 2-sided α value of 0.05, we calculated that we need to include at least 16 noncarriers of the *CYP2C19*2/*3* mutant allele. Thus, the needed study population was estimated as a total of 80 patients including 16 noncarriers and 24 carriers of the *CYP2C19* variant allele for each treatment group. Continuous variables are expressed as mean±SD, and their differences were tested using the Student unpaired *t*, Wilcoxon signed rank, or Mann-Whitney *U* tests. Categorical variables are expressed as frequencies and percentages, and χ^2 statistics or Fisher exact test was used for their comparisons (if an expected frequency was <5). We calculated Hardy-Weinberg equilibrium proportions using the Pearson goodness-of-fit χ^2 statistics to test a possible deviation of the *CYP2C19* genotype distribution. To adjust potential confounding variables in comparison of the end points, logistic regression analysis was performed including age, sex, body mass index, diabetes mellitus, hypertension, hypercholesterolemia, current smoking, chronic kidney disease, previous myocardial infarction, calcium channel blocker, left ventricular ejection fraction ≥45%, multivessel disease, American College Cardiology/American Heart Association (ACC/AHA) lesion type B2/C, and total stent length. Statistical analyses were performed using SPSS version 13 (SPSS Inc, Chicago, Ill) and conducted at the 0.05 significance level.

Results

Patient Characteristics and Follow-Up

Because we recruited the study subjects from the cohort of the ACCEL trials, platelet function measurements at 30-day follow-up was available for all enrolled subjects (Figure 1). Although there were 4 cases of transient headache and 2 cases of palpitation in the triple group in the early phase of treatment, all regimens were generally well tolerated and no patients discontinued the study regimen during 30 days. Genetic distributions of the *CYP2C19* polymorphisms were similar to the published East Asian frequencies,[26] and did not significantly deviate from Hardy-Weinberg equilibrium (*CYP2C19*2*: P=0.33 and *CYP2C19*3*: P=0.80, respectively). As expected,[27–29] carriage of the *CYP2C19* mutant allele (**2* or **3*) was relatively high, 67.2% of total (n=90) (see the Appendix in the online-only Data Supplemental Table 1): 63 carriers with 1 mutant allele (47.0%: n=31 in the high-MD group and n=32 in the triple group) and 27 carriers with 2 mutant alleles (20.2%: n=12 in the high-MD group and n=15 in the triple group), and 44 wild-type homozygotes (wt/wt) (32.8%: n=22 in the high-MD group and n=22 in the triple group). There were no differences in preprocedural platelet measures between carriers of the *CYP2C19*2* versus **3* variant allele (see the Appendix in the online-only Data

Table 1. Baseline Clinical and Procedural Characteristics of the Study Population

Variables	Wild-Type Homozygote			Mutant-Type Variant		
	High-MD Group (n=22)	Triple Group (n=22)	*P*	High-MD Group (n=43)	Triple Group (n=47)	*P*
Demographic characteristics, n (%)						
Age, y	60.2±7.9	63.4±9.1	0.230	64.3±9.9	63.4±9.6	0.662
Male	14 (63.6)	14 (63.6)	1.000	31 (72.1)	29 (61.7)	0.296
Body mass index, kg/m^2	25.1±3.2	23.6±3.1	0.115	25.5±3.0	24.8±4.2	0.344
Clinical characteristics, n (%)						
Diabetes mellitus	6 (27.3)	5 (22.7)	0.728	11 (25.6)	15 (31.9)	0.508
Hypertension	15 (68.2)	12 (54.5)	0.353	23 (53.5)	26 (55.3)	0.862
Hypercholesterolemia	5 (22.7)	5 (22.7)	1.000	15 (34.9)	8 (17.0)	0.052
Current smoking	11 (50.0)	7 (31.8)	0.220	15 (34.9)	18 (38.3)	0.737
Chronic kidney disease	0 (0)	4 (18.2)	0.108	3 (7.0)	4 (8.5)	1.000
Previous PCI	2 (9.1)	6 (27.3)	0.240	9 (20.9)	12 (25.5)	0.606
Previous myocardial infarction	2 (9.1)	4 (18.2)	0.664	4 (9.3)	8 (17.0)	0.282
Previous bypass surgery	0 (0)	1 (4.5)	1.000	1 (2.3)	1 (2.1)	1.000
Previous stroke	2 (9.1)	1 (4.5)	1.000	2 (4.7)	2 (4.3)	1.000
Clopidogrel use, n (%)			1.000			0.844
300-mg loading	17 (77.3)	17 (77.3)		31 (72.1)	33 (70.2)	
Chronic therapy (≥7 days)	5 (22.7)	5 (22.7)		12 (27.9)	14 (29.8)	
Concomitant medications, n (%)						
Statin	17 (77.3)	21 (95.5)	0.185	37 (86.0)	39 (83.0)	0.688
β-Blocker	15 (68.2)	15 (68.2)	1.000	32 (74.4)	36 (76.6)	0.810
ARB or ACEI	14 (63.6)	16 (72.7)	0.517	26 (60.5)	29 (61.7)	0.904
Nitrate	16 (72.7)	11 (50.0)	0.122	33 (76.7)	31 (66.0)	0.259
Calcium channel blocker	8 (36.4)	5 (22.7)	0.322	17 (39.5)	13 (27.7)	0.233
Proton pump inhibitor	1 (4.5)	1 (4.5)	1.000	0 (0)	2 (4.3)	0.495
Laboratory characteristics						
LV ejection fraction ≤45%	2 (9.1)	4 (18.2)	0.664	3 (7.0)	5 (10.6)	0.716
Hemoglobin, g/dL	13.6±1.1	13.1±1.5	0.234	13.6±1.5	13.3±1.4	0.315
Platelet count, ×10^3/mm^3	282±68	281±80	0.966	254±56	248±60	0.680
Hb A1$_C$, %	6.2±1.3	6.1±1.1	0.794	6.4±1.1	6.4±1.1	0.959
GFR (MDRD, mL/min/1.73 m^2)	106.4±28.4	98.0±32.3	0.364	98.0±31.8	101.4±36.1	0.639
Total cholesterol, mg/dL	172.2±46.6	177.5±43.2	0.702	175.4±42.4	165.9±37.5	0.259
Lesion and procedural characteristics, n (%)						
Target artery			0.504			0.419
Left anterior descending	16 (72.7)	12 (54.5)		24 (55.8)	26 (55.3)	
Left circumflex	3 (13.6)	1 (4.5)		5 (11.6)	10 (21.3)	
Right coronary	3 (13.6)	7 (31.8)		14 (32.6)	10 (21.3)	
Left main	0 (0)	2 (9.1)		0 (0)	1 (2.1)	
Multivessel disease	6 (27.3)	12 (54.5)	0.066	24 (55.8)	23 (48.9)	0.514
ACC/AHA lesion type B2/C	17 (77.3)	20 (90.9)	0.412	33 (76.7)	44 (93.6)	0.034
Intravascular ultrasound guidance	19 (86.4)	20 (90.9)	1.000	32 (74.4)	41 (87.2)	0.121
Usage of drug-eluting stent	22 (100.0)	21 (95.5)	1.000	41 (95.3)	45 (95.7)	1.000
Multivessel intervention	4 (18.2)	8 (36.4)	0.310	16 (37.2)	13 (27.7)	0.333
Stent diameter, mm	3.0±0.3	3.2±0.4	0.279	3.2±0.4	3.1±0.4	0.602
Stents per patient	1.5±0.7	1.9±1.1	0.146	1.5±0.7	1.8±1.1	0.165
Total stent length, mm	30.3±18.4	43.8±27.2	0.061	34.2±19.6	38.1±23.9	0.397

PCI indicates percutaneous coronary intervention; ARB, angiotensin receptor blocker; ACE, angiotensin-converting enzyme; LV, left ventricular; Hb A1$_C$, hemoglobin A1$_C$; GFR, glomerular filtration rate; and ACC/AHA, American College of Cardiology/American Heart Association. Data are presented as mean±SD for continuous variables and absolute numbers (percentages) for dichotomous variables.

Table 2. Platelet Reactivity by Light Transmittance Aggregometry

	Wild-Type Homozygote			Mutant-Type Variant		
Variables	High-MD Group (n=22)	Triple Group (n=22)	*P*	High-MD Group (n=43)	Triple Group (n=47)	*P*
Maximal platelet aggregation						
5 μmol/L ADP						
Preprocedure	47.7±16.4	50.2±15.8	0.600	54.0±15.1	54.2±12.6	0.949
30-day follow-up	31.1±12.9	26.7±15.2	0.304	42.9±18.1	28.4±13.9	<0.001
20 μmol/L ADP						
Preprocedure	60.5±15.1	62.4±13.8	0.661	66.8±11.3	66.8±12.2	0.990
30-day follow-up	41.9±16.4	36.0±19.2	0.276	55.4±15.9	40.5±16.7	<0.001
Late platelet aggregation						
5 μmol/L ADP						
Preprocedure	40.3±20.3	41.0±17.9	0.913	47.1±20.1	45.8±16.8	0.742
30-day follow-up	19.0±13.4	14.9±13.4	0.318	30.7±22.2	16.7±11.5	<0.001
20 μmol/L ADP						
Preprocedure	52.2±21.1	54.2±18.4	0.734	61.0±16.8	60.9±16.0	0.986
30-day follow-up	26.9±18.8	21.2±19.0	0.321	43.2±22.0	26.4±17.4	<0.001

Data are presented as mean±SD.

Supplemental Tables 2 and 3). The triple group consisted of 22 wild-type homozygotes and 47 mutant-type variants (Figure 1). The high-MD group consisted of 22 wild-type homozygotes and 43 mutant-type variants.

Baseline characteristics were well matched between the treatment groups according to *CYP2C19* genotyping, except for a higher rate of ACC/AHA lesion type B2/C in the triple versus high-MD group of mutant-type variants (Table 1). Preprocedural values of platelet measures were similar between the treatment groups according to metabolizer and genotype status (Tables 2 and 3) (see the Appendix in the online-only Data Supplemental Tables 2 and 3). There were no differences in preprocedural platelet reactivity after 300-mg loading and chronic therapy of clopidogrel (see the Appendix in the online-only Data Supplemental Table 4). In addition, adjusting statistically did not change any of the end points, based on platelet function assays. During the follow-up period, no major ischemic and bleeding events were observed in any group.

Primary End Points

In wild-type homozygotes of the *CYP2C19* allele, both treatment groups showed remarkable reductions in Agg_{max} values at 30-day follow-up compared with preprocedural values (all values, $P<0.001$). After any of the tested concentrations of ADP stimuli, ΔAgg_{max} did not differ significantly between the triple versus high-MD group (Figure 2A). ΔAgg_{max} with 5 μmol/L ADP stimuli was 23.6±21.6% in the triple group and 16.6±15.4% in the high-MD group ($P=0.224$), whereas it was 26.4±22.2% and 18.6±14.9% with 20 μmol/L ADP stimuli, respectively ($P=0.174$).

In mutant-type variants of the *CYP2C19* allele, both treatment regimens also reduced significantly Agg_{max} values at 30-day follow-up compared with preprocedural values (all values, $P<0.001$). Triple antiplatelet therapy showed greater values of ΔAgg_{max} in the addition of 5 μmol/L (25.8±16.8% versus 11.1±19.8%, $P<0.001$) and 20 μmol/L ADP (26.3±16.0% versus 11.5±16.3%, $P<0.001$), compared with high-MD clopidogrel (Figure 2B).

Secondary End Points

Compared with preprocedural values of Agg_{late}, Agg_{late} values at 30-day follow-up were also significantly reduced by both regimens irrespective of *CYP2C19* genotyping (all values, $P<0.001$). For wild-type homozygotes of the *CYP2C19* allele, ΔAgg_{late} in the triple group was not significantly higher than that in the high-MD group (Figure 3A). Values of ΔAgg_{late} after 5 μmol/L ADP stimuli were

Table 3. Platelet Reactivity by VerifyNow $P2Y_{12}$ Assay

	Wild-Type Homozygote			Mutant-Type Variant		
Variables	High-MD Group (n=22)	Triple Group (n=22)	*P*	High-MD Group (n=43)	Triple Group (n=47)	*P*
$P2Y_{12}$ reaction unit						
Preprocedure	272.5±75.5	253.8±76.7	0.420	278.3±69.7	296.5±53.2	0.167
30-Day follow-up	149.7±65.4	129.4±76.4	0.348	214.1±68.5	191.6±78.4	0.153
Percent inhibition						
Preprocedure	20.2±18.7	22.4±22.9	0.733	14.0±16.2	11.1±10.8	0.319
30-Day follow-up	65.2±12.8	70.5±7.6	0.317	33.5±19.2	45.8±21.2	0.005

Data are presented as mean±SD.

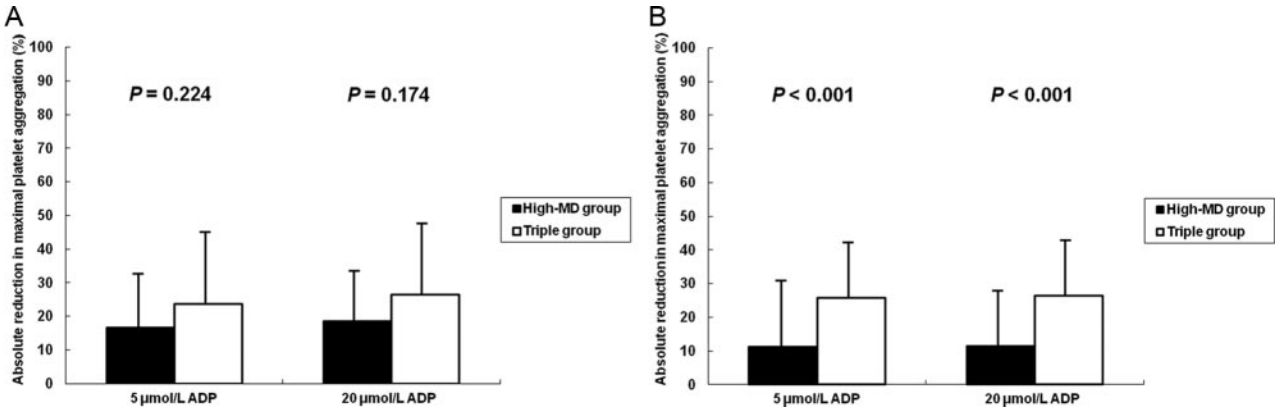

Figure 2. Absolute reductions in maximal platelet aggregation according to wild-type homozygotes (A) and mutant-type variants (B). Results are expressed as mean (boxes)±SD (error bars).

26.1±21.3% in the triple group and 21.4±19.3% in the high-MD group (P=0.445), whereas it was 33.0±24.0% and 25.3±19.6% after 20 μmol/L ADP stimuli, respectively (P=0.246). For mutant-type variants of the *CYP2C19* allele, the triple group showed greater values of ΔAgg_{late} than the high-MD group (Figure 3B): 29.1±18.9% versus 16.4±23.2% after the addition of 5 μmol/L ADP (P=0.005) and 34.5±19.0% versus 17.8±20.9% after the addition of 20 μmol/L ADP (P<0.001).

At 30-day follow-up, both groups presented significantly lower PRU and higher percent inhibition as compared with preprocedural values irrespective of *CYP2C19* genotyping (all values, P<0.001). In wild-type homozygotes of the *CYP2C19* allele, ΔPRU did not significantly differ according to the regimens (124.4±84.6 in the triple group versus 122.7±72.1 in the high-MD group, P=0.945) (Figure 4A). However, triple antiplatelet therapy considerably enhanced ΔPRU more than high-MD clopidogrel in mutant-type variants of the *CYP2C19* allele (104.8±74.6 versus 64.2±75.7, P=0.012) (Figure 4B).

Both regimens could reduce significantly the rate of HPPR at 30-day follow-up compared with preprocedural values irrespective of *CYP2C19* genotyping (all values, P<0.001). Among wild-type homozygotes of the *CYP2C19* allele, no differences between the triple versus high-MD group were seen at preprocedure (63.6% versus 45.5%, P=0.226) and 30-day follow-up (4.5% versus 13.6%, P=0.607) (Figure 5A). Among mutant-type variants of the *CYP2C19* allele, there was no difference for preprocedural rate of HPPR between the triple versus high-MD group (68.1% versus 65.1%, P=0.765) (Figure 5B). However, the triple group demonstrated the lower rate of HPPR at 30-day follow-up as compared with the high-MD group (6.4% versus 37.2%, P<0.001).

Effect of *CYP2C19* Genotyping Within Treatment Groups

In the high-MD group, even though differences did not reach statistical significance, wild-type homozygotes showed a trend toward higher values of 5 and 20 μmol/L ADP–stimulated ΔAgg_{max} than mutant-type variants (16.6±15.4% versus 11.1±19.8%, P=0.263 and 18.6±14.9% versus 11.5±16.3%, P=0.092, respectively). ΔAgg_{late} values after 5 and 20 μmol/L ADP stimuli also showed trends toward higher values in wild-type homozygotes compared with mutant-type variants (21.4±19.3% versus 16.4±23.2%, P=0.292, and 25.3±19.6% versus 17.8±20.9%, P=0.169, respectively). ΔPRU in wild-type homozygotes was significantly greater than that of mutant-type variants (122.7±72.1 versus 64.2±75.7, P=0.004). Wild-type homozygotes showed the lower rate of HPPR at 30-day follow-up compared with mutant-type variants (13.6% versus 37.2%, P=0.048).

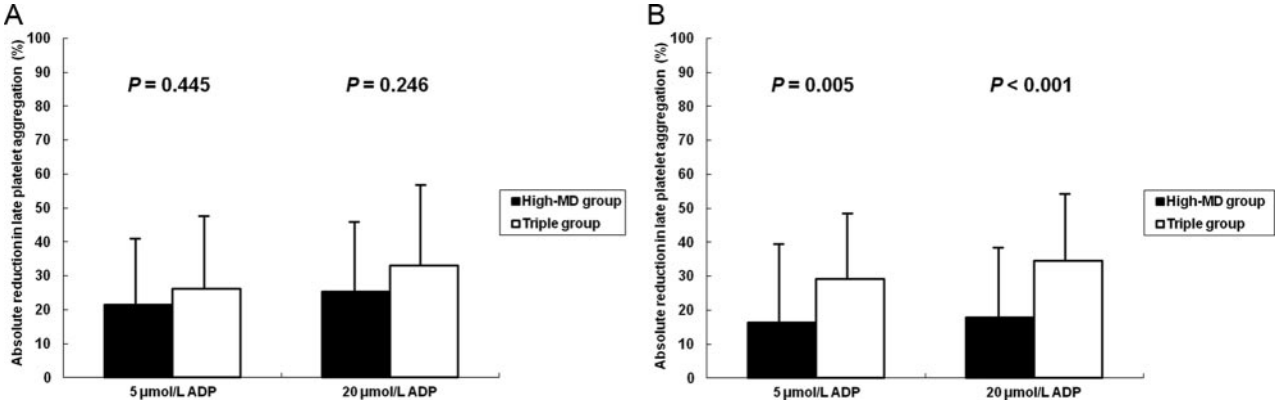

Figure 3. Absolute reductions in late platelet aggregation according to wild-type homozygotes (A) and mutant-type variants (B). Results are expressed as mean (boxes)±SD (error bars).

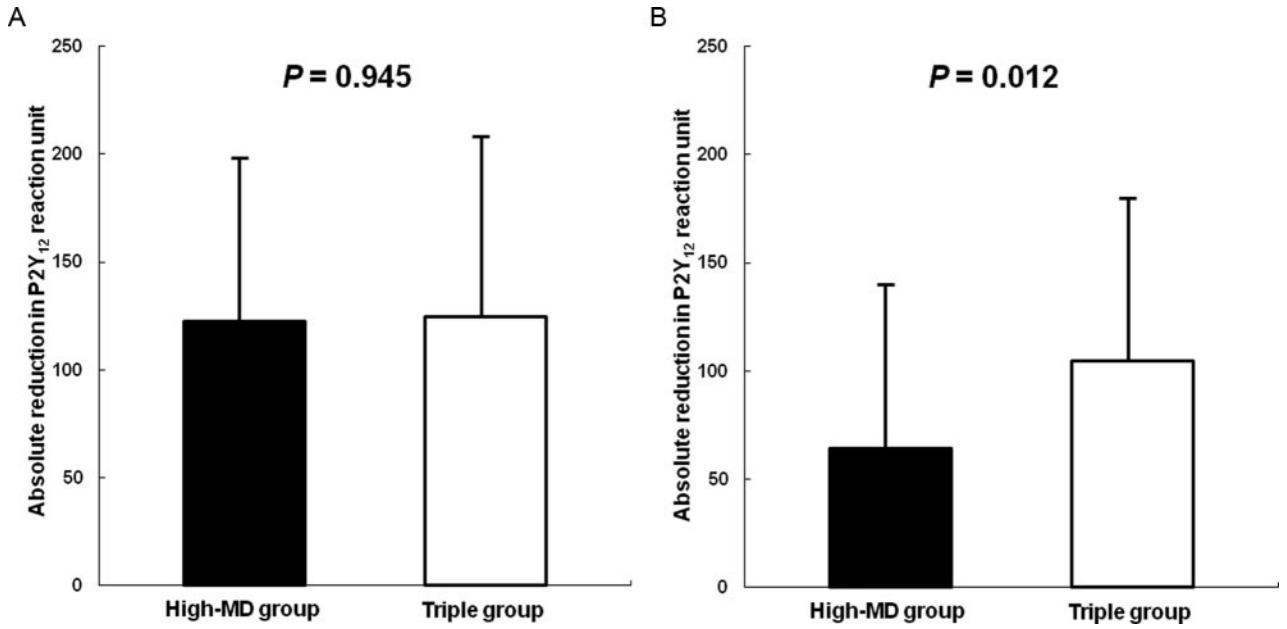

Figure 4. Absolute reductions in $P2Y_{12}$ reaction unit according to wild-type homozygotes (A) and mutant-type variants (B). Results are expressed as mean (boxes)±SD (error bars).

In the triple group, wild-type homozygotes did not differ with mutant-type variants in terms of 5 and 20 μmol/L ADP–stimulated ΔAgg_{max} (23.6±21.6% versus 25.8±16.8%, $P=0.641$, and 26.4±22.2% versus 26.3±16.0%, $P=0.980$, respectively). ΔAgg_{late} values also were not different between wild-type homozygotes and mutant-type variants: 26.1±21.3% versus 29.1±18.9% after 5 μmol/L ADP stimuli ($P=0.556$) and 33.0±24.0% versus 34.5±19.0% after 20 μmol/L ADP stimuli ($P=0.788$). In addition, wild-type homozygotes showed similar values of ΔPRU (124.4±84.6 versus 104.8±74.6, $P=0.334$) and the 30-day rate of HPPR (4.5% versus 6.4%, $P=1.000$), compared with mutant-type variants.

Discussion

To the best of our knowledge, this ACCEL-POLYMORPHISM study is the first to demonstrate the impact of adjunctive cilostazol on intensified platelet inhibition according to *CYP2C19* genotyping in high-risk patients undergoing elective PCI. Major findings of the present study are (1) adjunctive cilostazol to dual antiplatelet therapy can achieve acceptable platelet inhibition and reduce the risk of HPPR in both noncarriers and carriers of the *CYP2C19* mutant allele; (2) additive platelet inhibition by adjunctive cilostazol may not be influenced by *CYP2C19* genotyping; and (3) antiplatelet response to high-MD clopidogrel may be influenced by carriage of the *CYP2C19* mutant allele.

Because all biological processes reflect the combined influence of multiple clinical, environmental, and genetic effects, antiplatelet response to a prodrug clopidogrel also can be influenced by various factors.[32] Because a small proportion of the ingested clopidogrel finally binds to $P2Y_{12}$ receptor, small changes in absorption, metabolism, and $P2Y_{12}$ receptor density can considerably affect residual platelet reactivity.[33] Furthermore, because HPPR increases the risk of ischemic events,[6–9] there remains a critical need to achieve adequate platelet inhibition in high-risk patients. Although concomitant disease processes, drug-drug interaction, and clinical factors are associated with increased on-treatment platelet reactivity,[12,13,32] genetic polymorphisms, especially the *CYP2C19* variant carriage, have been identified to be major predictors of HPPR and adverse clinical events in PCI-treated patients.[14–17] Antiplatelet regimens to overcome the loss-of-function effect of the *CYP2C19* mutant allele have been under investigation.[34] The present study suggests that enhanced platelet inhibition by adjunctive cilostazol may not be influenced by *CYP2C19* polymorphism. The pathway related with active conversion of cilostazol can explain this observation. The major active metabolites of cilostazol are OPC-13015 (dehydrocilostazol) and OPC-13213 (monohydroxy-cilostazol).[25] OPC-13015 is mainly produced by the *CYP3A4* system, and it is 3 times more potent than cilostazol. On other hand, OPC-13213 is produced by the *CYP3A4/5* and *CYP2C19* pathways, and it is 3 times less potent than cilostazol. Therefore, the active metabolite of cilostazol may be consistently produced with little effect from the *CYP2C19* variant, which may be related with consistently achieved platelet inhibition irrespective of *CYP2C19* genotyping.

Recent studies have suggested a possibility that enhanced responsiveness to clopidogrel is associated with a higher risk of major bleeding.[35,36] Thus, it is imperative to maximize efficacy and maintain safety by defining unidentified therapeutic window for $P2Y_{12}$ inhibition.[32] Although novel and more potent $P2Y_{12}$ inhibitors including prasugrel and ticagrelor can overcome the limitations of clopidogrel, the benefits of these agents were associated with the increased rates of major bleeding compared with clopidogrel.[37,38] Interestingly, there are no reports of the increased risk of major or

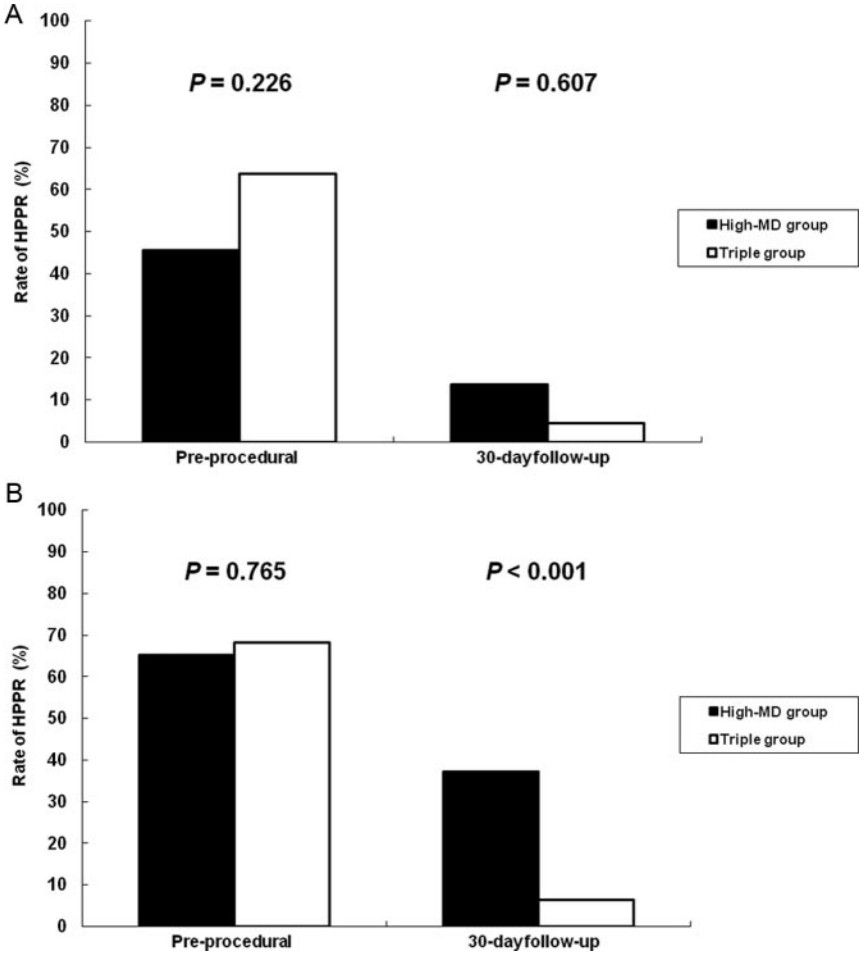

Figure 5. Rate of HPPR according to wild-type homozygotes (A) and mutant-type variants (B). HPPR indicates high posttreatment platelet reactivity (5 μmol/L ADP–induced maximal platelet aggregation >50%).

fatal bleeding by adjunctive cilostazol.[39–41] This finding might be explained by an endothelium-targeted antithrombotic therapy, which reduces the number of partially activated platelets by interacting with activated endothelial cells.[20] In addition, a previous report documented that adjunctive cilostazol to other antiplatelet regimen did not prolong bleeding time.[42] Cilostazol also has the relatively short recovery time of platelet function.[20] Although it is hard to establish because of the lack of large clinical data, adjunctive cilostazol might be an option if there are worrisome risks of bleeding with novel and more potent $P2Y_{12}$ inhibitors.

Because atherothrombosis is initiated and propagated by cross-talk of multiple causes, only intensified platelet inhibition cannot guarantee the escape from atherothrombosis. As aforementioned, cilostazol is known as a unique selective dual inhibitor of PDE3 and adenosine uptake, which causes elevation of cyclic AMP (cAMP) in various cardiovascular systems.[20] Therefore, cilostazol not only inhibits platelet aggregation[20] but also has favorable pleiotropic effects on the diverse processes in preclinical studies.[22–24] Adjunctive cilostazol can reduce aspirin and clopidogrel resistance in patients with vascular disease[18,19,43] Cilostazol not only can inhibit oxidative stress and inflammatory burden[23,44] but also can protect from endothelial senescence and dysfunction.[22,24] Enhancement of endothelial nitric oxide synthase by cilostazol underlies its vasodilating property,[22] which is used for intermittent claudication and cerebral infarction.[45] In addition, cilostazol can inhibit smooth muscle cell proliferation after vascular stenting,[21,46] and protect myocardium against ischemia-reperfusion injury.[47] Finally, cilostazol also can elevate circulating adenosine levels,[20] which is suggested as a mechanism for superiority of ticagrelor to prasugrel in terms of cardiovascular mortality.[48]

The clinical impact of triple antiplatelet therapy has been proven mostly for an East Asian population. Because the *CYP2C19* variant is more prevalent in East Asians than whites (≈60% versus ≈30%),[14–17,29] the benefit of this regimen may be somewhat related with ethnic singularity. Because the effect of clopidogrel dose-up may be more influenced by the *CYP2C19* variant carriage, adjunctive cilostazol can be a more useful intensified antiplatelet therapy in East Asian patients compared with high-MD clopidogrel. In addition, cilostazol also might provide valuable effects on endothelium protection and antiatherogenic milieu. Therefore, triple antiplatelet therapy conceptually can be an optimal antiplatelet regimen for PCI-treated patients in high-risk clinical or lesion subsets. However, this concept would be acceptable in clinical practice if global scaled, large, prospective studies can verify this conceptual efficacy and safety.

Study Limitations

First, the follow-up period was short and the number of patients studied was small. In addition, variations of anti-

platelet response during initial clopidogrel maintenance therapy might have influenced absolute changes of platelet measures. Second, preprocedural use of clopidogrel (300-mg loading versus chronic therapy) was relatively heterogeneous. However, preprocedural platelet reactivity was similar between the 2 treatments. Third, the present study including East Asians only showed laboratory data and could not suggest any result of clinical outcomes caused by enrollment of patients undergoing uneventful PCI and the number of cohorts. The present study also may not suggest the optimal duration of intensified antiplatelet therapy in high-risk patients. Fourth, we performed *CYP2C19* genotyping only, and we could not absolutely exclude the impact of other genetic polymorphisms. Finally, the present study is a post hoc analysis of subjects drawn from prospective trials.

Conclusion

Among high-risk patients undergoing elective PCI, adjunctive cilostazol can achieve consistently intensified platelet inhibition and reduce the risk of HPPR irrespective of *CYP2C19* genotyping.

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Disclosures

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CLINICAL PERSPECTIVE

Recent data have shown that increased platelet reactivity despite clopidogrel therapy is associated with a higher risk of ischemic events in patients who undergo percutaneous coronary intervention (PCI). Although clinical factors and drug-drug interactions reduce the antiplatelet response to clopidogrel, the hepatic cytochrome P450 (*CYP*) mutant alleles, especially the *CYP2C19* isoenzyme, have shown a strong association with increased platelet reactivity and the risk of major adverse cardiovascular events. Therefore, antiplatelet regimens that retain antiplatelet efficacy in the presence of the *CYP2C19* polymorphism would benefit high-risk PCI-treated patients. Adjunctive cilostazol to dual antiplatelet therapy (or "triple antiplatelet therapy") can be an attractive option for this genotypic risk profile. Accumulating data have verified the antiplatelet effects of cilostazol. Furthermore, cilostazol is converted into active metabolites by the *CYP3A* system and therefore should not lose efficacy in the presence of *CYP2C19* isoenzyme mutations. The present study enrolled high-risk patients undergoing elective PCI to evaluate platelet reactivity reductions between preprocedure and 30-day follow-up. Additive platelet inhibition by double-dose clopidogrel was diminished in carriers of the *CYP2C19* variant allele. However, adjunctive cilostazol therapy resulted in consistent platelet inhibition irrespective of *CYP2C19* genotyping. These results support the hypothesis that triple antiplatelet therapy may be an optimal antiplatelet regimen for PCI-treated patients in high-risk clinical, lesion, or genotyping subsets, which requires further testing in global scaled, large, prospective studies to verify the efficacy and safety of this antiplatelet regimen.

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Cytochrome 2C19 Polymorphism and Response to Adjunctive Cilostazol Versus High Maintenance-Dose Clopidogrel in Patients Undergoing Percutaneous Coronary Intervention

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SUPPLEMENTAL MATERIAL

Supplemental Table 1. Allelic and genotypic distributions of the *CYP2C19* variant

Allele	Frequency (%)	Genotype	Distribution, n (%)	Predicted function*
***1**	56.3	***1/*1**	44 (32.8)	Normal
***2**	34.0	***1/*2**	47 (35.1)	Decreased
***3**	9.7	***1/*3**	16 (11.9)	Decreased
		***2/*2**	18 (13.4)	Decreased
		***2/*3**	8 (6.0)	Decreased
		***3/*3**	1 (0.7)	Decreased

CYP indicates the hepatic cytochrome P450.

Supplemental Table 2. Platelet reactivity according to the *CYP2C19* genotypes in the high maintenance-dose group

	Wild-type	**Mutant-type Heterozygote**		**Mutant-type Homozygotes**	
	****1/*1***	****1/*2***	****1/*3***	****2/*2***	****2/*3***
	(n = 22)	**(n = 23)**	**(n = 8)**	**(n = 8)**	**(n = 4)**
5 μmol/L ADP-Agg_{max} (%)					
Pre-procedure	47.7 ± 16.4	53.2 ± 15.0	54.2 ± 12.6	54.0 ± 15.1	54.2 ± 12.6
30-day follow-up	31.1 ± 12.9	42.9 ± 18.1	28.4 ± 13.9	42.9 ± 18.1	28.4 ± 13.9
20 μmol/L ADP-Agg_{max} (%)					
Pre-procedure	60.5 ± 15.1	66.8 ± 11.3	66.8 ± 12.2	66.8 ± 11.3	66.8 ± 12.2
30-day follow-up	41.9 ± 16.4	55.4 ± 15.9	40.5 ± 16.7	55.4 ± 15.9	40.5 ± 16.7
5 μmol/L ADP-Agg_{late} (%)					
Pre-procedure	40.3 ± 20.3	47.1 ± 20.1	45.8 ± 16.8	47.1 ± 20.1	45.8 ± 16.8
30-day follow-up	19.0 ± 13.4	30.7 ± 22.2	16.7 ± 11.5	30.7 ± 22.2	16.7 ± 11.5
20 μmol/L ADP-Agg_{late} (%)					
Pre-procedure	52.2 ± 21.1	61.0 ± 16.8	60.9 ± 16.0	61.0 ± 16.8	60.9 ± 16.0
30-day follow-up	26.9 ± 18.8	43.2 ± 22.0	26.4 ± 17.4	43.2 ± 22.0	26.4 ± 17.4
$P2Y_{12}$ reaction unit					
Pre-procedure	272.5 ± 75.5	278.3 ± 69.7	296.5 ± 53.2	278.3 ± 69.7	296.5 ± 53.2
30-day follow-up	149.7 ± 65.4	214.1 ± 68.5	191.6 ± 78.4	214.1 ± 68.5	191.6 ± 78.4
% inhibition					
Pre-procedure	20.2 ± 18.7	14.0 ± 16.2	11.1 ± 10.8	14.0 ± 16.2	11.1 ± 10.8
30-day follow-up	65.2 ± 12.8	33.5 ± 19.2	45.8 ± 21.2	33.5 ± 19.2	45.8 ± 21.2

CYP indicates the hepatic cytochrome P450; Agg_{max}, maximal platelet aggregation; Agg_{late}, late platelet aggregation.

Supplemental Table 3. Platelet reactivity according to the *CYP2C19* genotypes in the triple group

	Wild-type	**Mutant-type Heterozygote**		**Mutant-type Homozygotes**		
	****1/*1***	****1/*2***	****1/*3***	****2/*2***	****2/*3***	****3/*3***
	(n = 22)	**(n = 24)**	**(n = 8)**	**(n = 10)**	**(n = 4)**	**(n = 1)**
5 μmol/L ADP-Agg_{max} (%)						
Pre-procedure	50.2 ± 15.8	54.0 ± 15.1	54.2 ± 12.6	54.0 ± 15.1	54.2 ± 12.6	54.2
30-day follow-up	26.7 ± 15.2	42.9 ± 18.1	28.4 ± 13.9	42.9 ± 18.1	28.4 ± 13.9	28.4
20 μmol/L ADP-Agg_{max} (%)						
Pre-procedure	62.4 ± 13.8	66.8 ± 11.3	66.8 ± 12.2	66.8 ± 11.3	66.8 ± 12.2	66.8
30-day follow-up	36.0 ± 19.2	55.4 ± 15.9	40.5 ± 16.7	55.4 ± 15.9	40.5 ± 16.7	40.5
5 μmol/L ADP-Agg_{late} (%)						
Pre-procedure	41.0 ± 17.9	47.1 ± 20.1	45.8 ± 16.8	47.1 ± 20.1	45.8 ± 16.8	45.8
30-day follow-up	14.9 ± 13.4	30.7 ± 22.2	16.7 ± 11.5	30.7 ± 22.2	16.7 ± 11.5	16.7
20 μmol/L ADP-Agg_{late} (%)						
Pre-procedure	54.2 ± 18.4	61.0 ± 16.8	60.9 ± 16.0	61.0 ± 16.8	60.9 ± 16.0	60.9
30-day follow-up	21.2 ± 19.0	43.2 ± 22.0	26.4 ± 17.4	43.2 ± 22.0	26.4 ± 17.4	26.4
$P2Y_{12}$ reaction unit						
Pre-procedure	253.8 ± 76.7	278.3 ± 69.7	296.5 ± 53.2	278.3 ± 69.7	296.5 ± 53.2	296.5
30-day follow-up	129.4 ± 76.4	214.1 ± 68.5	191.6 ± 78.4	214.1 ± 68.5	191.6 ± 78.4	191.6
% inhibition						
Pre-procedure	22.4 ± 22.9	14.0 ± 16.2	11.1 ± 10.8	14.0 ± 16.2	11.1 ± 10.8	45.8
30-day follow-up	70.5 ± 7.6	33.5 ± 19.2	45.8 ± 21.2	33.5 ± 19.2	45.8 ± 21.2	54.2

CYP indicates the hepatic cytochrome P450; Agg_{max}, maximal platelet aggregation; Agg_{late}, late platelet aggregation.

Supplemental Table 4. Platelet reactivity after 300-mg loading and standard chronic therapy of clopidogrel

Variables	300-mg loading (n = 98)	Chronic therapy (n = 36)	*P*
5 μmol/L ADP-Agg_{max} (%)	52.6 ± 14.8	52.1 ± 14.5	0.869
20 μmol/L ADP-Agg_{max} (%)	65.2 ± 13.2	64.6 ± 11.9	0.790
5 μmol/L ADP-Agg_{late} (%)	45.2 ± 18.4	42.6 ± 19.6	0.462
20 μmol/L ADP-Agg_{late} (%)	58.9 ± 17.9	57.0 ± 17.7	0.585
$P2Y_{12}$ reaction unit	282.2 ± 69.2	272.9 ± 63.2	0.410
% inhibition	15.2 ± 17.4	16.0 ± 14.8	0.798

Agg_{max} indicates maximal platelet aggregation; Agg_{late}, late platelet aggregation.