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Interaction analysis between genetic polymorphisms and pharmacodynamic effect in patients treated with adjunctive cilostazol to dual antiplatelet therapy: results of the ACCEL-TRIPLE (Accelerated Platelet Inhibition by Triple Antiplatelet Therapy According to Gene Polymorphism) study

In-Suk Kim,[1] Young-Hoon Jeong,[2,3] Yongwhi Park,[2] Seong-Eun Yoon,[2] Tae Jung Kwon,[2] Jeong Rang Park,[2] Seok-Jae Hwang,[2] Eun-Ha Koh,[4] Choong Hwan Kwak,[2] Jin-Yong Hwang[2] & Sunjoo Kim[4]

[1]Department of Laboratory Medicine, Pusan National University Hospital, Busan, [2]Division of Cardiology, Department of Internal Medicine, Gyeongsang National University Hospital, Jinju, Korea, [3]Sinai Center for Thrombosis Research, Baltimore, Maryland, USA and [4]Department of Laboratory Medicine, Gyeongsang National University Hospital, Jinju, Korea

Correspondence
Dr Young-Hoon Jeong MD PhD, Sinai Center for Thrombosis Research, Hoffberger Building, Suite 56, 2401 W. Belvedere Ave, Baltimore, MD 21215, USA.
Tel.: +1 41 0601 4795
Fax: +1 41 0601 4796
E-mail: yjoeng@lifebridgehealth.org

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Compared with standard dual antiplatelet therapy, adjunctive cilostazol to dual antiplatelet therapy ('triple antiplatelet therapy') has a potential to reduce ischemic event occurrence after percutaneous coronary intervention.
- The pharmacokinetic and pharmacodynamic effects of clopidogrel have been significantly influenced by the enzyme activity of the *ABCB1 C3435T* and the *CYP2C19* system.
- For the pharmacokinetics of cilostazol, genetic polymorphisms of the *CYP3A5* and *CYP2C19* have been associated with the substantial interindividual variability in healthy volunteers.

WHAT THIS STUDY ADDS

- Loss-of-function polymorphism of the *CYP2C19* gene, but not the *ABCB1 C3435T* and *CYP3A5*3* genes, affects the antiplatelet effect of triple antiplatelet therapy.
- Most of extensive and intermediate East Asian metabolizers (0 or 1 *CYP2C19* loss-of-function allele) show adequate platelet inhibition when treated with triple antiplatelet therapy after percutaneous coronary intervention.
- However, carriage of 2 *CYP2C19* loss-of-function alleles is still associated with the risk of high platelet reactivity (defined by by 5 μM ADP-induced maximal platelet aggregation >46%), which clinical impact needs to be validated in future clinical trials.

AIMS

Although adjunctive cilostazol to dual antiplatelet therapy can reduce the risks of clinical events after percutaneous coronary intervention (PCI), whether genetic polymorphism can influence the pharmacodynamics of this regimen has not been evaluated.

METHODS

One hundred and twenty-seven patients treated with PCI and taking triple antiplatelet therapy (≥1 month) were enrolled. Platelet reactivity was assessed by conventional aggregometry and the VerifyNow P2Y12 assay. High on-treatment platelet reactivity (HPR) was defined as 5 μM ADP-induced maximal platelet reactivity (Agg_{max}) >46%. *CYP3A5*3*, *CYP2C19*2/*3* and *ABCB1* 3435C > T were genotyped.

RESULTS

*CYP3A5**3 and *ABCB1* 3435C > T variants did not affect the antiplatelet effect of triple antiplatelet therapy. For non-carriers, one and two carriers of the *CYP2C19* loss-of-function (LOF) allele, Agg_{max} consecutively increased after the addition of 5 μM [mean (95% confidence intervals): 24.6% (20.8 to 28.5%) *vs.* 28.7% (25.4 to 32.0%) *vs.* 32.3% (25.8 to 38.7%), $P = 0.062$, respectively] and 20 μM ADP [34.2% (29.3 to 39.0%) *vs.* 41.7% (37.8 to 45.6%) vs. 44.9% (37.9 to 51.9%), $P = 0.007$, respectively]. Likewise, late platelet reactivity and P2Y12 reaction units proportionally changed according to the number of *CYP2C19* LOF alleles. HPRs were observed in 9.2% of subjects: 6.3%, 7.4% and 20.0% with 0, 1 and 2 carriers of *CYP2C19* LOF allele(s) ($P = 0.099$). In multivariate analysis, carriage of two *CYP2C19* LOF alleles was a significant predictor for the prevalence of HPR (odds ratio 5.78, 95% CI 1.21, 27.78, $P = 0.028$).

CONCLUSION

Among PCI-treated patients, the effect of triple antiplatelet therapy is influenced by the *CYP2C19* LOF allele. Its clinical benefit needs to be validated according to the *CYP2C19* metabolic phenotype in future clinical trials. [Adjunctive Cilostazol Versus High Maintenance dose ClopidogrEL in Acute Myocardial Infarction Patients According to CYP2C19 Polymorphism (ACCEL-AMI-2C19), NCT00915733 and Adjunctive Cilostazol Versus High Maintenance-dose Clopidogrel According to Cytochrome 2C19 Polymorphism (ACCEL-2C19), NCT01012193].

Introduction

Dual antiplatelet therapy with aspirin and clopidogrel reduces ischaemic complications following percutaneous coronary intervention (PCI) or acute coronary syndrome (ACS) [1, 2]. Although this combination has been widely adopted in clinical practice, the recurrence of post PCI adverse cardiovascular events has still been observed in a proportion of patients. Numerous clinical factors can contribute to the development of cardiovascular events [3], and clopidogrel hyporesponsiveness and/or high on-treatment platelet reactivity (HPR) has been suggested as an important trigger of post PCI ischaemic event [4].

Cilostazol, a selective and reversible inhibitor of phosphodiesterase type 3 (PDE 3), has a unique property of platelet inhibition and vascular protection [5, 6]. A growing body of studies has demonstrated that adjunctive cilostazol to standard dual antiplatelet therapy (triple antiplatelet therapy) can reduce the risk of post PCI ischaemic events compared with standard antiplatelet therapy [6–15]. Although its beneficial role in recovery of endothelial dysfunction has been suggested as the underlying mechanism [7–9, 11], reduction of thrombotic events such as myocardial infarction and stent thrombosis [10, 12–14] may be related to a platelet-centric mechanism. To date, several translational studies demonstrated that adding cilostazol can enhance inhibition of adenosine diphosphate (ADP)-induced platelet aggregation [16–19].

On-clopidogrel platelet reactivity measured by multiple platelet function testing has been significantly associated with the enzyme activities of intestinal transporter and the hepatic cytochrome P-450 (CYP) system, especially the CYP2C19 isozyme [4]. Single nucleotide polymorphisms (SNPs) occurring in these genes can change the antiplatelet effect of clopidogrel [20–22]. Likewise, cilostazol is metabolized by the hepatic metabolism via the CYP enzyme, primarily CYP3A4/5 and, to a lesser extent, CYP2C19 [23]. Yoo *et al*. demonstrated that with regard to the pharmacokinetics of cilostazol, genetic polymorphisms of the *CYP3A5*3* and *CYP2C19*2/*3* can explain the substantial interindividual variability in healthy volunteers [24, 25]. However, there have been no data regarding the influence of genetic polymorphisms on the pharmacodynamics of adding cilostazol to dual antiplatelet therapy, especially the antiplatelet effect.

This analysis was performed to assess the achieved antiplatelet effect of triple antiplatelet therapy and the effect of SNPs on the pharmacodynamics among post PCI patients.

Methods

Patient population and study design

A total of 127 PCI-treated patients with available genotyping and platelet measures together were enrolled into the ACCEL-TRIPLE (Accelerated Platelet Inhibition by Triple Antiplatelet Therapy According to Gene Polymorphism) study (Figure 1). Patients were recruited from the Gyeongsang National University Hospital between January 2008 and June 2009. One hundred and two patients (80.3%) were collected from the triple therapy group in the ACCEL [Adjunctive Cilostazol *vs.* high maintenance dose (MD) ClopidogrEL] studies, which were performed to compare the magnitude of platelet inhibition by adjunctive cilostazol *vs.* high MD clopidogrel in high risk patients: HPR, diabetes, complex lesion PCI and acute myocardial infarction [17, 18, 26]. Patients receiving triple antiplatelet therapy were treated for 1 month. A minority ($n = 25$, 19.7%) of patients received post PCI triple antiplatelet therapy for 1 month at the attending physician's discretion. A minority ($n = 25$, 19.7%) of the patients was recruited from high risk patients who started adding cilostazol after PCI at the attending physician's discretion. Patient compliance to antiplatelet therapy was assessed by questionnaire, interview and tablet counting at the follow-up visit. Because pharmacokinetic and pharmacodynamic responses may vary profoundly during the initial days or weeks after antiplatelet therapy [16], only patients in the steady-state phase of triple antiplatelet therapy were included (≥1 month of cilostazol 100 mg twice a day, clopidogrel 75 mg once a day and aspirin 200 mg once a day).

Patients were eligible for enrolment if they were ≥18 years of age and had been treated with PCI for symptomatic coronary artery disease. The contraindications to triple antiplatelet therapy were known allergies to antiplatelet therapy, haemodynamic instability, active bleeding and bleeding diatheses, oral anticoagulation therapy with coumadin, left ventricular ejection fraction <30%, a leucocyte count <3000 mm^3 and/or a platelet count <100 000 mm^3, an aspartate aminotransferase (AST) concentration or an alanine aminotransferase (ALT) concentration ≥three times the upper normal limit, noncardiac disease with a life expectancy <1 year and the 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 measurements

Blood samples were obtained 2–4 h after the last intake of triple antiplatelet therapy. Blood samples were collected using the double-syringe technique and the first 2 to 4 ml of blood was discarded to avoid spontaneous platelet activation. The platelet functions were measured by light transmittance aggregometry (LTA) and the VerifyNow P2Y12 assay (Accumetrics Inc., San Diego, California). We have previously presented the correlation between the two methods at our laboratory [27].

LTA was performed according to a standard protocol and has been described in detail elsewhere [27]. Blood samples were drawn into Vacutainer tubes containing

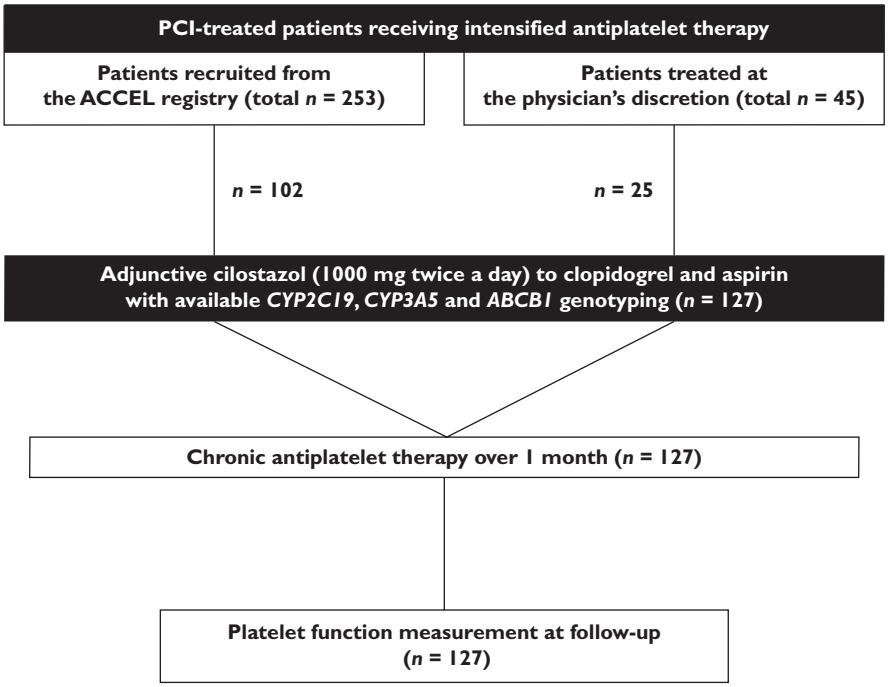

Figure 1

Flow diagram of the ACCEL-TRIPLE study. *ABCB1*, ATP-binding cassette sub-family B member 1; ACCEL, Adjunctive Cilostazol Versus High Maintenance-Dose Clopidogrel; *CYP*, cytochrome P450; PCI, percutaneous coronary intervention

0.5 ml of 3.2% sodium citrate (Becton-Dickinson, San Jose, California) and processed within 2 h. Platelet rich plasma (PRP) was obtained as a supernatant fluid after centrifuging the blood at 120 *g* for 10 min. The remaining blood was further centrifuged at 1200 *g* for 10 min 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 Corporation, Beaumont, Texas). Light transmission was adjusted to 0% with PRP and to 100% with PPP for each measurement. Platelet function tests were performed after the addition of 5 and 20 μM ADP and the curves were recorded for 10 min. Platelet reactivity was determined at maximal aggregation (Agg_{max}) and late aggregation at 5 min (Agg_{late}).

The VerifyNow P2Y12 assay is a whole blood, point-of-care system that has been developed to assess responsiveness to P2Y12 antagonists [27]. Blood was drawn into a Greiner Bio-One 3.2% citrate Vacuette tube (Greiner Bio-One, Kremsmünster, Austria). The assay device contains fibrinogen-coated polystyrene beads and 20 μM ADP, which also contains 22 nM PGE_1 to reduce the non-specific contribution of other pathways. The results are reported in P2Y12 reaction units (PRU).

Gene analysis and phenotype

We performed genotyping of the two *CYP2C19* LOF alleles (**2* and **3*), *CYP3A5*3*, and *ABCB1 3435*C > T variants on the basis of previous studies [20–25]. The base numbering and allele definitions follow the nomenclature of the Human *CYP* Allele Nomenclature Committee [28]. Genomic DNA was extracted from leucocytes of whole blood specimens with a commercially available kit (QIAamp DNA Blood Mini Kit, Qiagen, Hilden, Germany). All genotyped SNPs were in Hardy-Weinberg equilibrium ($P > 0.05$).

Because the frequencies of the *CYP2C19*4, *5, *6, *7* and **8* LOF alleles are extremely rare in East Asians [29], genotyping for the *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. Polymerase chain reaction (PCR) was carried out by using the same primers. 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 (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 s, 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.

We classified every *CYP2C19* phenotype by established nomenclature and its reported effect on enzymatic function according to published reports, extensive (EM), intermediate (IM) and poor (PM) metabolizers. For *ABCB1 3435*C > 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). In terms of the *CYP3A5* phenotype, carriers of two *CYP3A5*3* LOF alleles were defined as reduced metabolizers [30].

Endpoints and definitions

The primary endpoint was Agg_{max} according to metabolic phenotypes. The secondary endpoints were (i) Agg_{late}, (ii) PRU and (iii) the prevalence and predictor of HPR according to metabolic phenotype. According to the consensus of the Working Group [4], HPR was defined as 5 μM ADP-induced Agg_{max} >46%.

Sample size calculation and statistical analysis

Under the assumption that carriage of the *CYP2C19* LOF variant may influence platelet inhibition by triple antiplatelet therapy, we calculated the number of enrolled patients. If there was a 20% relative difference of ADP-induced Agg_{max} between the *CYP2C19* LOF carriers *vs.* non-carriers, and the *CYP2C19* LOF allele was observed in 60% of East Asians [29, 31], at least 120 patients (72 carriers and 48 non-carriers of the *CYP2C19* LOF variant) were needed to guarantee a power of 90% to detect a significant difference with a two-sided α-level of 0.05 and SD of 0.3.

Continuous variables, presented as mean ± SD, were compared using Student's unpaired *t*-test or Mann-Whitney *U* test. Categorical variables, presented as numbers or percentages, were compared using chi-square test or Fisher's exact test as appropriate. Platelet measures and baseline characteristics according to metabolic phenotype were analyzed using one way analysis of variance (ANOVA). After demonstration of significant differences among variables by one way ANOVA, *post hoc* comparisons among the groups were made with the Student-Newman-Keuls procedure for multiple comparisons. To consider the gene–dose relationship, the Jonckheere-Terpstra test also was used. To determine predictors of HPR, a logistic regression analysis was performed using known variables, and odds ratio (OR) and 95% 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 18.0 (SPSS Inc., Chicago, Illinois).

Results

Patient characteristics

Because of the characteristics of this analysis, the cohorts showed complete compliance with triple antiplatelet therapy for at least 30 days. No major cardiovascular and bleeding events were observed in any patient during follow-up period. One patient in the triple therapy group suffered from thrombolysis in myocardial infarction minor bleeding due to entry site aneurysm and haematoma. Although there were four cases of transient headache (3.1%) and two cases of palpitation (1.6%) in the triple therapy group in the early phase of treatment, all regimens were generally well tolerated. Baseline characteristics of the enrolled patients are shown in Table 1. The average age was 62.9 (SD 10.9) years, and about 70% of patients were men. Patients were comprised of relatively high risk cohorts: ACS 74.8%, diabetes 35.4%, and drug-eluting stent implantation 96.8%.

Table 2 shows distributions according to allele, genotype and metabolic phenotype. There was a high prevalence of the *CYP2C19* LOF phenotype (IMs and PMs: 42.5% and 19.7%, respectively). Moreover, there were 22 carriers of the *CYP2C19*3* LOF allele (17.3%). There were no differences in platelet measures between the *CYP2C19*2 vs. *3* LOF allele whether these were present in IMs or PMs (data not shown). Baseline characteristics did not differ across the *CYP3A5* and *ABCB1 3435*C > T phenotype (data not shown), and they were well matched between the *CYP2C19* groups, except for a higher prevalence of diabetes in IMs and PMs (Table 1).

Platelet function measurements according to metabolic phenotype

The means of 5 and 20 μM ADP-induced Agg_{max} were 27.9 ± 13.5% and 39.5 ± 16.2%, respectively. Agg_{late} values after the addition of 5 and 20 μM ADP were 15.7 ± 12.5% and 25.0 ± 18.0%, respectively. The PRU value from the VerifyNow P2Y12 assay was 172 ± 87. Phenotypes of the *CYP3A5* and *ABCB1* alleles did not affect the levels of platelet reactivity. Compared with non-carriers, carriers of the *CYP2C19* LOF allele showed significant differences of platelet measures (data not shown).

The number of the *CYP2C19* LOF carriage alleles increased proportionally Agg_{max} after the addition of 5 μM [24.6 ± 13.3% (95% CI 20.8, 28.5%) *vs.* 28.7 ± 12.2% (95% CI 25.4, 32.0%) *vs.* 32 ± 15.7% (95% CI 25.8, 38.7%), $P = 0.062$] and 20 μM ADP [34.2 ± 16.7% (95% CI 29.3, 39.0%) *vs.* 41.7 ± 14.2% (95% CI 37.8, 45.6%) *vs.* 44.9 ± 17.0% (95% CI 37.9, 51.9%), $P = 0.007$] (Table 3, Figure 2). Values of 5 and 20 μM ADP-induced Agg_{late} were increased depending on the number of the *CYP2C19* LOF alleles ($P = 0.021$ and 0.002 after adjustment for diabetes, respectively) (Table 3, Figure 3). PRU consecutively increased according to the number of the *CYP2C19* LOF alleles ($P < 0.001$ after adjustment for diabetes) (Table 3, Figure 4).

Prevalence and predictors of HPR

Twelve patients (9.4%) of all cohorts receiving triple platelet therapy met the criteria of HPR. The *CYP3A5*3* and *ABCB1 3435*C > T polymorphisms were not correlated with the increased risk of HPR (Table 3). The *CYP2C19* PMs

Table 1

Baseline characteristics according to *CYP2C19* metabolic phenotype

Variables *n* (%)	Total population (*n* = 127)	Extensive metabolizer (*n* = 48)	Intermediate metabolizer (*n* = 54)	Poor metabolizer (*n* = 25)	*P* value
Age (years)	62.9 ± 10.9	61.3 ± 12.3	63.4 ± 9.8	65.1 ± 10.3	0.351
Male	89 (70.1)	33 (68.8)	38 (70.4)	18 (72.0)	0.795
BMI (kg m^{-2})	24.3 ± 3.1	24.0 ± 3.0	24.7 ± 3.3	24.1 ± 3.2	0.531
Clinical presentation					
Stable angina	32 (25.2)	10 (20.8)	16 (29.6)	6 (24.0)	0.259
Unstable angina	40 (31.5)	14 (29.2)	16 (29.6)	10 (40.0)	
NSTEMI	29 (22.8)	12 (25.0)	10 (18.5)	7 (28.0)	
STEMI	26 (20.5)	12 (25.0)	12 (22.2)	2 (8.0)	
Risk factors					
Diabetes mellitus	45 (35.4)	11 (22.9)	23 (42.6)	11 (44.0)	0.045
Hypertension	68 (53.5)	23 (47.9)	29 (53.7)	16 (64.0)	0.229
Hypercholesterolaemia	32 (25.2)	11 (22.9)	11 (20.4)	10 (40.0)	0.214
Current smoking	56 (44.1)	21 (43.8)	23 (42.6)	12 (18.0)	0.810
Chronic kidney disease	17 (13.4)	7 (14.6)	8 (14.8)	2 (8.0)	0.599
History					
Previous MI	67 (52.8)	29 (60.4)	26 (48.1)	12 (48.0)	0.279
Previous CABG	4 (3.1)	1 (2.1)	1 (1.9)	2 (8.0)	0.310
Previous stroke	4 (3.1)	1 (2.1)	2 (3.7)	1 (4.0)	0.737
Concomitant medications					
Statin	116 (91.3)	46 (95.8)	46 (85.2)	24 (96.0)	0.832
***CYP* 3A4 metabolized**	102 (80.3)	37 (77.1)	42 (77.8)	23 (92.0)	0.227
β-adrenoceptor blocker	107 (84.7)	39 (81.3)	46 (85.2)	22 (88.0)	0.512
Angiotensin blockade	106 (91.3)	48 (100.0)	45 (83.3)	23 (92.0)	0.438
Calcium channel blocker	22 (17.3)	8 (16.7)	12 (22.2)	2 (8.0)	0.533
Proton pump inhibitor	4 (3.1)	2 (4.2)	2 (3.7)	0 (0.0)	0.508
LV ejection fraction ≤45%	22 (17.3)	12 (25.0)	7 (13.0)	3 (12.0)	0.116
Haemoglobin (g dl^{-1})	12.9 ± 1.4	13.0 ± 1.7	12.6 ± 1.3	13.0 ± 1.2	0.254
Platelet count (× 10^3 mm^{-3})	285.0 ± 91.4	283.4 ± 86.7	288.2 ± 100.8	281.0 ± 81.7	0.940
Hb A1$_C$ (%)	6.5 ± 1.2	6.3 ± 0.9	6.5 ± 1.4	6.6 ± 1.3	0.716
GFR (MDRD (ml min^{-1} 1.73 m^{-2})	85.3 ± 23.5	82.6 ± 23.6	87.0 ± 25.1	86.8 ± 19.8	0.609
Total cholesterol (mg dl^{-1})	135.6 ± 26.4	136.9 ± 25.2	133.6 ± 27.4	137.3 ± 27.0	0.778
Target artery					
Left anterior descending	69 (54.3)	28 (58.3)	28 (51.9)	13 (52.0)	0.352
Right coronary	33 (26.0)	12 (25.0)	13 (24.1)	8 (32.0)	
Left circumflex	21 (16.5)	6 (12.5)	11 (20.4)	4 (16.0)	
Left main	4 (3.1)	2 (4.2)	2 (3.7)	0 (1.0)	
Intervention method					
Drug-eluting stent	123 (96.8)	46 (95.8)	53 (98.1)	24 (96.0)	0.838
Bare-metal stent	2 (1.6)	1 (2.1)	0 (0.0)	1 (4.0)	
Ballooning only	2 (1.6)	1 (2.1)	1 (1.9)	0 (0.0)	
Multivessel intervention	37 (29.1)	11 (22.9)	19 (35.2)	7 (28.0)	0.510
Stent diameter (mm)	3.1 ± 0.4	3.2 ± 0.4	3.1 ± 0.4	3.1 ± 0.3	0.487
Stents per patient	2.1 ± 1.2	1.9 ± 1.1	2.2 ± 1.3	2.2 ± 1.2	0.531
Total stent length (mm)	50.0 ± 32.9	47.0 ± 28.0	51.7 ± 36.3	51.8 ± 35.1	0.742

Values are expressed as mean ± SD or as number of patients (%). BMI, body mass index; CABG, coronary artery bypass grafting; CYP, cytochrome P450; GFR, glomerular filtration rate; Hb A1$_C$, haemoglobin A1$_C$; LV, left ventricular; MDRD, Modification of Diet in Renal Disease; MI, myocardial infarction; NSTEMI, non-ST-segment elevation myocardial infarction; PCI, percutaneous coronary intervention; STEMI, ST-segment elevation myocardial infarction.

tended to have a higher prevalence of HPR than the *CYP2C19* EMs and IMs: 6.3%, 7.4% and 20.0% in 0, 1 and 2 carriers of *CYP2C19* LOF allele(s) ($P = 0.099$ after adjustment for diabetes) (Figure 5). To determine the predictors of HPR, a logistic multivariate regression analysis was performed using the known covariates, gender, age, body mass index (BMI), the *CYP2C19* phenotype, ACS presentation at index PCI, smoking status, hypertension, diabetes mellitus, chronic kidney disease, left ventricular ejection fraction ≤45% and use of a CYP3A4-metabolized statin, calcium channel blocker and proton pump inhibitor. Carriage of two *CYP2C19* LOF alleles (PMs) was only a significant predictor for HPR while taking triple antiplatelet therapy (OR 5.78, 95% CI 1.21, 27.78, $P = 0.028$).

Discussion

The present study demonstrates the interaction between candidate polymorphisms and the pharmacodynamic

Table 2

Distributions according to allele, genotype and metabolic phenotype

Gene	Allele	Frequency, %	Genotype	Distribution, *n* (%)	Predicted phenotype
CYP2C19	**1*	59.1	**1/*1*	48 (37.8)	Extensive metabolizer
	**2*	31.5	**1/*2*	42 (33.1)	Intermediate metabolizer
	**3*	9.4	**1/*3*	12 (9.4)	Intermediate metabolizer
			**2/*2*	15 (11.8)	Poor metabolizer
			**2/*3*	8 (6.3)	Poor metabolizer
			**3/*3*	2 (1.6)	Poor metabolizer
CYP3A5	**1*	24.8	**1/*1*	10 (7.9)	Normal metabolizer
	**3*	75.2	**1/*3*	43 (33.9)	Normal or reduced metabolizer
			**3/*3*	74 (58.3)	Reduced metabolizer
*ABCB1 3435*C > T	*C*	65.4	*CC*	52 (40.9)	High expression
	T	34.6	*CT*	62 (48.8)	Intermediate expression
			TT	13 (10.2)	Low expression

CYP, cytochrome P450: ABCB1, ATP-binding cassette sub-family B member 1.

effect of triple antiplatelet therapy in high-risk PCI-treated patients. Observed findings were as follows: (i) on the basis of consensus-defined HPR, more than 90% of the patients showed adequate platelet inhibition during triple antiplatelet therapy, (ii) about 60% of the patients carried the *CYP2C19* LOF allele (**2* or **3*), which are representative of an East Asian population [28], (iii) SNPs of the *CYP2C19* gene, but not the *ABCB1 3435*C > T and *CYP3A5* genes could affect the antiplatelet effect of triple antiplatelet therapy and (iv) a proportion of the *CYP2C19* PMs still exhibited low platelet inhibition with triple antiplatelet therapy (~20%).

During antiplatelet therapy, persistent HPR can increase the risk of occurrence of ischaemic events, whereas enhanced platelet inhibition may increase the risk of bleeding events [32]. Therefore, it may be important to identify the major attributable factors to antiplatelet response. Although clinical risk factors can affect the effect of antiplatelet therapy and the density of the platelet P2Y12 receptor [4, 33], enzymatic activity of genes encoding for intestinal absorption and hepatic metabolism has shown a significant and consistent association with the effect of antiplatelet therapy or clinical outcomes [20–25].

ADP-induced platelet inhibition by triple antiplatelet therapy may be mainly derived from the additive increase of cyclic adenosine monophosphate by both clopidogrel and cilostazol [16–18]. For clopidogrel response, the *CYP2C19* LOF allele has been consistently linked with pharmacokinetic and pharmacodynamic profiles [4, 20–22], and the *ABCB1 3435*C > T and the *CYP3A5* polymorphism also have reduced the antiplatelet response of clopidogrel in some cases [20, 21, 30]. Meanwhile, for the cilostazol effect, the *CYP3A5* and *CYP2C19* LOF alleles could explain the substantial variability in the pharmacokinetics of cilostazol in healthy subjects [24, 25]. However, in the present study, the *ABCB1 3435*C > T and *CYP3A5*3* variant did not significantly influence the effect of triple antiplatelet therapy.

In addition to cilostazol itself, its metabolites also can inhibit platelet activation with different potency, which may implicate that the pharmacodynamics of cilostazol can be different from the pharmacokinetics of cilostazol. OPC-13015 (dehydro-cilostazol) and OPC-13213 (monohydroxy-cilostazol) are the main metabolites of cilostazol [23]. OPC-13015 is mainly produced by the *CYP3A4* system, whereas OPC-13213 is generally produced by the *CYP3A5* and *CYP2C19* pathways. Because of its different potency (OPC-13015 : cilostazol : OPC-13213 = 9:3:1), the pharmacodynamics of cilostazol are less influenced by the *CYP3A5* and *CYP2C19* LOF polymorphisms. Therefore, the association between the antiplatelet effect of triple antiplatelet therapy and the *CYP2C19* LOF allele carriage in the present study may be mainly explained by the interaction between the *CYP2C19* LOF allele and clopidogrel.

In the ACCEL-DOUBLE (Accelerated Platelet Inhibition by a Double Dose of Clopidogrel According to Gene Polymorphism) study [34], we reported 21.1% prevalence of HPR (5 μM ADP-induced Agg_{max} >50%) in PCI-treated patients taking a double dose of clopidogrel (150 mg day^{-1}). If we adapted the consensus-defined criteria of HPR (5 μM ADP-induced Agg_{max} >46%), the frequency of HPR reached 29.4%: 13.0%, 33.3% and 55.0% in 0, 1 and 2 carriers of the *CYP2C19* LOF allele(s) ($P < 0.001$). These findings may explain partly the modest effect of double dose clopidogrel on the risk of HPR and clinical outcomes in the GRAVITAS (Gauging Responsiveness with A VerifyNow assay-Impact on Thrombosis And Safety) trial [35]. In this ACCEL-TRIPLE study, ~ 20% of the *CYP2C19* PMs were stratified into HPR even though they were receiving triple antiplatelet therapy. However, because the prevalence of the *CYP2C19* PMs among African and Caucasian is much less (<5%) [31, 36], the estimated risk may be low in these populations. Moreover, some studies have suggested that East Asians could have a different cutoff of HPR (≥275 PRU) for predicting post PCI ischaemic events [37, 38]. If we apply this cutoff of HPR for the ACCEL-TRIPLE study, most of the patients achieved adequate platelet inhibition (~95%).

Table 3

Platelet reactivity and rate of HPR according to metabolic phenotype

	CYP2C19 Extensive metabolizer (*n* = 48)	Intermediate metabolizer (*n* = 54)	Poor metabolizer (*n* = 25)	*CYP3A5* *1/*1 (*n* = 10)	*1/*3 (*n* = 43)	*3/*3 (*n* = 74)	*ABCB1 3435*C > T CC (*n* = 52)	CT (*n* = 62)	TT (*n* = 13)
light transmittance aggregometry									
5 μM ADP-agg$_{max}$ (%)									
Mean ± SD	24.6 ± 13.3	28.7 ± 12.2	32.3 ± 15.7	27.0 ± 14.9	28.4 ± 13.6	27.7 ± 13.5	27.4 ± 13.8	27.9 ± 13.7	29.7 ± 12.5
95% CI	20.8, 28.5	25.4, 32.0	25.8, 38.7	16.3, 37.6	24.2, 32.6	24.5, 30.8	23.5, 31.2	24.4, 31.4	22.1, 37.3
***P* value (ANOVA)**		**0.062**			**0.934**			**0.855**	
***P* value (Jonckheere-Terpstra)**		**0.016**			**0.678**			**0.538**	
5 μM ADP-Agg$_{late}$ (%)									
Mean ± SD	12.1 ± 12.2	16.4 ± 11.1	21.0 ± 14.0	14.9 ± 10.2	15.9 ± 13.8	15.6 ± 12.1	15.4 ± 13.2	16.2 ± 12.6	14.1 ± 9.2
95% CI	8.6, 15.7	13.3, 19.4	15.2, 26.7	7.6, 22.2	11.7, 20.2	12.8, 18.4	11.8, 19.1	13.0, 19.4	8.5, 19.7
***P* value (ANOVA)**		**0.021**			**0.972**			**0.846**	
***P* value (Jonckheere-Terpstra)**		**0.002**			**0.933**			**0.859**	
20 μM ADP-Agg$_{max}$ (%)									
Mean ± SD	34.2 ± 16.7	41.7 ± 14.2	44.9 ± 17.0	41.4 ± 19.6	40.2 ± 16.5	38.8 ± 15.7	39.3 ± 16.5	38.7 ± 16.3	44.1 ± 14.6
95% CI	29.3, 39.0	37.8, 45.6	37.9, 51.9	27.3, 55.4	35.1, 45.3	35.2, 42.5	34.7, 43.9	34.5, 42.8	35.3, 52.9
***P* value (ANOVA)**		**0.007**			**0.845**			**0.551**	
***P* value (Jonckheere-Terpstra)**		**0.003**			**0.601**			**0.596**	
20 μM ADP-Agg$_{late}$ (%)									
Mean ± SD	18.5 ± 17.4	27.1 ± 16.8	32.9 ± 18.1	28.8 ± 21.4	24.3 ± 19.7	24.8 ± 16.7	25.0 ± 18.6	24.1 ± 17.6	28.7 ± 18.4
95% CI	13.4, 23.5	22.5, 31.6	25.4, 40.4	13.5, 44.1	18.2, 30.4	21.0, 28.7	19.8, 30.2	19.7, 28.6	17.6, 39.8
***P* value (ANOVA)**		**0.002**			**0.775**			**0.713**	
***P* value (Jonckheere-Terpstra)**		**<0.001**			**0.990**			**0.696**	
VerifyNow P2Y12 assay									
P2Y12 reaction units									
Mean ± SD	125 ± 76	188 ± 75	226 ± 88	164 ± 75	173 ± 92	172 ± 86	172 ± 88	169 ± 86	186 ± 91
95% CI	103, 147	168, 209	190, 262	111, 218	145, 201	152, 192	147, 196	147, 191	131, 241
***P* value (ANOVA)**		**<0.001**			**0.958**			**0.824**	
***P* value (Jonckheere-Terpstra)**		**<0.001**			**0.975**			**0.880**	
HPR (5 μM ADP-induced Agg$_{max}$ >46%)									
***n* (%)**	3 (6.3)	4 (7.4)	5 (20.0)	1 (10.0)	5 (11.6)	6 (8.1)	7 (13.5)	4 (6.5)	1 (7.7)
***P* value**		**0.099**			**0.620**			**0.279**	

CYP, cytochrome P450; ABCB1, ATP-binding cassette sub-family B member 1; ADP, adenosine diphosphate; Agg$_{max}$, maximal platelet aggregation; Agg$_{late}$, late platelet aggregation at 5 min; HPR, high on-treatment platelet reactivity.

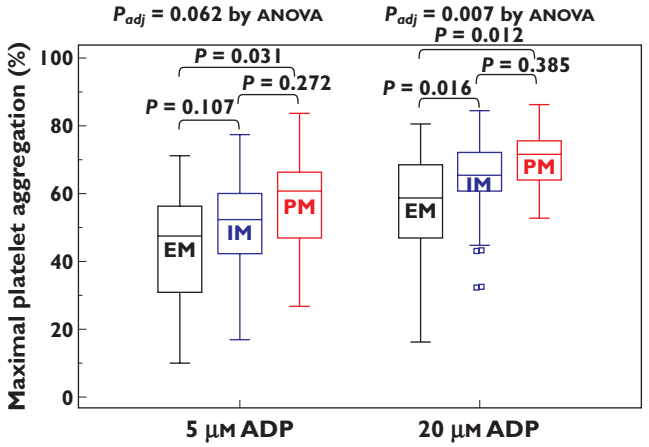

Figure 2

Maximal platelet aggregation according to *CYP2C19* metabolic phenotype. EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; ADP, adenosine diphosphate. The central box represents the values between the lower and upper quartiles and the middle line is the median. The vertical line extends from the minimum to the maximum value, excluding outside values, which are displayed as separate points

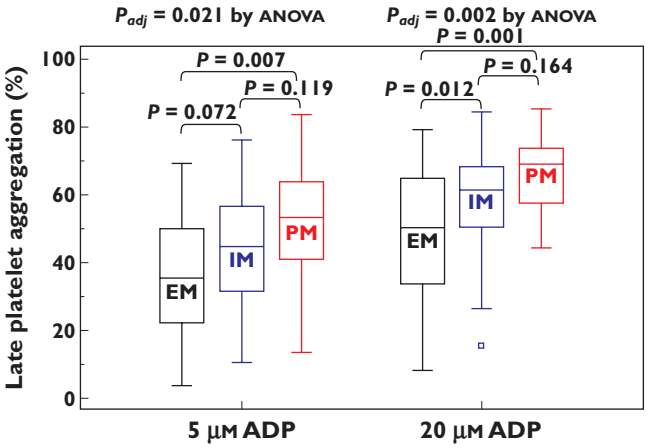

Figure 3

Late platelet aggregation according to *CYP2C19* metabolic phenotype. EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; ADP, adenosine diphosphate. The central box represents the values between the lower and upper quartiles and the middle line is the median. The vertical line extends from the minimum to the maximum value, excluding outside values, which are displayed as separate points

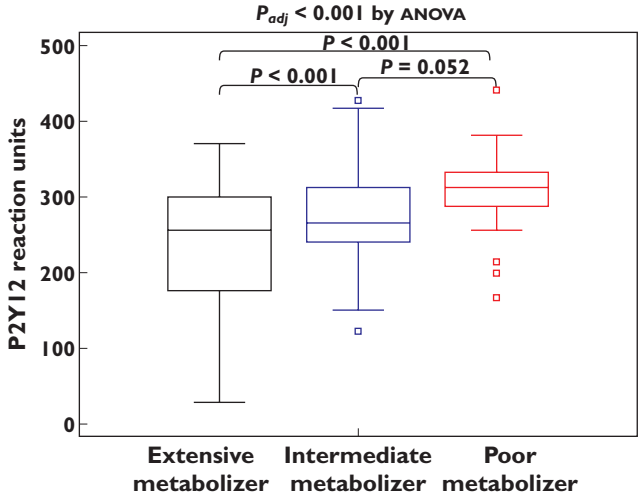

Figure 4

P2Y12 reaction units according to *CYP2C19* metabolic phenotype. The central box represents the values between the lower and upper quartiles and the middle line is the median. The vertical line extends from the minimum to the maximum value, excluding outside values, which are displayed as separate points

Compared with clopidogrel, new P2Y12 receptor antagonists have shown the consistently predictable and potent pharmacokinetic and pharmacodynamic profiles and impacting clinical outcomes, irrespective of genetic polymorphisms [21, 39, 40]. However, P2Y12 receptors play a limited role in ADP-induced platelet aggregation [41] and post PCI ischaemic event recurrence has still been observed during potent P2Y12 receptor inhibitor administration. Furthermore, clinical introduction of these regimens is inevitably accompanied by the increased risk of major bleeding and bleeding risk seems higher in Asians according to the subanalysis [42]. More interestingly, adjunctive cilostazol with dual antiplatelet therapy did not increase the risk of major bleeding in several registries and prospective trials [7–15]. The endothelium-targeted antithrombotic effect and reversible antiplatelet properties (such as ticagrelor) of cilostazol may explain the safe profile for bleeding [5]. In addition, pleiotropic effects of cilostazol including endothelium [6–9, 11, 12, 15, 43], inflammation and ischaemia-reperfusion injury [44, 45] may also influence clinical outcomes in PCI-treated patients. Therefore, when we take into account both post-PCI efficacy and safety, adjunctive cilostazol with standard antiplatelet therapy can be a considerable antiplatelet regimen.

However, this concept of adjunctive cilostazol has not been supported by the result of the CILON-T (Influence of CILostazol-based triple antiplatelet therapy ON ischaemic complication after drug-eluting stenT implantation) prospective trial [46]. This study enrolled relatively low risk patients treated with a drug-eluting stent (~10%: positive cardiac enzyme), and the 6 month composite of cardiac death, nonfatal myocardial infarction and ischaemic stroke was quiet low (~2.8%). In addition, adjunctive cilostazol could not reduce the risk of restenosis. Although the GRAVITAS and CURRENT-OASIS 7 (double dose *vs.* standard dose clopidogrel and high dose *vs.* low dose aspirin in individuals undergoing percutaneous coronary intervention for acute coronary syndromes) trials including PCI-treated patients compared the efficacy and safety of the same regimens (standard dose *vs.* double dose clopi-

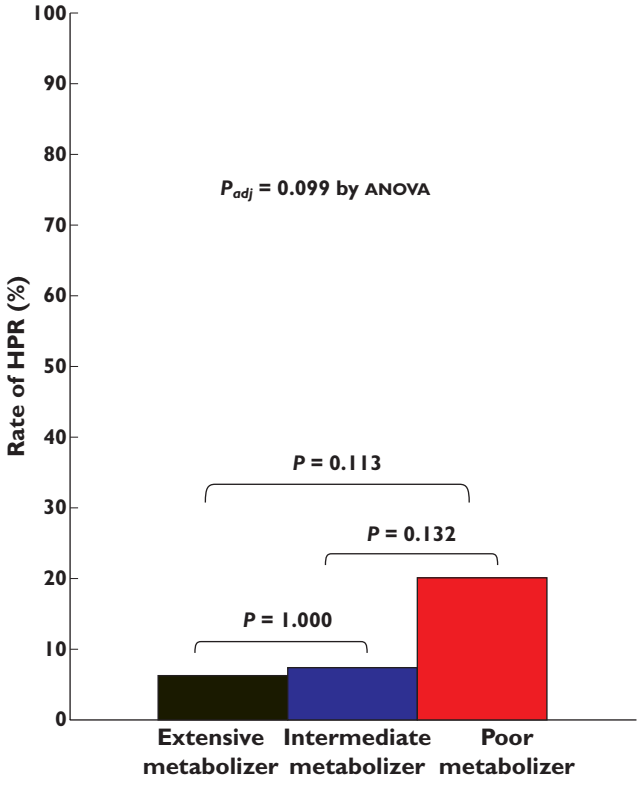

Figure 5

Rate of HPR according to *CYP2C19* metabolic phenotype. HPR indicates high on-treatment platelet reactivity (5 μ ADP-induced maximal platelet aggregation >46%)

dogrel) [36, 47], the latter only could suggest the beneficial effect of double dose clopidogrel in reduction of ischaemic events. Contrary to the GRAVITAS trial, the CURRENT-OASIS 7 trials enrolled ACS patients with high risk profiles, and started double dose therapy before coronary angiography, implicating that clinical outcome in antiplatelet therapy trials can be changed depending on the study design. Future clinical trials using cilostazol need to incorporate a study design including high risk patients and evaluation of front-loaded effect.

To date, there have been no definite guidelines of P2Y12 blockade in the *CYP2C19* PMs. The GIFT (Genotype Information and Functional Testing) demonstrated that doubling of clopidogrel dose cannot overcome the influence of *CYP* LOF allele, especially in PMs, based on pharmacodynamic and clinical outcomes [48]. New potent P2Y12 inhibitors can be used as alternative drugs in PMs [49, 50]. However, exploratory analysis including non-ST elevation ACS patients showed no difference in the occurrence of ischaemic events with prasugrel *vs.* clopidogrel in carriers of the *CYP2C19* LOF allele (HR 0.98, 95% CI 0.80, 1.20) [49]. The present study also suggested that adding cilostazol may be a useful regimen in PMs. Pharmacodynamic profiles of several antiplatelet regimens currently have been extrapolated into clinical scenarios. Because clinical benefits of theses regimens cannot be explained by their antiplatelet effect alone, superiority of one regimen over another must be determined based on large-scale clinical trials.

The present study has several limitations. First, because this observational, single centre study included only a small number to compare the genetic influences on platelet reactivity, it may be underpowered to elucidate significant differences of clinical efficacy and safety. Second, this study was performed using candidate gene analysis and other genetic variants may be relevant in risk stratification. Third, the sample size was calculated solely based on the hypothesized influence of *CYP2C19* alleles without considering the other two genetic variants. However, the results of this analysis justified our assumption. Finally, this study enrolled East Asians only, and the pharmacodynamics of triple antiplatelet therapy may be different in other ethnic groups. Contrary to other clinical risk factors, there are remarkable differences in BMI and the *CYP2C19* genotype between Caucasians and East Asians. Among East Asians, low BMI (about 24 kg m^{-2} in this study) can increase the antiplatelet effect of clopidogrel, whereas the influence of the *CYP2C19* LOF allele can decrease the effect. In addition, carriage of the *CYP2C19*17* gain-of-function allele seems uncommon among the Korean population (~2%) [37]. Taken together, the antiplatelet effect of clopidogrel appears to be low in East Asians, and response to triple antiplatelet therapy may be somewhat greater in Western populations [16].

In conclusion, among PCI-treated high risk patients, the antiplatelet effect of triple antiplatelet therapy is influenced by carriage of the *CYP2C19* LOF allele. Its clinical benefit in PMs needs to be validated in future large scale trials.

Competing Interests

Dr Jeong received honoraria for lectures from Sanofi-Aventis, Daiichi Sankyo Inc and Otsuka. Other authors declared no conflict of interest.

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