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Comparison of antiplatelet treatment in patients with clopidogrel nonresponders with or without carriage of *CYP2C19* polymorphism

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Background/Aims: Several interventions exist for overcoming high platelet reactivity (HPR) on clopidogrel therapy. The goal of this study was to identify strategies that improve inhibition of platelet reactivity in clopidogrel nonresponders with or without loss of function *CYP2C19* genotypes, resulting in platelet reactivity similar to that in responders.

Methods: A total of 376 patients with stenting for coronary artery disease underwent platelet function testing in three centers. Blinded platelet function tests were performed after 75 mg daily clopidogrel treatment for 28 days. In total, 183 nonresponders were genotyped, were randomized to four treatment groups with each treatment lasting approximately 28 days, and underwent repeated measurements of platelet reactivity after treatment.

Results: With 75 mg of daily clopidogrel, nonresponders had significantly higher HPR than did responders (multiple electrode aggregometry [MEA, arbitrary platelet aggregation unit]: mean, 71.4; 95% confidence intervals [CI], 68.6 to 74.3; and mean, 27.5; 95% CI, 26.0 to 28.9, respectively; $p < 0.001$). Ticagrelor or ticlopidine treatment in nonresponders resulted in platelet reactivity similar to that in responders in intermediate metabolizers (mean, 24.0; 95% CI, 19.6 to 28.4; $p > 0.05$; and mean, 30.0; 95% CI, 24.7 to 37.5; $p > 0.05$, respectively) and poor metabolizers (mean, 23.2; 95% CI, 18.0 to 28.3; $p > 0.05$; and mean, 30.3; 95% CI, 24.5 to 6.0; $p > 0.05$, respectively). However, in extensive metabolizers, only ticagrelor treatment showed platelet reactivity similar to that in responders (mean, 26.1; 95% CI, 24.1 to 28.0; $p > 0.05$).

Conclusions: Among clopidogrel nonresponders with cardiovascular disease on 75 mg daily clopidogrel, ticagrelor resulted in a comparable degree of platelet inhibition in all nonresponders compared with 150 mg daily clopidogrel or triple therapy with clopidogrel and cilostazol, irrespective of phenotype.

Keywords: Stable cardiovascular disease; Clopidogrel; Cytochrome 2C19 polymorphism

INTRODUCTION

Clopidogrel, an oral thienopyridine antiplatelet drug, has been reported to be effective when combined with aspirin in preventing atherothrombotic events in patients with acute coronary syndrome (ACS), particular when percutaneous coronary intervention (PCI) is performed [1].

Dual antiplatelet therapy with aspirin and standard 75 mg maintenance doses of clopidogrel has become the cornerstone of medical regimens for prevention of ischemic events in patients undergoing PCI with stent placement. However, variability in pharmacodynamic response to clopidogrel is well-recognized, and patients with high platelet reactivity (HPR) have an increased risk of adverse cardiovascular events [2].

Clopidogrel biotransformation is mainly mediated by the hepatic cytochrome P450 (CYP) enzymes. Recent studies have shown that both heterozygous and homozygous loss of function (LOF) allele carriers have a marked decrease in platelet response with the standard 75 mg maintenance dose of clopidogrel [3,4], and higher rates of adverse cardiovascular events compared with non-carriers [5].

Guidance is needed regarding optimal treatment strategies in patients with high clopidogrel on-treatment platelet reactivity with or without *CYP2C19* LOF polymorphism.

Although various P2Y12 inhibitors that might be unaffected by the *CYP2C19* genotype are available, these medications may be expensive and not globally accessible, particularly in Asia. In contrast, clopidogrel, cilostazol, and ticlopidine are widely available.

Therefore, we conducted repeated measurements of platelet reactivity in patients with HPR with or without *CYP2C19* LOF polymorphism to identify the optimal treatment strategy among 150 mg maintenance doses of clopidogrel, 75 mg daily clopidogrel, 100 mg twice-daily cilostazol, 250 mg twice-daily ticlopidine, and 90 mg twice-daily ticagrelor.

METHODS

Patients were considered eligible to be enrolled if they had coronary artery disease with stenting and were on daily 75 mg clopidogrel. To be eligible, patients needed to have an indication for the use of clopidogrel (a
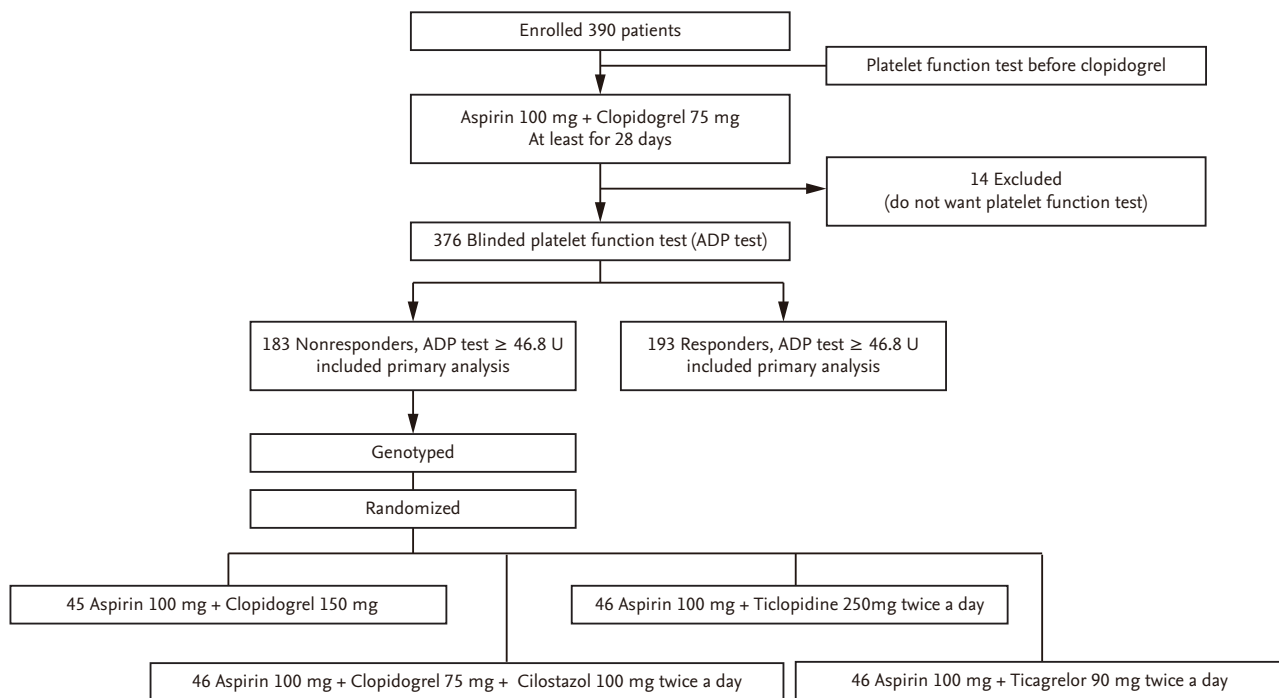
Figure 1. Patient flow diagram. ADP, adenosine diphosphate.

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myocardial infarction and/or PCI ≥ 4 weeks and ≤ 6 months prior to enrollment) and had to have a clinically stable state. In total, 390 patients from three sites were screened. A patient enrollment flow diagram is shown in Fig. 1. All patients were requested to continue daily 100 mg aspirin during the study. Key exclusion criteria were the use of anticoagulants or proton pump inhibitors, current smoking, prior stent thrombosis, heightened risk of bleeding, end-stage renal or hepatic disease, or a procedure or hospitalization scheduled for the 12 subsequent weeks.

Study protocol

Baseline clinical evaluation was performed at the time of enrollment, and each patient underwent blood sampling for blinded platelet function testing. Blood sampling was done 2 hours after but not more than 3 hours after the last drug dose.

Platelet function was assessed using multiple electrode aggregometry (MEA) (Multiplate analyzer, Dynabyte, Munich, Germany). This system detects changes in electrical impedance due to adhesion and aggregation of platelets on two independent electrode-set surfaces in the test cuvette. A 1:2 dilution of whole blood treated with the anticoagulant hirudin and 0.9% NaCl was stirred at 37°C for 3 minutes in the test cuvettes, 6.4 μM adenosine diphosphate (ADP) and 9.4 nM prostaglandin E1 were added, and increase in electrical impedance was recorded continuously for 6 minutes. Mean values of two independent determinations were expressed as the area under the curve (AUC) of the aggregation tracing. Impedance measured with MEA was transformed to arbitrary aggregation units (U) which were plotted against time (U·min). We reported AUC in units (U). Good reproducibility of MEA measurements has been reported previously (< 6% variability) [6]. All measurements were obtained by laboratory personnel who were unaware of results of phenotyping or clinical outcomes of patients. Laboratory imprecision, measured as a coefficient of variation (CV) of MEA measurements, was determined by assessing samples five times from healthy subjects (controls) and from ACS patients on dual antiplatelet therapy. The mean CV of ADP MEA measurement was 3.9% in controls and 5.8% in ACS patients.

Genotyping was performed at the Green Cross Reference Laboratory in Korea. The base numbering and allele definitions followed the nomenclature of the Human *CYP* Allele Nomenclature Committee, and the *CYP2C19* genotypes for *CYP2C19*2* (rs4244285, c. 681G>A, p. P227P) and *CYP2C19*3* (rs4986893, c. 636G>A, p. W212X) were determined using the polymerase chain reaction (PCR)–SNaPshot method (Applied Biosystems, Foster City, CA, USA) using genomic DNA isolated from leukocytes of peripheral venous blood with an extraction kit (QIAamp® DNA Blood Mini Kit, Qiagen, Hilden, Germany). The genomic DNA region containing one of the two single nucleotide polymorphisms (SNPs) was amplified with PCR separately. The PCR product was processed as per the ABI SNaPshot protocol using primers designed for fluorescent dideoxy nucleotide termination. SNP analysis was carried out using the ABI 3100 genetic analyzer. In this study, *CYP2C19* genotyping of HPR patients revealed three phenotypes: extensive metabolizers (**1/*1*), intermediate metabolizers (**1/*2* and/or **1/*3*), and poor metabolizers (**2/*2*, **2/*3*, and/or **3/*3*).

After blinded platelet function testing, patients were classified as responders or nonresponders. Nonresponder status has been specified based on a platelet aggregation value of 46.8 U or greater [7]. We used 47 U as the cutoff value for nonresponders, because platelet reactivity measured using the MEA kit shows the resulting numerical value as an integer. All the nonresponders underwent genotyping and repeated measurements of platelet reactivity after sequential maintenance doses of the four treatment strategies. Treatment strategies were 150 mg daily clopidogrel, triple antiplatelet therapy with clopidogrel 75 mg once and cilostazol 100 mg twice daily, ticlopidine (Clid®, Yuyu Pharmaceutical Inc., Seoul, Korea) 250 mg twice daily, or ticagrelor 90 mg twice daily, each lasting approximately 28 ± 4 days. All responders were included in the primary analysis without genotyping. At the end of each treatment period, ischemia, bleeding, and other adverse events were ascertained. After the last study drug treatment period, patients were recommended to follow the treatment strategy showing the best results.

This study was conducted in a manner consistent with ethical principles based on the Declaration of Helsinki. Informed consent was obtained from all participants. This study was approved by the Institutional Review Board of each participating hospital.

End points

The primary endpoint was platelet reactivity during the study drug administration (platelet reactivity index [PRI]). PRI was obtained at each site with encrypted point-of-care, MEA, and reported as an arbitrary unit (U). Patients suffering from fatal cardiovascular or cerebrovascular events who needed medical treatment or admission and those who met the criteria for bleeding requiring medical attention were assessed at each visit. Adverse events and serious adverse events were documented.

Adherence

Standard pill count was used to assess adherence. At each visit, the number of remaining pills in the containers was counted. Adherence was defined by the number of pills taken (dispensed pills–returned pills) relative to the theoretical number of prescribed doses.

Statistical analysis

Continuous data were represented as mean ± standard deviation (SD), and categorical data were represented as counts and percentages unless otherwise specified. The analytic dataset consisted of all patients who had successfully completed platelet function testing. No imputation was applied for missing data, and platelet function data were analyzed per protocol. Comparisons of baseline characteristics between responders and nonresponders were done using Student t test or Pearson chi-square test as appropriate. Comparisons of baseline characteristics among responders and nonresponders with the four genotypes were done using either one-way analysis of variance (ANOVA) followed by Dunnett's multiple-comparison test if significant differences were detected, or Pearson chi-square/Kolmogorov tests depending on the type of variable. Association of the different treatment strategies with platelet reactivity among the nonresponders (with heterozygotes and homozygotes tested separately and combined) and responders was evaluated by using a repeated mixed model with patient response as a variable effect and treatment strategy as a fixed effect. Least square means (LSMs) and differences between means were calculated. A linear mixed model was also used to test equality of differences in LSMs among the treatment strategies in each genotype with patient response as a variable effect and treatment strategy as a

fixed effect, followed by the Tukey-Kramer test to compare LSM of each treatment strategy in responders after 75 mg daily clopidogrel intake. Differences in the LSMs were calculated and reported with 95% confidence intervals (CIs). Proportions of nonresponders in each treatment strategy were compared using the Bonferroni test. Multiple logistic regression analysis was performed to determine independent risk factors for distinguishing responders from nonresponders with the $CYP2C19 *1/*1$ genotype. Comparisons of clinical events between the responder group and each of the nonresponder groups were done using either the Student t test or ANOVA as appropriate.

To attain at least 80% power to detect a 13.7 U difference in the paired U means of the treatment strategies with ADP-induced platelet aggregation (which corresponds to the observed difference seen in a similar study that compared the results of 20 μM ADP-induced platelet aggregation with 75 mg clopidogrel or ticlopidine in homozygous nonresponders) [8], and maintaining an overall α of 0.0125 for the study, at least 29 homozygous patients were required. In this calculation, the standard deviation of the difference was assumed to be 20%. Based on an estimate of the proportion of Korean patients carrying mutant alleles of $CYP2C19*1/*1$ (50%), $CYP2C19*1/*2$ and $*1/*3$ (36.5%), and $CYP2C19*2/*2$, $*2/*3$, and $*3/*3$ (13.5%) [9], the total sample sizes needed were at least 108, 75, and 29, respectively. We enrolled 30% more patients than the calculated sample size considering follow-up loss, and because we did not know the exact proportion of nonresponders with the mutant allele $CYP2C19*1/*1$, we enrolled an additional 30% patients with this genotype. All statistical analyses were performed with the software SAS version 9.2 (SAS Inc., Cary, NC, USA). All analyses were two-tailed, and a p value of less than 0.05 was considered as the threshold for statistical significance.

RESULTS

In total, 376 patients were screened, and blood samples were obtained from all patients for blinded platelet function testing to identify response to clopidogrel. Their mean ± SD age was 64.8 years, 61.7% were male, and 33.5% had a history of myocardial infarction. A total of 193 pa-

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Table 1. Patient baseline characteristics

Characteristic	Overall (n = 373)	Responders (n = 193)	Nonresponders (n = 183)	p value
Age, yr	64.8 ± 11.6	63.8 ± 11.5	66.1 ± 11.9	0.0671
Male sex	232 (61.7)	122 (63.2)	110 (60.1)	0.5361
Weight, kg	162.4 ± 8.1	162.7 ± 7.7	162.1 ± 8.6	0.5389
Height, cm	67.5 ± 15.9	66.8 ± 16.5	68.1 ± 15.2	0.4212
Body mass index[a], kg/m²	25.1 ± 3.7	24.6 ± 3.3	25.7 ± 4.0	0.0046
SBP, mmHg	127.4 ± 18.6	127.6 ± 19.1	127.2 ± 18.3	0.8257
DBP, mmHg	77.8 ± 11.2	78.9 ± 11.1	76.7 ± 11.3	0.0505
Heart rate, /min	74.5 ± 12.3	74.5 ± 11.6	74.4 ± 12.9	0.9356
Hypertension	255 (67.8)	134 (69.4)	121 (66.1)	0.4923
Diabetes mellitus	131 (34.8)	61 (31.6)	70 (38.3)	0.1765
Hyperlipidemia	252 (67.6)	122 (63.5)	130 (71.0)	0.1222
Family history	25 (6.7)	13 (6.7)	14 (7.7)	0.7314
History of MI	125 (33.5)	65 (33.7)	63 (34.4)	0.8785
History of PCI	312 (83.6)	168 (87.1)	144 (78.7)	0.0311
History of CABG	36 (9.7)	14 (7.3)	22 (12.0)	0.1163

Values are presented as mean ± SD or number (%).
SBP, systolic blood pressure; DBP, diastolic blood pressure; MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft.
[a]Calculated as weight in kilograms divided by height in meters squared.

Table 2. Patient baseline characteristics on phenotypes

Characteristic	Responder (n = 193)	Non-responder (n = 183)			p value[a]
		EM (n = 49)	IM (n = 97)	PM (n = 37)	
Age, yr	66.8 ± 16.5	71.8[b] ± 8.8	64.05 ± 11.1	66.4 ± 13.9	< 0.0001
Male sex	122 (63.2)	31 (63.3)	56 (56.5)	23 (62.2)	0.8120
Weight, kg	162.7 ± 7.7	161.6 ± 7.4	161.7 ± 7.9	162.6 ± 10.4	0.6144
Height, cm	66.8 ± 16.5	73.7[b] ± 15.4	64.9 ± 13.8	66.3 ± 13.2	0.0221
Body mass index[c], kg/m²	24.6 ± 3.3	28.4[b] ± 4.2	24.4 ± 3.5	25.1 ± 3.4	< 0.0001
SBP, mmHg	127.6 ± 19.1	131.8 ± 19.9	127.9 ± 19.6	121.2 ± 14.6	0.2311
DBP, mmHg	78.9 ± 11.1	78.0 ± 12.5	77.8 ± 11.1	71.0 ± 9.5	0.0021
Heart rate, /min	74.5 ± 11.6	76.0 ± 13.3	73.1 ± 12.9	73.9 ± 13.9	0.7123
Hypertension	134 (69.4)	35 (71.4)	64 (64.5)	22 (59.5)	0.6932
Diabetes mellitus	61 (31.6)	22 (44.9)	34 (34.8)	14 (37.8)	0.5193
Hyperlipidemia	122 (63.5)	39 (79.6)	66 (71.9)	25 (67.6)	0.2034
Family history of CAD	13 (6.7)	1 (2.0)	4 (2.8)	9 (24.3)	0.0032
History of MI	65 (33.7)	11 (22.5)	35 (36.8)	17 (46.0)	0.3012
History of PCI	168 (87.1)	37 (75.5)	82 (84.4)	25 (67.6)	0.0394
History of CABG	14 (7.3)	5 (10.2)	12 (15.8)	5 (13.5)	0.1467

Values are presented as mean ± SD or number (%).
EM, extensive metabolizers; IM, intermediate metabolizers: PM, poor metabolizers; SBP, systolic blood pressure; DBP, diastolic blood pressure; CAD, coronary artery disease; MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft.
[a]p values are calculated by analysis of variance, Pearson chi-square test, or Fisher exact test as appropriate.
[b]p < 0.05, Dunnett's test compared with responder group.
[c]Calculated as weight in kilograms divided by height in meters squared.

tients were responders, whereas 183 were nonresponders (49 extensive metabolizers, CYP2C19*1/*1; 97 intermediate metabolizers, 71 CYP2C19*1/*2 and 26 CYP2C19*1/*3; and 37 poor metabolizers, CYP2C19*2 and/or *3). Clinical characteristics did not differ between the responder and nonresponder groups (Tables 1 and 2). Adherence rates for the treatment strategies 150 mg daily clopidogrel, triple therapy, ticlopidine 250 mg twice daily, or ticagrelor 90 mg twice daily among nonresponders on the study drug were 97.6%, 98.2%, 98.7%, and 98.1%, respectively.

Response to interventions

When treated with a standard clopidogrel maintenance dose of 75 mg daily, platelet reactivity values in nonresponders were higher than those in responders on average (mean ADP test, 71.4 U, 95% CI, 68.6 to 74.3; and 27.5 U, 95% CI, 26.0 to 28.9, respectively; $p < 0.001$); this observation held true in all three phenotypes (65.7 U, 95% CI, 60.9 to 70.5 in extensive metabolizers; 70.8 U, 95% CI, 64.8 to 76.9 in intermediate metabolizers; and 81.2 U, 95% CI, 74.1 to 88.2 in poor metabolizers; $p < 0.001$ for all comparisons). Each treatment strategy resulted in an approximate 22.3% to 63.4% absolute reduction in platelet reactivity, with some but not statistically significant reversal between the first two strategies. Among nonresponders, interventions with 150 mg clopidogrel, triple therapy, ticlopidine, or ticagrelor produced significant reductions in platelet reactivity on average (55.5 U,

95% CI, 52.4 to 58.7; 58.0 U, 95% CI, 54.6 to 61.3; 32.8 U, 95% CI, 30.2 to 35.4; and 26.1 U, 95% CI, 24.1 to 28.1; $p < 0.001$ for all comparisons); this observation also held true in extensive metabolizers (49.2 U, 95% CI, 44.7 to 53.8; 55.4 U, 95% CI, 49.0 to 61.8; 37.9 U, 95% CI, 33.1 to 42.6; and 31.9 U, 95% CI, 28.7 to 35.1; $p < 0.001$), in intermediate metabolizers (54.1 U, 95% CI, 46.9 to 61.2; 56.7 U, 95% CI, 48.6 to 64.8; 30.0 U, 95% CI, 24.7 to 37.5; and 24.0 U, 95% CI, 19.6 to 28.4; $p < 0.001$), and in poor metabolizers (69.5 U, 95% CI, 61.5 to 77.5; 66.6 U, 95% CI, 58.2 to 75.0; 30.3 U, 95% CI, 24.5 to 36.0; and 23.2 U, 95% CI, 18.0 to 28; $p < 0.001$) (Table 3, Fig. 2). When we compared only platelet reactivity between responders and nonresponders with or without LOF alleles, ticagrelor similarly produced significant reductions in platelet reactivity on average (Table 4). For nonresponders on 75 mg clopidogrel, proportions of nonresponders after each treatment were significantly reduced to 64.4% (116/180) with 150 mg clopidogrel, to 68.4% (121/177) with triple therapy, to 22.9% (25/175) with ticlopidine, and to 8.0% (24/168) with ticagrelor treatment. The risk ratios for nonresponder status with 150 mg daily clopidogrel, triple therapy, ticlopidine, or ticagrelor were 0.64 (95% CI, 0.58 to 0.72), 0.68 (95% CI, 0.62 to 0.76), 0.23 (95% CI, 0.17 to 0.30), and 0.08 (95% CI, 0.05 to 0.14), respectively. Proportions of nonresponders after the four above interventions, respectively based on genotype were 53.1%, 60.4%, 27.7%, and 8.9% in CYP2C19*1/*1; 64.3%, 69.6%,
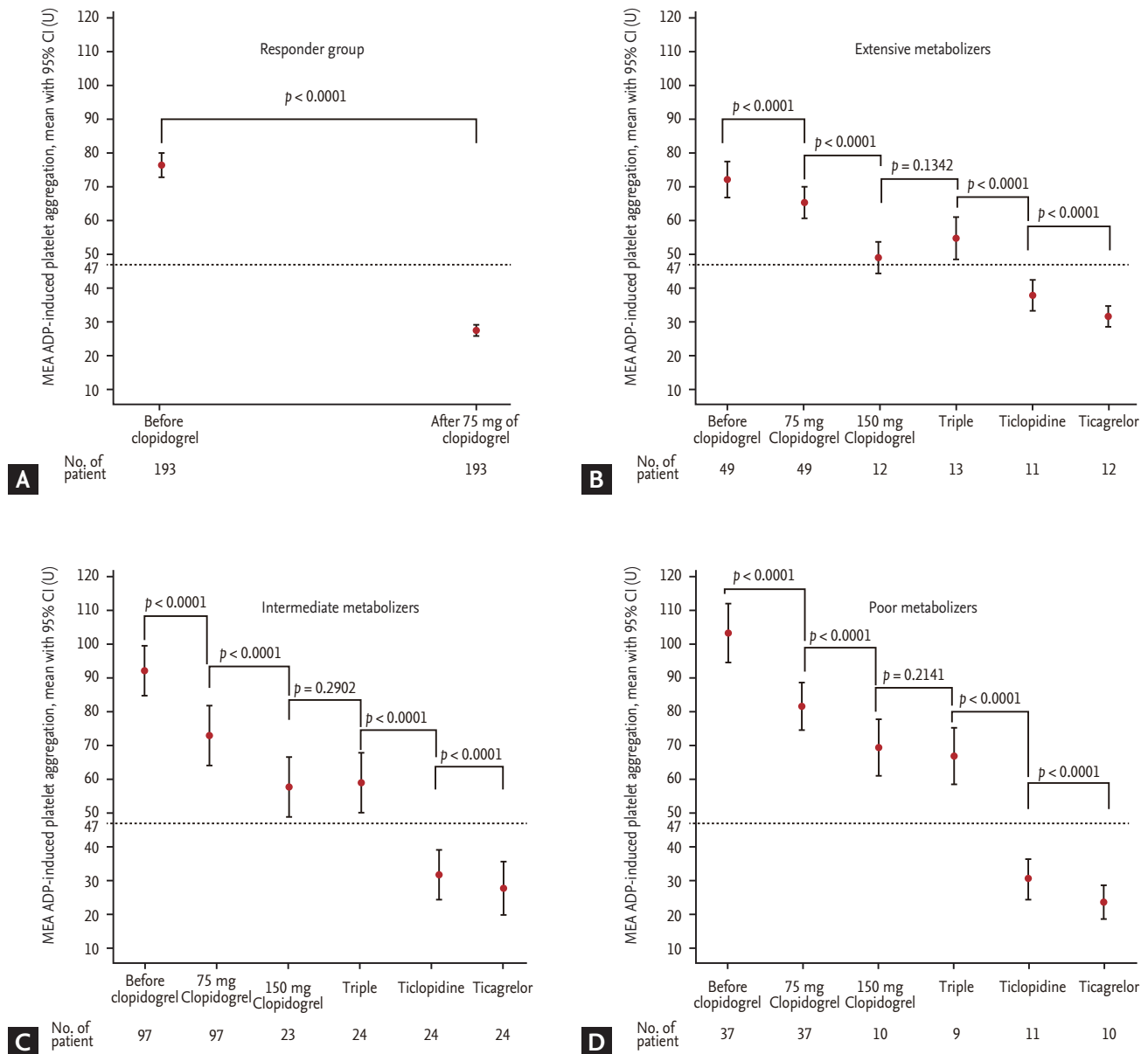
Table 3. On-treatment platelet reactivity

	Before clopidogrel	75 mg Clopidogrel	150 mg Clopidogrel	Triple	Ticlopidine	Ticagrelor	p value[a]
Responder	76.3 (72.8–79.8)	27.5 (26.0–28.9)					< 0.001
No. of patients	193	193					
Nonresponders	90.2 (86.6–93.9)	71.4 (68.6–74.3)	55.5 (52.4–58.7)	58.0 (54.6–61.3)	32.8 (30.2–35.4)	26.1 (24.1–28.0)	< 0.001
No. of patients	183	183	45	46	46	46	
EM	72.9 (67.5–78.4)	65.7 (60.9–70.5)	49.2 (44.7–53.8)	55.4 (49.0–61.8)	37.9 (33.1–42.6)	31.9 (28.7–35.1)	< 0.001
No. of patients	49	49	12	13	11	12	
IM	97.9 (88.6–103.2)	70.8 (64.8–76.9)	54.1 (46.9–61.2)	56.7 (48.6–64.8)	30.0 (24.7–37.5)	24.0 (19.6–28.4)	< 0.001
No. of patients	97	97	23	24	24	24	
PM	108.4 (94.8–112.1)		69.5 (61.5–77.5)	66.6 (58.2–75.0)	30.3 (24.5–36.0)	23.2 (18.0–28.3)	< 0.001
No. of patients	37	37	10	9	11	10	

Values are presented as mean (adenosine diphosphate-induced platelet reactivity index, 95% confidence interval).

EM, extensive metabolizers; IM, intermediate metabolizers: PM, poor metabolizers.

[a]p value represents result of before clopidogrel versus the result after antiplatelet treatment.

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Figure 2. On-treatment platelet reactivity across phenotypes and treatments. Dotted line represents cutoff value of nonresponder. (A) Platelet reactivity on responder group. (B) Platelet reactivity on extensive metabolizers. (C) Platelet reactivity on intermediate metabolizers. (D) Platelet reactivity on poor metabolizers. MEA, multiple electrode aggregometry; ADP, adenosine diphosphate; CI, confidence interval.

16.2%, and 4.6% in CYP2C19*1/*2; 56.0%, 60.0%, 24%, and 13% in *CYP2C19*1/*3*; and 86.1%, 82.9%, 28.6%, and 11.8% in homozygotes (Fig. 3).

Among patients treated with 150 mg daily clopidogrel or triple therapy, although the response to triple therapy was somewhat higher than that to 150 mg daily clopidogrel without statistically significant differences ($p > 0.05$),

the proportion of nonresponders and platelet reactivity in nonresponders with 75 mg daily clopidogrel showed a significant reduction. However, the responses were still inferior to those seen with ticlopidine or ticagrelor treatment in all genotypes in nonresponders ($p < 0.001$ for all).

Table 4. On-treatment platelet reactivity (responder vs. nonresponder with or without LOF alleles)

	Before clopidogrel	75 mg Clopidogrel	150 mg Clopidogrel	Triple	Ticlopidine	Ticagrelor	p value[a]
Responder	76.3 (72.8–79.8)	27.5 (26.0–28.9)					< 0.001
No. of patients	193	193					
Nonresponders	90.2 (86.6–93.9)	71.4 (68.6–74.3)	55.5 (52.4–58.7)	58.0 (54.6–61.3)	32.8 (30.2–35.4)	26.1 (24.1–28.0)	< 0.001
No. of patients	183	183	45	46	46	46	
Without LOF alleles	72.9 (67.5–78.4)	65.7 (60.9–70.5)	49.2 (44.7–53.8)	55.4 (49.0–61.8)	37.9 (33.1–42.6)	31.9 (28.7–35.1)	< 0.001
No. of patients	49	49	12	13	11	12	
With LOF alleles	107.5 (82.5–110.2)	77.1 (68.1–82.2)	61.8 (53.1–69.6)	60.5 (52.1–65.7)	30.7 (24.7–37.4)	23.9 (18.7–28.8)	< 0.001
No. of patients	134	134	33	33	36	34	

Values are presented as mean (adenosine diphosphate-induced platelet reactivity index, 95% confidence interval).

LOF, loss of function.

[a]p value represents results of before clopidogrel versus the result after antiplatelet treatment.

Responses after interventions in nonresponders with extensive metabolizer and intermediate metabolizer phenotypes vs. those in responders, on 75 mg daily clopidogrel

When compared with patients in the responder group treated with 75 mg daily clopidogrel, patients in the nonresponder group treated with 150 mg clopidogrel or triple therapy showed higher LSMs of platelet reactivity in all genotype ($p < 0.001$ for all comparisons). Interventions with ticlopidine or ticagrelor in nonresponders resulted in platelet reactivity that was as good as the response to standard clopidogrel dosing in responder patients with an intermediate metabolizer phenotype. Treatment with ticagrelor resulted in a reduction of platelet reactivity levels in nonresponders with standard clopidogrel dosing regardless of genotype, which was similar to the results with standard clopidogrel dosing in responders (Fig. 4).

Clinical events

There were no deaths, cerebrovascular events needing medical treatment or admission, or Thrombolysis In Myocardial Infarction major or minor bleeding events. Among responders taking 75 mg clopidogrel, one patient experienced a cardiac ischemic event. No patients with bleeding events met the criteria for bleeding requiring medical attention. Among nonresponders, one patient experienced a cardiac ischemic event with 75 mg clopidogrel. A bleeding event requiring medical attention occurred in one patient in the 150 mg daily clopido-grel group, two patients in the triple therapy group, and two patients in the ticagrelor group. No adverse events or serious adverse events that met end points occurred at a frequency greater than 3% per treatment group, and there were no significant hematologic, gastrointestinal, or musculoskeletal disorders in nonresponders in the different intervention groups.

DISCUSSION

Pharmacodynamic studies have demonstrated wide interindividual variability in the platelet inhibitory response to clopidogrel [10], and an observational study linked poor pharmacodynamic responses to cardiovascular events with the standard dose of clopidogrel [11]. HPR is associated with an increased risk of adverse events after coronary artery stenting [2], with this risk specifically linked to the presence of the LOF alleles CYP2C19*2 and CYP2C19*3 [12,13].

Interindividual variability in platelet response to clopidogrel, in addition to genetic variants of enzymes in metabolic pathways, results in high on-clopidogrel platelet reactivity [14]. In addition, it has been reported that clinical factors (noncompliance, under-dosing, poor absorption, drug-drug interactions, diabetes mellitus, and increased body mass index) and cellular factors (expression of receptors for clopidogrel and platelet turnover) may influence platelet response to clopidogrel [11,15]. In this regard, we evaluated and analyzed all non-

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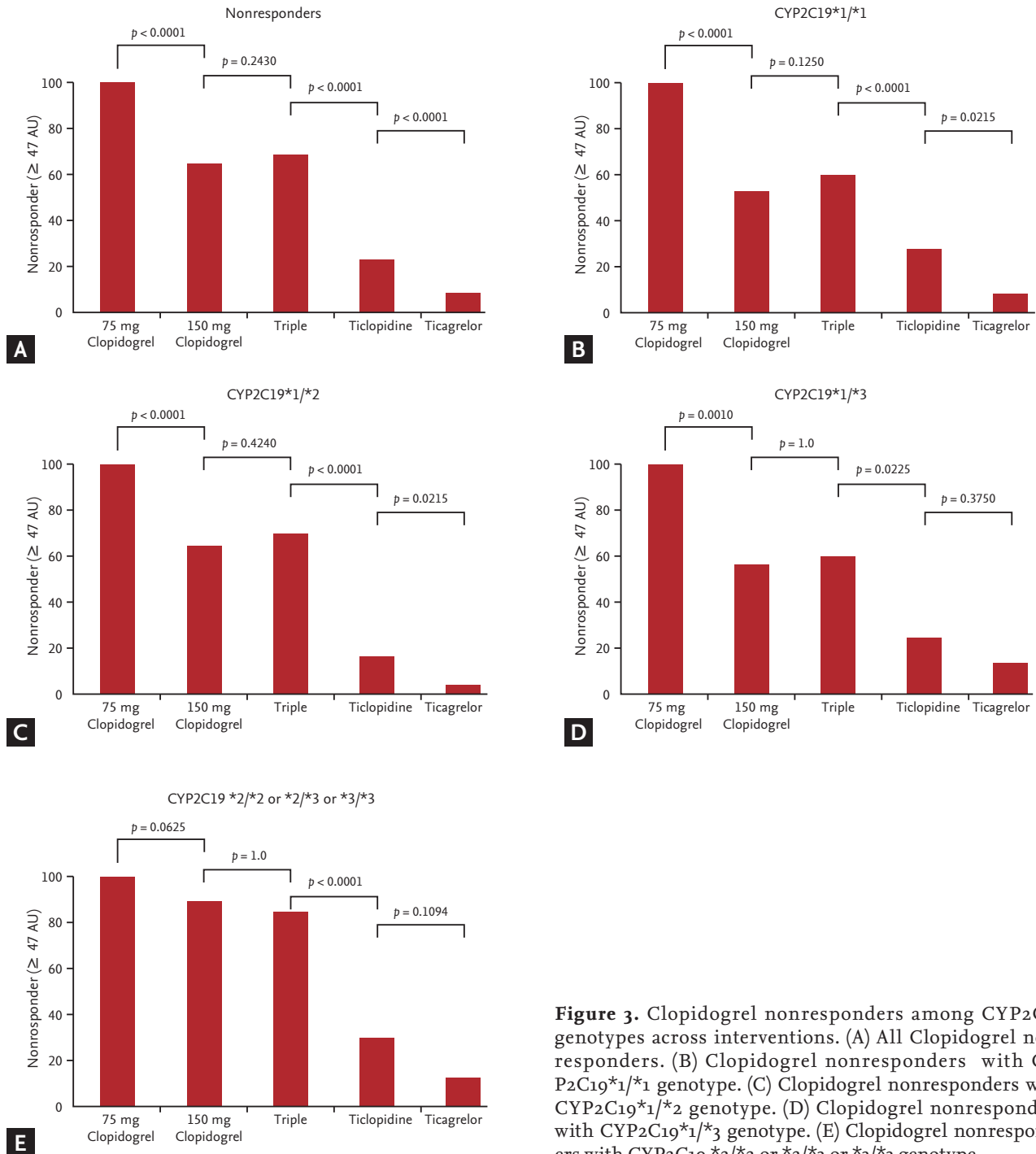
Figure 3. Clopidogrel nonresponders among CYP2C19 genotypes across interventions. (A) All Clopidogrel nonresponders. (B) Clopidogrel nonresponders with CYP2C19*1/*1 genotype. (C) Clopidogrel nonresponders with CYP2C19*1/*2 genotype. (D) Clopidogrel nonresponders with CYP2C19*1/*3 genotype. (E) Clopidogrel nonresponders with CYP2C19 *2/*2 or *2/*3 or *3/*3 genotype.

responders with or without the *CYP2C19* LOF alleles.

We found that the four interventions to reduce HPR with clopidogrel maintenance treatment resulted in more significant reductions in platelet reactivity in responders than in nonresponders with a standard dose of 75 mg clopidogrel. In nonresponders with *CYP2C19* LOF alleles, ticlopidine or ticagrelor resulted in similar platelet inhibition as in responders, but 150 mg daily clopidogrel or triple therapy did not. Among all nonresponders with or without *CYP2C19* LOF alleles, only patients on
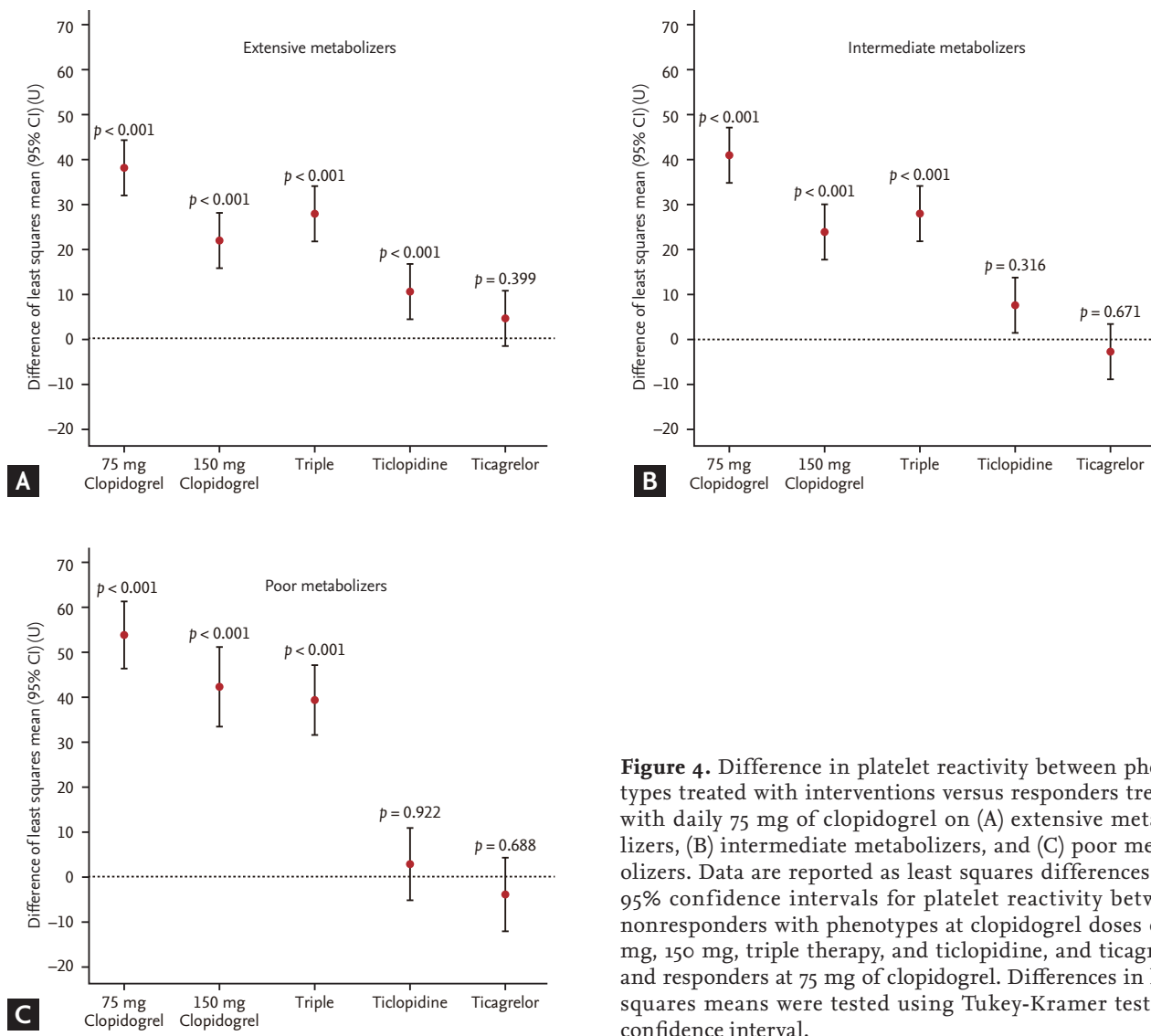
Figure 4. Difference in platelet reactivity between phenotypes treated with interventions versus responders treated with daily 75 mg of clopidogrel on (A) extensive metabolizers, (B) intermediate metabolizers, and (C) poor metabolizers. Data are reported as least squares differences and 95% confidence intervals for platelet reactivity between nonresponders with phenotypes at clopidogrel doses of 75 mg, 150 mg, triple therapy, and ticlopidine, and ticagrelor and responders at 75 mg of clopidogrel. Differences in least squares means were tested using Tukey-Kramer test. CI, confidence interval.

ticagrelor were able to achieve on-treatment platelet reactivity similar to that achieved with 75 mg daily clopidogrel in responder patients with cardiovascular disease.

Several studies have evaluated methods of overcoming clopidogrel resistance. Different loading doses of clopidogrel (up to a total dose of 900 mg) based on the CYP2C19 genotype achieved a degree of platelet inhibition comparable with that observed in response to the standard 300 mg loading dose in noncarrier patients [16]. However, high maintenance doses of clopidogrel (e.g., 150 mg daily) did not show this degree of platelet inhibition [17]. Mega et al. [18] have reported that raising the daily dose of clopidogrel up to 225 mg led to platelet inhibition in carriers of the CYP2C19 *2 alleles, similar to

that seen in noncarriers except for homozygotes. In this report, increasing clopidogrel up to 300 mg daily also did not show superior reduction of platelet inhibition in patients who were homozygotes (poor metabolizers) [18].

Our study also found that increasing the maintenance dose from 75 to 150 mg in nonresponder patients did not (on average) inhibit platelet reactivity to the levels seen in responder patients taking 75 mg clopidogrel.

Cilostazol, a phosphodiesterase type III inhibitor, has been shown to be a more effective inhibitor of platelet aggregation than 75 mg daily clopidogrel in most studies. Cilostazol inhibits phosphodiesterase activity, suppresses cyclic adenosine monophosphate degradation, and activates vasodilation-stimulated protein

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phosphorylation. Thus, cilostazol acts on pathways downstream of clopidogrel action without involvement of the P2Y12 receptor [9,19]. Moreover, cilostazol is metabolized mainly by the *CYP3A* pathway and, to a lesser extent, the *CYP2C19* pathway, the major clopidogrel-metabolizing pathway [20].

Potential mechanisms contributing to the benefits of adjunctive cilostazol might include not only consistent platelet inhibition but also other pleiotropic effects beyond pure P2Y12 receptor inhibition [21]. These pharmacologic aspects of cilostazol may explain why cilostazol is superior to dual antiplatelet therapy including a 75 mg daily maintenance dose of clopidogrel.

One study revealed superior efficacy of cilostazol in the inhibition of platelet reactivity compared to high-dose clopidogrel (e.g., 150 mg/day) in patients with clopidogrel resistance (ACCEL-RESISTANCE study) [22].

On the other hand, there have been no reports showing platelet inhibition with cilostazol in nonresponders similar to that seen in responders or *CYP2C19* noncarriers. A previous study reported that triple therapy did not show as much of a reduction in platelet reactivity as in noncarriers, although triple therapy significantly reduced on-treatment platelet reactivity compared with dual antiplatelet therapy [23]; however, a recent randomized clinical trial failed to show a significant clinical benefit of triple versus dual antiplatelet therapy [24].

We implemented a consecutive change in drug treatment from 150 mg daily clopidogrel to triple therapy in the same group of nonresponders, regardless of the presence or absence of the *CYP2C19* LOF allele. Triple therapy, although showing significant reduction in platelet inhibition compared with dual antiplatelet therapy, did not result in superior efficacy of platelet inhibition compared with high-dose clopidogrel (e.g., 150 mg/day); further, it showed inferior results compared with 75 mg clopidogrel in responders.

Another thienopyridine derivative, ticlopidine, exerts a protective effect against stent thrombosis and major adverse cardiac events [25]. Ticlopidine is also a prodrug metabolized by multiple CYPs, including *CYP2C19* [26]. The pathways that activate ticlopidine have not been identified, but it is known that ticlopidine is not primarily metabolized through the *CYP2C19* pathway which is the main clopidogrel-metabolic pathway [27]; however, ticlopidine is probably effective even in patients with

CYP2C19 polymorphism. A cross-over study compared poor responsiveness after taking clopidogrel or ticlopidine, and found that poor responsiveness to either clopidogrel or ticlopidine at steady state was common [28].

In one study, switching from clopidogrel to ticlopidine significantly decreased platelet aggregation in patients who were *CYP2C19* homozygotes, similar to the results of the present study [8]. It has been reported that heightened platelet reactivity based on frequency of the *CYP2C19* LOF polymorphism is strikingly higher in Asians than in Caucasians [29].

In Koreans, it was reported that the proportion of patients carrying at least one *CYP2C19*2* allele was 53%, and that these patients had significantly higher clopidogrel on-treatment platelet reactivity than noncarriers [29]. Considering the presence of the *CYP2C19*3* LOF allele, which is almost nonexistent in Caucasian patients, frequency of *CYP2C19* LOF allele carriers exceeds 60% in the Korean population [30]. In the Japanese, the percentage of *CYP2C19*2* LOF carriers is reported to be approximately 42% [8].

Although its tolerability is poorer than that of clopidogrel [25-27,29,30], ticlopidine may be a good choice for intervention in high on-clopidogrel reactivity in carriers of *CYP2C19* loss-function polymorphisms, particularly in Asians if adequate blood tests are done during follow-up.

Prasugrel and ticagrelor are the newer P2Y12 ADP receptor blockers that achieve significantly higher levels of platelet inhibition than clopidogrel [31]. In contrast to clopidogrel, the effects of prasugrel and ticagrelor are less affected by variants in the *CYP2C19* gene (and correspondingly, no association between the *CYP2C19* genotype and clinical events has been observed in patients treated with these agents) [32], and thus, these medications represent other alternative treatment strategies in such patients.

Ticagrelor is a new antiplatelet agent chemically known as a cyclopentyl triazolopyrimidine, with properties that distinguish it from the thienopyridines. Ticagrelor, irrespective of the *CYP2C19* genotype also demonstrated superior pharmacodynamic effects compared with clopidogrel on inhibition of platelet reactivity [33]. In the present study, ticagrelor treatment showed similar platelet inhibition in responders and nonresponders with or without the LOF *CYP2C19* alleles.

There were several limitations in our analyses. First, we used only a platelet function test for the analysis. A prospective trial has established that HPR diagnosed using MEA is an independent risk factor for early stent thrombosis, whereas profound inhibition of platelet aggregation induced by ADP is an independent risk factor for bleeding. Moreover, the study assessed the predictive value of HPR in clopidogrel-treated patients and used the upper quintile of the AUC values in order to establish the cutoff MEA value [12]. Second, we focused on platelet reactivity as the primary outcome of interest. While platelet reactivity is a well-validated predictor of poor clinical outcomes, large-scale and long-term trials powered for clinical outcomes will be necessary to assess adverse events and establish more preferred treatment regimens. Third, because there is no reliable cutoff value of HPR in East Asian patients, we used Dr. Sibbing's cutoff HPR value.

In conclusion, this study demonstrates that among patients with stable angina, ticlopidine or ticagrelor treatment in nonresponder patients including all carriers of CYP2C19*2 and/or *3 alleles, compared with 150 mg daily clopidogrel or triple therapy, achieved similar platelet reactivity as that in responders. In particular, ticagrelor resulted in a comparable degree of platelet inhibition in all nonresponders irrespective of genotype. These data help to define how patients with higher on-clopidogrel platelet reactivity and with different *CYP2C19* genotypes respond to different evidence-based treatment strategies, and provide useful information to guide further clinical studies.

KEY MESSAGE

1. Post-percutaneous coronary intervention patients include clopidogrel nonresponders.
2. This study suggests that a combination of ticlopidine and ticagrelor is a good alternative to conventional maintenance therapy for clopidogrel nonresponders.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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