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LETTERS TO THE EDITOR

Influence of *CYP2C19*2* and **3* loss-of-function alleles on the pharmacodynamic effects of standard- and high-dose clopidogrel in East Asians undergoing percutaneous coronary intervention: the results of the ACCEL-DOUBLE-2N3 study

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Carriage of the cytochrome P450 (*CYP*) *2C19* loss-of-function (LoF) polymorphism has been associated with high on-treatment platelet reactivity (HPR) and an increased risk of ischemic event occurrence in clopidogrel-treated patients undergoing a percutaneous coronary intervention (PCI) [1]. Although there are multiple *CYP2C19* alleles associated with null function (i.e. **2*–**8*), the treatment strategy to overcome the LoF allele effect was mostly based on the *CYP2C19*2* LoF allele [2]. There is a marked interethnic difference in the frequency of poor metabolizers between East Asians (13–30%) and Caucasians (< 5%). The *CYP2C19*2* (rs4244285, c.681G>A in exon 5, splice site mutation) and **3* (rs4986893, c.636G>A in exon 4, a premature stop codon) alleles account for the LoF polymorphism in East Asians, whereas most of the LoF polymorphism in Caucasians consist of the *CYP2C19*2* allele with extremely rare **3* carriage. The effect of the *CYP2C19*3* allele on the enzyme activity appeared greater than the *CYP2C19*2* allele in several previous studies [3,4].

In this pilot study, we analyzed the association between platelet reactivity and the *CYP2C19* genotype in East Asians during standard- and high-dose clopidogrel, and compared the gene-dose effect of the *CYP2C19*2* and **3* alleles.

Baseline platelet function measurement was performed during standard-dose clopidogrel therapy (n = 155) (Data S1): for elective PCI, patients received a 300-mg clopidogrel load at least 12 h before PCI or ingested a daily 75-mg clopidogrel therapy (≥ 5 days), and acute myocardial infarction patients were treated with emergent PCI after a 600-mg clopidogrel load, followed by daily 75-mg clopidogrel administration (≥ 5 days). Immediately before discharge, patients started high-dose clopidogrel administration (150-mg daily). Follow-up platelet measurement was performed after patients took high-dose clopidogrel during at least 30 days.

The platelet function test (AggRAM aggregometer; Helena Laboratories Corp., Beaumont, TX, USA) and genotyping were performed (Data S1). The primary endpoint was the level of 20 μM ADP-induced platelet aggregation (PA) during clopidogrel treatment according to the *CYP2C19* metabolizer status. Secondary endpoints were ΔPA between standard- and high-dose clopidogrel, and the frequency of HPR according to the C*YP2C19* metabolizer status. We also evaluated differences in these parameters across the *CYP2C19* genotype. HPR was defined as 20 μM ADP-induced PA ≥ 59% [1].

To adjust the influence of covariates on PA, a multivariate linear and binary logistic regression analysis was performed using known relevant clinical characteristics as fixed covariates [5] (Data S1). $P < 0.05$ was considered to indicate a significant difference, and all statistical analyzes were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA).

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Ninety-nine patients (63.9%) carried at least one *CYP2C19* LoF allele, including 73 intermediate (1 LoF allele: 47.1%) and 26 poor metabolizers (2 LoF alleles: 16.8%). Baseline characteristics according to the predicted *CYP2C19* metabolizer status were overall well matched (Table S1).

During treatment with standard-dose clopidogrel, the *CYP2C19* LoF carriage status and clinical covariates could explain 6.3% and 11.0% of the interindividual variability in 20 μM ADP-induced PA, respectively (Table S2). When treated with high-dose clopidogrel, the contribution of the *CYP2C19* LoF carriage and clinical covariates to 20 μM ADP-induced PA was increased to 14.5% and 14.3% of the variability, respectively.

Platelet reactivity and the prevalence of HPR increased in a gene-dose manner during standard- and high-dose clopidogrel treatments, which was more prominent during high-dose clopidogrel treatment. A general linear model analysis demonstrated that the level of 20 μM ADP-induced PA during standard-dose clopidogrel increased proportionally across the *CYP2C19* metabolizer status ($mean_{adj}$: 56.0 ± 6.2%, 60.6 ± 6.4%, and 66.3 ± 6.0% for carriers of 0, 1, and 2 LoF allele[s], respectively; $P < 0.001$). During high-dose clopidogrel, the increase in the level of 20 μM ADP-induced PA was greater across *CYP2C19* metabolizers compared with standard-dose clopidogrel ($mean_{adj}$: 41.1 ± 7.3%, 49.1 ± 7.1%, and 60.1 ± 6.5% for carriers of 0, 1, and 2 LoF allele[s], respectively; $P < 0.001$). The frequency of HPR proportionally increased according to the number of the *CYP2C19* LoF allele carriage in a logistic regression model analysis, which was more prominent during treatment with high-dose clopidogrel.

Depending on the *CYP2C19* genotype, overall baseline characteristics did not differ. The level of PA and the frequency of HPR increased across the *CYP2C19* genotype during standard- and high-dose clopidogrel treatments. After adjustment with clinical covariates, the level of 20 μM ADP-induced PA during standard-dose clopidogrel increased gradually across the *CYP2C19* genotype ($mean_{adj}$: 56.0 ± 6.2%, 59.4 ± 6.2%, 64.1 ± 5.8%, 65.8 ± 6.3%, and 67.3 ± 5.5% for carriers of *CYP2C19 *1/*1*, **1/*2*, **1/*3*, **2/*2* and **2/*3,* respectively; $P < 0.001$) (Fig. 1A). During high-dose clopidogrel, the difference in the level of 20 μM ADP-induced PA became greater between the *CYP2C19* genotypes compared with standard-dose clopidogrel ($mean_{adj}$: 41.1 ± 7.3%, 47.7 ± 5.9%, 53.4 ± 8.5%, 57.1 ± 4.9%, and 65.8 ± 5.5% for carriers of *CYP2C19 *1/*1*, **1/*2*, **1/*3*, **2/*2* and **2/*3,* respectively; $P < 0.001$).

The influence of the *CYP2C19*3* allele on the level of PA appeared greater than the *CYP2C19*2* allele during standard- (β coefficient: 7.8% vs. 7.1%) and high-dose clopidogrel treatments (β coefficient: 12.7% vs. 9.7%). The *CYP2C19*1/*3* carriers showed greater PA compared with subjects with the *CYP2C19*1/*2* gene, and the *CYP2C19*2/*3* vs. **2/*2* carriers exhibited an enhanced level of PA (Table S3). The adjusted frequency of HPR increased gradually across the *CYP2C19* genotype (Fig. 1B), which became prominent during treatment with high-dose clopidogrel.

This is the first study to demonstrate that the influence of the *CYP2C19*3* vs. **2* allele carriage on platelet reactivity appears greater during both treatments with standard- and high-dose clopidogrel in PCI-treated East Asians. In this population on clopidogrel, the *CYP2C19* LoF carriage was a major determinant of on-treatment platelet reactivity, which contributed according to clopidogrel dosage.

East Asians with the same genotype as Caucasians have shown lower CYP2C19 activity [3,6]. For example, Korean subjects carrying the *CYP2C19*1/*1* gene had higher a metabolic ratio than Swedish subjects (1.48 vs. 0.58; $P < 0.000001$), indicating lower CYP2C19 activity in Koreans [6]. However, the contribution of the *CYP2C19* LoF allele to platelet reactivity appears similar during clopidogrel treatment. In the Western population, the *CYP2C19*2* LoF allele alone was accountable for 6.2% of the variability in 20 μM ADP-induced PA during standard-dose clopidogrel treatment [7], which was similar to the results of the current study (6.3%).

Several previous studies have suggested that the enzyme activity in the *CYP2C19*3* heterozygous is lower than that of the *CYP2C19*2* heterozygous [3,4]. To date, there are no definite explanations available for this observation. Although several subtypes of the *CYP2C19*2* gene (*CYP2C19*2A-D*) have been detected, the enzyme activity has not been determined for all subtypes. Linkage with other loci can be another consideration. Another plausible explanation may be an incomplete loss of enzyme activity by the *CYP2C19*2* mutation [8]. Feng *et al*. [8] reported that the enzyme activity in the *CYP2C19*2/*2* genotype was inducible by rifampicin. Because the pharmacodynamic and clinical influences of the *CYP2C19* genotype according to clopidogrel dosing may be important in the tailored antiplatelet treatment, especially ethnicity-based choice, the present study facilitates the need of a more specific approach of clopidogrel treatment based on the *CYP2C19* genotype (**2* vs. **3*).

This study is an exploratory study involving a small-sized cohort from a single-center experience. However, the number of poor metabolizers treated with high-dose clopidogrel in the present study ($n = 26$) was greater than that analyzed in the GIFT study ($n = 9$) [9]. We did not find a difference in on-clopidogrel PA between stable vs. acute coronary syndrome patients, which may be relatively different to other studies. Probably it is due to the time between entry at hospital and blood sample.

In conclusion, this pilot study is the first to demonstrate that the influence of the *CYP2C19*3* allele on the clopidogrel response may be more relevant compared with the *CYP2C19*2* allele. Our findings suggest that the

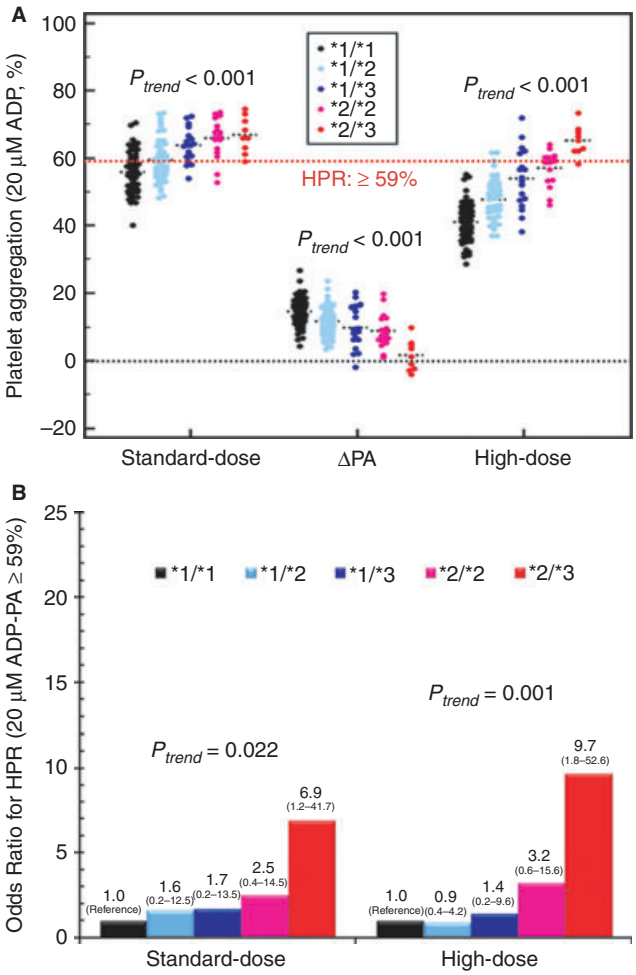

Fig. 1. 20 μM ADP-induced platelet aggregation (A) and odds ratio of HPR (B) during standard- and high-dose clopidogrel according to the *CYP2C19* genotype (adjusted*). *Platelet aggregation was adjusted with age ≥ 65 year old, gender, body mass index ≥ 25 kg m^{-2}, index ACS presentation, diabetes, hypertension, current smoking, chronic kidney disease, left ventricular ejection fraction ≤ 45%, calcium channel blocker, proton pump inhibitor and clopidogrel treatment strategy at the baseline. ΔPA represents the difference of platelet aggregation between standard- and high-dose clopidogrel. Dotted lines between platelet aggregation markers indicate the median values. Odds ratios are vs. *CYP2C19*1/*1* carriers and are adjusted for clinical covariates. ADP indicates adenosine diphosphate; PA, platelet aggregation; HPR, high on-clopidogrel platelet reactivity; *CYP,* cytochrome P450; ACS, acute coronary syndrome; LoF, loss-of-function.

type of LoF single nucleotide polymorphism should be taken into consideration when choosing alternative antiplatelet therapies to overcome its influence.

Addendum

Y.-H. Jeong and K. A. Abadilla designed the study, analyzed the data and wrote the manuscript; U. S. Tantry and Y. Park developed the hypothesis and designed the study; J.-S. Koh, C. H. Kwak and J.-Y. Hwang analyzed the data; P. A. Gurbel wrote the manuscript.

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Disclosure of Conflict of Interest

Dr Jeong has received honoraria for lectures from Sanofi-Aventis, Daiichi Sankyo/Lilly, Nanosphere, Haemonetics and Otsuka; and research grants or support from Dong-A Pharmaceuticals, Han-mi Pharmaceuticals, Boehringer-Ingelheim, Otsuka, Accumetrics, and Haemonetics. Dr Gurbel has received research funding from Astra Zeneca, Daiichi Sankyo/Lilly, Pozen, Bayer Healthcare, Sanofi-Aventis, CSL Pharmaceuticals, Accumetrics, Nano-sphere and Haemoscope; and Honoraria from Merck, Daiichi Sankyo/Lilly, Boerhinger Ingleheim, Johnson and Johnson, AstraZeneca and Discovery Channel. The other authors report no conflicts.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Study Population, Platelet Function Measurements and Genotyping, and Statistical Analysis.
Table S1. Baseline characteristics according to the *CYP2C19* metabolizer status.
Table S2. Percentage of Variability Related to On-treatment Platelet Reactivity.
Table S3. Difference of 20 μM ADP-induced platelet aggregation according to the *CYP2C19* loss-of-function allele carriage (adjusted*).

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Coagulation-related gene expression profile in glioblastoma is defined by molecular disease subtype

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High-grade brain tumors, especially glioblastoma multiforme (GBM), present an extreme therapeutic challenge, as patients rarely survive beyond 14 months after diagnosis [1].

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Morphological hallmarks of these lesions encompass their infiltrative growth characteristics, pseudopalisading necrosis, striking endothelial cell proliferation and occlusive intravascular thrombosis [2,3]. These exclusively intracranial tumors are also associated with a high risk (10–30%) of peripheral venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE), which can be fatal [4]. The nature of the circulating procoagulant activity responsible for these events is controversial, and the proposed role of tissue factor (TF)-bearing microparticles (TF-MPs) [5] has recently been questioned [6].

We have previously suggested that coagulopathy in cancer is affected by oncogenic mutations, and their impact on the expression of haemostasis-related genes [7,8]. For example, oncogenic amplification (40%) and