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Genetic determinants of clopidogrel responsiveness in Koreans treated with drug-eluting stents

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ABSTRACT

Background: Variations of genes encoding cytochrome enzymes, drug transporters, and paraoxonase have recently been reported to be associated with clopidogrel response variability besides the well-known CYP2C19 loss-of-function (LOF) alleles. We determined whether newly reported genetic variations are associated with clopidogrel on-treatment platelet reactivity (OPR) in Korean patients.

Methods: OPR was measured in 1264 consecutive patients who underwent percutaneous coronary intervention using the VerifyNowP2Y12 assay system and genotyping of PON-1 Q192R, ABCB1 C3435T, CYP1A2*1F, CYP2B6*6, CYP2C19*2, CYP2C19*3, CYP2C19*17, CYP3A4 (IVS10+12G>A), and CYP3A5*3 was performed. We applied two different cutoffs, i.e. 240 P2Y12 reaction units (PRU) and 253 PRU, to define high OPR.

Results: Mean OPR of the entire population was 231±83 PRU. Genetic variations of ABCB1 and PON-1 genes as well as that of CYP1A2, 2B6, 3A4, and 3A5 were not associated with clopidogrel response variability. As for CYP2C19, patients were classified into 4 metabolism genotypes: 0.6% ultrarapid (UM), 40.3% extensive (EM), 48.8% intermediate (IM), and 10.3% poor metabolizers (PM). After adjustment for possible confounders, CYP2C19 metabolism genotype was associated with a significant increase in OPR: effect on OPR-difference: +27 PRU, $p=0.015$ for EM, +53 PRU, $p<0.001$ for IM, and +74 PRU, $p=0.006$ for PM compared with UM. In multivariable analysis, the CYP2C19 genotype was the only independent predictor of high-OPR among genetic variations using two different cutoffs.

Conclusions: Among genes postulated to be involved in clopidogrel metabolism, only the CYP2C19 genotype is associated with response variability and emerged as an independent predictor of high-OPR using two different cutoffs. PON-1 and ABCB1 genetic variants do not affect clopidogrel OPR in Korean patients.

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1. Introduction

The inter-individual and inter-racial differences in response to clopidogrel have all been previously reported [1,2], and high on-treatment platelet reactivity (OPR) is associated with increased thromboembolic complications after stent implantation [3–5].

Clopidogrel response variability has been linked to its 2 bioactivation steps by hepatic cytochrome P450 (CYP) enzymes, enteric drug transporters, and paraoxonase enzymes [6–9]. The CYP2C19*2 and *3 loss of function (LOF) polymorphisms have been shown to be associated with high OPR by multiple investigators [6,8–10]. The CYP2C19 enzymes were found to play a key role in both steps of clopidogrel bioactivation [11]. Recently, the paraoxonase-1 (PON1) enzyme activity was found to be strongly linked to clopidogrel bioactivation resulting in genetic variation of the gene being associated with clopidogrel OPR and thrombotic clinical outcome [7]. However, subsequent studies by Sibbing et al., Fontana et al., and Trenk et al. could not reproduce the association between PON-1 polymorphism and clopidogrel response variability [12–14]. ABCB1 is a drug transporter gene, and the TT genotype of the ABCB1 C3435T polymorphism was shown to be associated with lower platelet inhibition after clopidogrel loading [15]. Two major limitations of previous reports are that first, only one or two genetic variations were analyzed in each study rather than all of the proposed genes, and second, no study was performed in a large cohort of pure Asian patients where frequency and effects are different. Taken together, conflicting results exist regarding which genetic variant influences clopidogrel response variability [12–14]. Therefore, this study was performed to determine whether the newly reported genetic variations are associated with clopidogrel OPR in a Korean cohort of patients.

2. Methods

2.1. Study population

The CROSS-VERIFY (measuring Clopidogrel Resistance tO aSsure Safety after percutaneous coronary intervention using VERIFYnow) is a dynamic cohort with on-going patient

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recruitment including all patients undergoing coronary angiography and/or percutaneous coronary intervention (PCI) who agreed to measurement of clopidogrel OPR with the VerifyNow P2Y12 assay after clopidogrel therapy at Seoul National University Hospital since June 1st, 2006. The Exclusion criteria were contraindication to aspirin, clopidogrel, or heparin; the use of intravenous glycoprotein IIb/IIIa inhibitor within 5 days before the platelet reactivity test; the concomitant use of cilostazol; uncontrolled malignancy; bleeding tendency; and ethnicity other than Korean heritage [2]. The enrollment period for the current study was from June 2006 through June 2010, and only patients with implantation of drug eluting stent (DES) were enrolled. Written informed consent was obtained before enrollment. The study complied with Declaration of Helsinki and was approved by the Institutional Review Board of Seoul National University Hospital. The authors comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

2.2. Platelet function test

The inhibitory effect of clopidogrel on platelet reactivity was measured using the VerifyNow P2Y12 assay (Accumetrics Inc., San Diego, CA). Blood sample was obtained 12 to 24 h after the final dose of clopidogrel in patients who had been on 75 mg of clopidogrel for more than 7 days, and 12 to 24 h after PCI in patients who were loaded with clopidogrel before catheterization. A loading dose of 300 mg clopidogrel was administered in patients who had been taking clopidogrel for less than 7 days; 600 mg was given to clopidogrel-naïve patients. Whole blood was anticoagulated in a sodium citrate bottle used exclusively for the VerifyNow P2Y12 assay. All patients took aspirin at 100 mg per day or 300 mg loading, if not taken previously.

The VerifyNow P2Y12assay reports the results as P2Y12 reaction units (PRU). This assay mimics turbidometric aggregation and utilizes disposable cartridges containing 20 μM ADP and 22 nM prostaglandin E1 (PGE1). Aggregation testing using ADP as a sole agonist activates $P2Y_1$ and $P2Y_{12}$ purinergic signaling, while adding PGE1 increases the specificity of the test for P2Y12 signaling [17,18]. Technical details and reliability of the assay have been reported previously [19,20]. The coefficient of variation for the test was 7.5% in our institution.

2.3. Definition of high-OPR

The median OPR is higher in Asians compared with Caucasians [1,2]. In our previous study, we reported a cut-off value of 253 PRU that could best predict thromboembolic complication in Koreans [21]. In Caucasians, Marucci et al. determined the optimal cutoff value in predicting 12-month cardiovascular death and nonfatal MI was OPR > 240 PRU [22,23]. Therefore, we incorporated both values and performed two analyses using once 253 PRU and the other 240 PRU to define high-OPR.

2.4. Genetic analysis

We examined 9 single nucleotide polymorphisms (SNPs) of 7CYP genes and 1 drug transporter gene, and 1 paraoxonase gene postulated to be involved in clopidogrel metabolism [24,25], i.e. CYP1A2, CYP2B6, CYP2C19, CYP3A4, CYP3A5, ABCB1, and PON-1 of 1264 patients who agreed with genetic analysis.

The genotyping of CYP1A2*1F (−163C > A, rs762551), CYP2C19*2 (P227P, rs4244285), CYP2C19*3 (W212X, rs4986893), CYP3A4 (IVS10 + 12G > A, rs2242480), CYP3A5 (CYP3A5*3, rs776746), ABCB1 (C3435T, rs1045642), and PON-1 (Q192R, rs662) was screened using the TaqMan fluorogenic 5′ nuclease assay (ABI, Foster City, CA). As for CYP2B6*6 (K262R, rs2279343) and CYP2C19*17 (rs12248560), the SNaPshot assay was performed according to the manufacturer's instructions (ABI PRISM SNaPShot Multiplex kit, Foster City, CA). Analysis was carried out using Genemapper software (version 4.0, Applied Biosystems Inc., Foster City, CA). Duplicate samples and negative controls were included to ensure accuracy of genotyping.

2.5. Statistical analysis

The mean OPR in Korean population was 241.9 ± 70.3 PRU [2]. In the genetic substudy of the CILON-T trial, we reported that frequencies of ultra-rapid (UM), extensive (EM), intermediate (IM), and poor metabolizer (PM) were 1.7%, 38.6%, 45.3%, and 14.4%, respectively [26]. Assuming a 10% difference between the various metabolizer groups, a total of 1098 patients (UM = 18, EM = 424, IM = 498, and PM = 158 patients) would be needed to achieve 95% power to detect a statistically significant difference between the groups using Tukey–Kramer multiple comparison test at a 0.05 significance level.

As for PON-1 genotypes, Bouman et al. reported significant differences in OPR between the 3 genotypes e.g. RR, QR, QQ carriers (58.3 ± 9.1% vs. 32.8 ± 13.1% vs. 11.3 ± 10.5%, $p < 0.001$) [7]. The reported genotype distributions in Korean were 20%, 40%, 40% for QQ, QR, and RR respectively. A total sample of 1005 patients (QQ = 201, QR = 402, and RR = 402) achieves 95% power to detect a 10% difference between the groups using the Tukey–Kramer multiple comparison test at a 0.05 significance level.

As for the ABCB1-genotypes, Mega et al. reported that TT-genotypes have 7.3% diminished platelet reactivity compared with non-TT genotypes [15]. The reported distributions of ABCB-1 genotype in Korean were 12.8%, 48.6% and 38.6% for TT, TC, and CC genotypes, respectively [27]. A total sample of 1098 patients (TT = 140, TC = 534, CC = 424) achieves 95% power to detect a difference of at least 7.3% using the Tukey–Kramer multiple comparison test at a 0.05 significance level.

The data were presented as numbers and frequencies for categorical variables, and as the mean ± the standard deviation for continuous variables. For comparison among groups, the Chi-square test (or Fisher's exact test when any expected cell count was <5 for a 2 × 2 table) was used for categorical variables and the unpaired Student's *t*-test or the 1-way analysis of variance was applied for continuous variables. The Chi-square test for goodness of fit was used to verify agreement with Hardy–Weinberg equilibrium using the Fisher's exact test. The general linear model analysis was applied to quantify the effect of genotypes on clopidogrel responsiveness by entering patients' genotype status as factor and clinical factors as covariates. In this case, the data were presented as the mean ± the standard error. The multivariable logistic regression analysis was performed to determine the independent association of gene polymorphisms with high OPR. Advanced age was defined as age ≥ 65 years, chronic kidney disease as glomerular filtration rate (GFR) < 60 ml/min/1.73 m^2, and obesity as BMI ≥ 25 kg/m^2. Two sided p values less than 0.05 were considered statistically significant. Statistical tests were performed using SPSS version 17 (SPSS Inc., Chicago, Illinois, USA), and the sample size calculation performed with PASS 11 (NCSS, Kaysvilee, Utah, USA).

3. Results

3.1. Baseline characteristics

A total of 1676 patients were initially enrolled in CROSS-VERIFY Cohort for the current study. We excluded 38 patients without DES implantation, 143 patients with concomitant use of cilostazol, 2 Caucasians, and 229 patients who did not agree with genetic analysis leaving 1264 patients available for further analysis.

The baseline characteristics of the study population are summarized in Table 1. The mean age was 64 ± 9 years, 68% were males, 68% had hypertension, and 33% had diabetes mellitus. Seven hundred and thirty-five patients (58%) presented with stable angina, 451 patients (36%) with unstable angina, 53 patients (4%) with Non-ST-elevation myocardial infarction (NSTEMI), and 29 patients (2%) with ST-elevation myocardial infarction (STEMI). The mean OPR of the entire study population was 231 ± 83 PRU.

As for the duration of clopidogrel treatment, 56% patients were clopidogrel naïve and received a loading dose, 11.1% had been taking clopidogrel for less than 1 week, 3.3% had been taking clopidogrel for 1–2 weeks, 5.6% for 2–4 weeks, and 22.2% at least 4 weeks. There was no statistically significant difference in mean OPR between the patients according to the different clopidogrel durations (clopidogrel-naïve: 233 ± 84 PRU, duration of <1 week: 224 ± 86 PRU, duration of 1–2 weeks: 226 ± 74 PRU, duration of 2–4 weeks: 218 ± 76 PRU, and duration of >4 weeks: 234 ± 83 PRU, ANOVA p = 0.446).

3.2. Genetics and clopidogrel on-treatment platelet reactivity

Genotyping results are shown in Supplement Table 1. Overall, the genotyping success rate was greater than 98%, and most of the alleles were within the Hardy–Weinberg equilibrium, except for CYP2C19*2, *3, *17 and PON-1.

The frequencies of genotypes for PON-1 QQ, QR and RR were 10.2%, 47.8% and 42.0%, respectively, and there was no difference in OPR between the different genotypes (227 ± 82 PRU for QQ, 229 ± 85 PRU for QR, 237 ± 82 PRU for RR genotypes, p = 0.159). As for ABCB1 C3435T, the distributions of CC, CT, and TT genotypes were 38.6%, 48.6%, and 12.8%, respectively. Among these genotypes, there were no differences in OPR (mean OPR: 233 ± 82, 232 ± 83, and 225 ± 89 PRU for CC, CT and TT genotypes respectively, p = 0.520). As for CYP2C19, the frequencies of genotypes for *17/*17, *1/*17, *1/*1, *2/*17, *3/*17 *1/*2, *1/*3, *2/*2, *2/*3, and *3/*3 were 0%, 0.6%, 40.3%, 0.4%, 0.2%, 32.3%, 15.9%, 8.7%, 0.1%, and 1.6%, respectively. Patients with the *1/*17 genotype were classified as ultrarapid metabolizer (UM), those with CYP2C19*1/*1 as extensive (EM), those with 2/*17, *3/*17 *1/*2, *1/*3 as intermediate (IM), and those with *2/*2, *2/*3, *3/*3 as poor (PM). The frequencies for UM, EM, IM, and PM were 7 (0.6%), 503 (40.3%), 609 (48.8%), and 129 (10.3%), respectively. The OPR increased significantly from UM to PM (175 ± 81 PRU for UM, 213 ± 80 PRU for EM, 239 ± 84 PRU for IM, and 266 ± 75 PRU for PM, ANOVA p < 0.001,

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