Original Article

Impact of CYP2C19 Polymorphism on Platelet Function Tests and Coagulation and Inflammatory Biomarkers in Patients Undergoing Percutaneous Coronary Intervention

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Aim: Carriers of the reduced-function CYP2C19 allele receiving dual antiplatelet therapy (DAPT) with aspirin and clopidogrel exhibit diminished platelet inhibition and an increased risk of events. The purpose of this study was to investigate the effects of CYP2C19 gene variants on platelet function tests and coagulation and inflammatory biomarkers in patients undergoing elective percutaneous coronary intervention (PCI).

Methods: This prospective, observational, multicenter study enrolled 104 consecutive Japanese patients undergoing elective PCI. We examined the CYP2C19 genotype, platelet function tests, the levels of coagulation and inflammatory biomarkers and the serum levels of high-sensitivity troponin T (hs-TnT) before, immediately after and one, two and 28 days after PCI.

Results: A total of 68 (65%) of the 104 enrolled patients were carriers of the CYP2C19 reduced-function allele. On-clopidogrel platelet aggregation (PA), measured using light transmittance aggregometry and the VerifyNow[®] P2Y12 system, and the platelet reactivity index (PRI) were significantly higher at all time points in the carriers than in the noncarriers ($p < 0.05$), whereas there were no differences in the levels of the coagulation and inflammatory biomarkers or serum hs-TnT. Simple and multiple logistic regression analyses identified on-clopidogrel PA and PRI as being significant predictors of carriers of the CYP2C19 reduced-function allele.

Conclusions: The present study suggests that platelet function tests, but not coagulation, inflammatory or cardiac biomarkers, are useful for identifying carriers of CYP2C19 reduced-function gene variants and monitoring the efficacy of DAPT in patients undergoing elective PCI.

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Key words: Angina, Coronary artery disease, Platelets, Thrombosis

Introduction

Platelet activation plays an important role in the onset of acute coronary syndrome (ACS)¹⁾. Disrup-

tion of coronary atherosclerotic plaque exposes the thrombogenic material in the lipid-rich core of the plaque to the blood, causing platelet adhesion and activation as well as thrombin generation. The activated platelets undergo morphological changes and release vasoactive and procoagulant substances, followed by conformational changes in the membrane glycoprotein IIb/IIIa receptor that allow for fibrinogen cross-linking and platelet aggregation. Dual antiplatelet therapy (DAPT) consisting of aspirin and clopidogrel is currently used to regulate activated platelets in

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disrupted coronary plaques in ACS patients and to reduce atherothrombotic complications in ACS patients undergoing percutaneous coronary intervention (PCI)²⁻⁹. Observational studies have demonstrated that a high level of on-clopidogrel platelet aggregation (PA) can result in adverse cardiovascular events after PCI, including stent thrombosis¹⁰⁻¹³. In this regard, carriers of at least one reduced-function cytochrome P450 2C19 (CYP2C19) allele treated with DAPT have been reported to have low levels of the active metabolites of clopidogrel, diminished platelet inhibition and a high rate of major adverse cardiovascular events among ACS patients undergoing PCI¹⁴.

Based on the clinical outcomes of patients with ACS undergoing PCI, recent studies have advocated the use of careful monitoring of the on-clopidogrel PA level using various platelet function assays^{15, 16}. In a previous study, we also demonstrated the efficacy of on-clopidogrel PA monitoring by showing the cutoff level of on-clopidogrel PA that can be used to discriminate carriers of the functional CYP2C19 reduced-function allele among patients with stable coronary artery disease (CAD) treated with clopidogrel¹⁷. However, it is not clear whether CYP2C19 gene variants influence serial changes in platelet function tests and blood coagulation and inflammatory biomarkers in stable CAD patients undergoing elective PCI. We therefore conducted a CALDERA-PCI (CYP2C19 gene ALteration and thienopyriDinE ResistAnce in PCI; NCT00876512) study, a prospective, observational, multicenter study that enrolled 104 consecutive Japanese patients with stable CAD scheduled to undergo elective PCI.

Aim

The present study was designed to further examine the effects of CYP2C19 gene variants on platelet function tests in stable CAD patients undergoing elective PCI. The tests included measurements of the level of on-clopidogrel PA [measured using light transmittance aggregometry (LTA) and the VerifyNow® P2Y12 system], the platelet reactivity index (PRI) that is the difference in vasodilator-stimulated phosphoprotein (VASP) fluorescence intensity between resting and activated platelets, and platelet adhesiveness. We also measured blood coagulation and inflammatory biomarkers [von-Willebrand factor (VWF), VWF-cleaving protease (ADAMTS13), soluble P-selectin (sP-selectin), soluble CD40 ligand (sCD40L), interleukin-6 (IL-6), soluble intercellular adhesion molecule-1 (sICAM-1) and high-sensitivity C-reactive protein (hs-CRP)] and a cardiac biomarker [serum high-sensi-

tivity troponin T (hs-TnT)].

Methods

Study Population

The CALDERA-PCI trial included 104 consecutive patients with stable CAD and angiographic evidence of $\geq 75\%$ organic coronary stenosis who were admitted to our institutions between October 2008 and November 2010. All patients were scheduled for elective PCI based on the presence of cardiac ischemia on stress thallium-201 single-photon emission computed tomography or a treadmill test and were treated with dual antiplatelet therapy consisting of both aspirin (100 mg/day) and clopidogrel (loading dose of 300 mg followed by 75 mg/day). We excluded patients who were treated with warfarin, steroids, thrombolytic agents, ticlopidine, sarpogrelate or cilostazol and those with deep vein thrombosis, atrial fibrillation, collagen disease, infection, hepatic dysfunction (serum aspartate aminotransferase or alanine aminotransferase levels of $\geq$ two times the upper limit of normal), renal dysfunction (a serum creatinine level of ≥ 2.0 mg/dL or the use of dialysis) or malignant diseases. We also excluded patients with ACS, defined as either acute myocardial infarction (with or without electrocardiographic evidence of ST-segment elevation) or unstable angina (class II or III according to Braunwald's classification). The risk factors for CAD were defined as current smoking (smoking within one year), diabetes mellitus (symptoms of diabetes in addition to a casual plasma glucose concentration of ≥ 200 mg/dL, a fasting plasma glucose concentration of ≥ 126 mg/dL, a 2-hour plasma glucose concentration of ≥ 200 mg/dL during a 75-g oral glucose tolerance test or the use of medications for diabetes mellitus), hypertension ($>140/90$ mmHg or the use of antihypertensive medications), dyslipidemia [a high-density lipoprotein (HDL) cholesterol level of <40 mg/dL, a low-density lipoprotein (LDL) cholesterol level of ≥ 140 mg/dL or a triglyceride level of ≥ 150 mg/dL or the use of medications for dyslipidemia] and a family history of myocardial infarction. The study protocol was approved by the Human Ethics Review Committee of Kumamoto University Graduate School of Medicine, Kumamoto Central Hospital and Saiseikai Kumamoto Hospital Cardiovascular Center, and a signed consent form was obtained from each subject.

Platelet Function Tests

Platelet function tests and assays for blood biomarkers of coagulation activation and inflammation were performed before, immediately after and one,

two and 28 days after elective PCI. Blood sampling before PCI was performed at least one week after aspirin treatment and at least 24 hours after clopidogrel loading just before elective PCI. The blood samples were collected using the double-syringe technique, in which the first 2 to 4 mL of blood was discarded to prevent spontaneous platelet activation.

The on-clopidogrel PA levels were measured using LTA (MCM HEMA TRACER 313M, MC Medical, Inc., Tokyo, Japan) and the VerifyNow® P2Y12 system (Ultegra rapid platelet function assay; Accumetrics Inc., San Diego, CA). The Vacutainer was inverted three to five times for gentle mixing and sent immediately to the laboratory. For LTA, the blood samples were drawn into Vacutainer tubes containing 0.5 mL of 3.2% sodium citrate (Becton-Dickinson, San Jose, CA) and processed within 60 minutes. LTA was performed at all time points using 20 $\mu\text{mol/L}$ of adenosine diphosphate (ADP) as an agonist. The relative maximum LTA response (maximum response to 20 $\mu\text{mol/L}$ ADP) and area under the curve (platelet aggregation area to 20 $\mu\text{mol/L}$ ADP) during a period of 10 minutes were recorded. For the VerifyNow® assay, the blood samples for each time point for the P2Y12 cartridge were drawn into two 1.8-mL blood collection tubes containing 3.2% sodium citrate and analyzed independently. Fibrinogen-coated microparticles are used in the P2Y12 cartridge to bind to available platelet receptors¹⁸. The results are reported in P2Y12 reaction units (PRU), which represent the amount of ADP-mediated aggregation specific to the platelet P2Y12 receptor. The PRU values were determined based on the rate and extent of platelet reactivity (by way of aggregation) in the ADP channel. Another separate channel contained fibrinogen-coated polystyrene beads and iso-thrombin receptor activating peptide (iso-TRAP) as an agonist, and the baseline value (BASE) was obtained. The %inhibition was calculated as: $[(\text{BASE-PRU})/\text{BASE}] \times 100$, which represents the difference between the pre- and posttreatment values.

The VASP phosphorylation state of whole blood was measured using a standardized flow cytometric assay, which is an adaptation of the method of Schwarz *et al.*¹⁹. The PRI (expressed as a percentage), which reflects the difference in VASP fluorescence intensity between resting (+ PGE1) and activated (+ ADP) platelets, was calculated from the median fluorescence intensity (MFI) of samples incubated with prostaglandin E1 (PGE1) or PGE1 and ADP according to the formula: $\text{PRI} = [(MFI_{(\text{PGE1})} - MFI_{(\text{PGE1} + \text{ADP})}) / MFI_{(\text{PGE1})}] \times 100$ ²⁰. Platelet adhesiveness was measured as previously described²¹.

Measurement of Blood Coagulation and Inflammatory Biomarkers

Venous blood samples were obtained before, immediately after and one, two and 28 days after elective PCI. At the time of sampling, the first 3 mL of blood was obtained for biochemical assessment, and the subsequent 4.5 mL of venous blood was collected in a sequential manner into an evacuated tube containing 0.5 mL of sodium citrate solution (0.13 mol/L, pH 7.5). All blood samples were immediately centrifuged at 1,700 g for 10 minutes at 4°C, and aliquots of the samples were stored at -80°C until the analysis.

The plasma VWF antigen levels were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Asserachrom VWF; Roche, Tokyo) according to the instructions supplied by the manufacturer. The plasma ADAMTS13 antigen levels were measured using sandwich ELISA with a polyclonal antibody against ADAMTS13, as previously reported²². The plasma levels of other biomarkers for coagulation activation, such as sP-selectin and sCD40L, and markers of inflammation, such as IL-6 and sICAM-1, were measured using ELISA kits (sP-selectin: Takara Shuzo Co, Tokyo, sCD40L: R&D Systems, Oxford, UK, IL-6: R&D Systems and sICAM-1: eBioscience; Vienna, Austria) according to the instructions provided by the supplier. The serum hs-TnT levels were also measured (Elecsys 2010 analyzer, Roche Diagnostics, Mannheim, Germany) as previously described²³.

Determination of the CYP2C19 Genotype and Phenotype

Genomic DNA was extracted from whole blood using the DNA Extractor WB kit (Wako Pure Chemical Industries, Osaka, Japan) and the modified protocol of Richards *et al.*²⁴. PCR restriction fragment length polymorphisms (RFLPs) for CYP2C19*2 (681G>A) and CYP2C19*3 (636G>A) were obtained as previously described²⁵. The CYP2C19 genotype was classified into three phenotypes: an extensive metabolizer (EM) carrying normal function alleles (CYP2C19*1/*1), an intermediate metabolizer (IM) carrying one loss-of-function allele (*1/*2, *1/*3) and a poor metabolizer (PM) carrying two loss-of-function alleles (*2/*2, *2/*3, *3/*3).

Assessment of Clinical Outcomes

After undergoing laboratory sampling and elective PCI, all patients received standard medical therapy and were followed for a maximum of six months or with visits until the occurrence of the clinical end point. The clinical end point was a major cardiac or cerebrovascular event, such as cardiac death, nonfatal

Table 1. Clinical characteristics of the study groups at baseline

Characteristics	CYP2C19 Reduced-function Allele		
	Carriers <i>n</i> = 68 (65%)	Noncarriers <i>n</i> = 36 (35%)	<i>p</i> value
Age, (yrs)	70.1 ± 9.3	66.8 ± 10.1	0.210
Female sex, <i>n</i> (%)	17 (25)	7 (19)	0.522
Body mass index (kg/m ²)	25.1 ± 3.4	25.9 ± 3.8	0.695
Current smokers, <i>n</i> (%)	10 (15)	12 (33)	0.304
Diabetes mellitus, <i>n</i> (%)	19 (29)	11 (31)	0.287
Hypertension, <i>n</i> (%)	56 (82)	31 (86)	0.622
Dyslipidemia, <i>n</i> (%)	44 (65)	25 (69)	0.627
Previous myocardial infarction	36 (53)	24 (67)	0.229
Clopidogrel treatment for more than 1 week prior to PCI, <i>n</i> (%)	35 (51)	19 (53)	0.899
Use of ISDNs, <i>n</i> (%)	22 (32)	9 (25)	0.435
Use of CCBs, <i>n</i> (%)	40 (59)	18 (50)	0.389
Use of ACE-Is or ARBs, <i>n</i> (%)	38 (56)	26 (72)	0.103
Use of β -blockers, <i>n</i> (%)	30 (44)	20 (56)	0.267
Use of Statins, <i>n</i> (%)	48 (71)	32 (89)	0.014
Use of CYP3A4-metabolized Statins, <i>n</i> (%)	20 (42)	13 (41)	0.838
Use of PPIs, <i>n</i> (%)	20 (29)	15 (42)	0.208
Use of omeprazole, <i>n</i> (%)	4 (20)	2 (13)	0.948
Hematocrit, (%)	39.9 ± 4.2	40.8 ± 3.6	0.285
Platelet count, (10 ³ / μ L)	22.0 ± 8.7	22.3 ± 5.4	0.942
Fibrinogen, (mg/dL)	353.8 ± 64.5	331.7 ± 17.5	0.598
Fasting blood glucose, (mg/dL)	123.1 ± 47.5	120.6 ± 52.8	0.393
Hemoglobin A1c, (%)	5.78 ± 0.99	6.05 ± 1.05	0.250
eGFR, (mL/min/1.73 m ²)	66.6 ± 16.7	69.0 ± 13.9	0.651
Number of narrowed coronary arteries ($\geq 75\%$)			
1, <i>n</i> (%)	56 (82)	30 (83)	0.900
2, <i>n</i> (%)	11 (16)	6 (17)	0.946
3, <i>n</i> (%)	1 (1)	0 (0)	1.000
B-type natriuretic peptide, (pg/mL)	48.6 ± 53.4	84.8 ± 164.4	0.346
Left ventricular ejection fraction, (%)	65.7 ± 9.3	62.2 ± 12.5	0.265

The data are presented as the mean $\pm$ SD or *n* (%).

CYP=cytochrome P450; ISDN=isosorbide dinitrate, CCB=calcium-channel blocker; ACE-I=angiotensin-converting enzyme antagonist; ARB=angiotensin II receptor antagonist; PPI=proton pump inhibitor; eGFR=estimated glomerular filtration rate

myocardial infarction, unstable angina pectoris or ischemic stroke.

Statistical Analysis

Based on the data described previously regarding the on-clopidogrel PA levels in the carriers and noncarriers of the CYP2C19 reduced-function allele²⁶, we hypothesized that the mean difference in the rate of inhibition of platelet aggregation between carriers and noncarriers of the CYP2C19 reduced-function allele would be 14% with a standard deviation of 20%. Therefore, in order to detect a statistically significant difference with a power of 90% and a two-sided α -level

of 0.05, a sample of 100 patients was required.

The data are expressed as the mean $\pm$ standard deviation. Categorical data are presented as frequencies and percentages. Differences between carriers and noncarriers were tested using the chi-square test (and Fisher's exact test) for categorical variables. Differences in continuous variables were analyzed using the unpaired *t*-test and Mann-Whitney *U* test. The statistical significance of changes in the time course of the platelet function tests and biomarkers between carriers and noncarriers was evaluated using a one-way analysis of variance for repeated measures. Associations between the presence of carriers of at least one

Table 2. Parameters for the platelet function tests and blood biomarkers in the study groups at baseline

Parameters	CYP2C19 Reduced-function Allele		
	Carriers <i>n</i> = 68 (65%)	Noncarriers <i>n</i> = 36 (35%)	<i>p</i> value
ADP-PRmax (%)	55.9 ± 9.8	47.0 ± 12.9	< 0.001
ADP-PR area (AU* min)	4067.4 ± 1247.7	2804.8 ± 1816.6	< 0.001
PRU	271.5 ± 75.4	212.1 ± 81.4	0.001
%Inhibition(%)	17.7 ± 17.6	35.8 ± 22.2	< 0.001
PRI (%)	62.7 ± 13.9	48.0 ± 20.4	< 0.001
Platelet Adhesiveness (%)	31.0 ± 17.3	30.6 ± 21.4	0.438
VWF (mU/mL)	1910.9 ± 715.0	1849.1 ± 767.4	0.485
ADAMTS13 (mU/mL)	886.8 ± 279.3	863.5 ± 281.4	0.734
sCD40L (pg/mL)	2259.0 ± 1023.0	2660.6 ± 1380.0	0.188
sP-selectin (ng/mL)	445.2 ± 256.1	499.7 ± 243.5	0.331
IL-6 (pg/mL)	2.20 ± 1.55	2.72 ± 3.08	0.733
sICAM-1 (ng/mL)	446.0 ± 144.9	467.6 ± 156.1	0.521
hs-CRP (mg/dL)	0.022 ± 0.034	0.027 ± 0.070	0.740
hs-TnT (pg/mL)	8.62 ± 8.29	12.5 ± 12.2	0.086

The data are presented as the mean ± SD.

ADP-PRmax = ADP-induced platelet reactivity maximum aggregation; ADP-PR area = ADP-induced platelet reactivity area; PRU = P2Y12 reaction unit; PRI = platelet reactivity index [the index of vasodilator-stimulated phosphoprotein (VASP) phosphorylation]; VWF = von-Willebrand factor; ADAMTS13 = the 13th member of the ADAMTS (a disintegrin-like domain and metalloprotease with thrombospondin type 1 motif) family; sCD40L = soluble CD40 ligand; sP-selectin = soluble P-selectin; IL-6 = interleukin-6; sICAM-1 = soluble Intercellular adhesion molecule-1; hs-CRP = high-sensitivity C-reactive protein; hs-TnT = high-sensitivity troponin T

reduced-function CYP2C19 allele and other significant parameters that have been shown to influence the platelet function in recent studies²⁷⁻³¹⁾ were analyzed using a multiple logistic regression analysis with the forced entry method. The Hosmer-Lemeshow statistic was applied to assess model calibration. A *p* value of < 0.05 denoted statistical significance. The statistical analyses were performed using the Statistical Package for Social Sciences version 18 software program (SPSS Inc., Tokyo, Japan).

Results

Patient Characteristics and the Frequency of the CYP2C19 Genotypes and Phenotypes

Of the 104 patients [80 men and 24 women, 68.7 ± 9.5 years of age (mean ± SD, range, 42-89)] who underwent PCI and were treated with aspirin and clopidogrel, the distribution of the CYP2C19 genotype was 36 (35%) patients, 41 (39%) patients, 14 (13%) patients, eight (8%) patients, three (3%) patients and two (2%) patients for *1/*1, *1/*2, *1/*3, *2/*2, *2/*3 and *3/*3, respectively. Therefore, 68 (65%) patients were carriers of at least one CYP2C19 reduced-function allele [IM: 55 (53%), PM: 13 (12%)]

and 36 (35%) patients were noncarriers (EM). The clinical characteristics of the carriers and noncarriers are listed in **Tables 1** and **2**. There were no significant differences between the carriers and noncarriers in baseline characteristics known to be associated with platelet aggregation. **Table 1** also lists the medication history in each group at the time of the baseline blood sampling. The proportions of patients receiving isosorbide dinitrate, calcium channel blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, β -blockers and proton pump inhibitors were identical in the two groups, while the use of statins was more frequent in the noncarriers than in the carriers.

Clinical Outcomes and Serial Changes in the Platelet Function Tests and Blood Levels of Coagulation and Inflammatory Biomarkers in the Carriers and Noncarriers

As shown in **Fig. 1**, platelet inhibition measured using LTA (20 μ mol/L ADP-maximal platelet reactivity (PRmax) and 20 μ mol/L ADP-PR area), the VerifyNow® P2Y12 system (PRU and %inhibition) and the PRI (VASP phosphorylation index), but not platelet adhesiveness, was diminished in the carriers com-

Fig. 1. Serial changes in the platelet function tests before, immediately after and one, two and 28 days after elective percutaneous coronary intervention in the carriers and noncarriers of the CYP2C19 reduced-function allele among the patients with stable coronary artery disease. The tests included measurements of the level of on-clopidogrel high platelet aggregation determined using light transmittance aggregometry [(A) 20 $\mu\text{mol/L}$ adenosine diphosphate (ADP)-induced maximal platelet reactivity (PRmax), (B) 20 $\mu\text{mol/L}$ ADP-induced PR area], the VerifyNow[®] assay system [(C) PRU, (D) %inhibition], (E) the platelet reactivity index (PRI) [the index of vasodilator-stimulated phosphoprotein (VASP) phosphorylation] and (F) platelet adhesiveness. * $p < 0.05$ vs noncarriers.

pared to that observed in the noncarriers at all time points. The levels of on-clopidogrel PA were significantly increased immediately after compared with before PCI. **Fig. 2** and **3** demonstrate the levels of var-

ious blood biomarkers for coagulation activation and inflammation at all time points. The plasma levels of VWF, ADAMTS13, sP-selectin, sCD40 ligand, IL-6, sICAM-1 and hs-CRP were identical between the car-

Fig. 2. Serial changes in the levels of various blood coagulation and inflammatory biomarkers [(A) von-Willebrand factor (VWF), (B) VWF-cleaving protease (ADAMTS13), (C) soluble CD40 ligand (sCD40L) and (D) soluble P-selectin (sP-selectin)] before, immediately after and one, two and 28 days after elective percutaneous coronary intervention in the carriers and noncarriers of the CYP2C19 reduced-function allele among the patients with stable coronary artery disease.

riers and noncarriers at all time points. The serum cardiac hs-TnT levels were increased significantly at one and two days after compared with before PCI in both groups, while the levels were identical between the two groups at all time points.

During the follow-up period, only one carrier patient developed ischemic stroke, while no major cardiac or cerebrovascular events were observed in the noncarriers.

Simple and Multiple Logistic Regression Analyses to Predict the Occurrence of Carriers of the CYP2C19 Reduced-Function Allele

We examined whether the above parameters measured before PCI could be used to predict carriers of the CYP2C19 reduced-function allele among stable CAD patients undergoing PCI on DAPT. A simple logistic regression analysis demonstrated that the platelet function parameters measured according to 20 μ mol/L of ADP-PRmax, 20 μ mol/L of ADP-PR area,

PRU, %inhibition and PRI before PCI were correlated with the presence of carriers of the CYP2C19 reduced-function allele among the examined patients (Table 3). However, the other platelet function parameter (platelet adhesion), the coagulation and inflammatory biomarkers (VWF, ADAMTS13, sP-selectin, sCD40L, IL-6, sICAM-1 and hs-CRP) and the serum hs-TnT levels were not correlated with a carriers status. A multiple logistic regression analysis identified the four on-clopidogrel PA parameters (20 μ mol/L of ADP-PRmax, 20 μ mol/L of ADP-PR area, PRU and %inhibition) and the PRI as being significant predictors of carriers of the CYP2C19 reduced-function allele among the stable CAD patients [odds ratio (OR) 13.17; 95% confidence interval (95%CI): 3.62-47.93; $p < 0.001$, OR 4.84; 95%CI: 1.59 to 14.77; $p = 0.006$, OR 4.85; 95%CI: 1.66 to 14.13; $p = 0.004$, OR 6.31; 95%CI: 1.98 to 20.08; $p = 0.002$, OR 6.32; 95%CI: 2.19 to 18.24; $p = 0.001$, respectively] (Table 4). These models were reliable when each parameter of the plate-

Fig. 3. Serial changes in the levels of various blood coagulation and inflammatory biomarkers [(A) interleukin (IL)-6, (B) soluble intercellular adhesion molecule 1 (sICAM-1), (C) high-sensitivity C-reactive protein (hs-CRP)] and a cardiac biomarker [(D) serum high-sensitivity troponin T (hs-TnT)] before, immediately after and one, two and 28 days after elective percutaneous coronary intervention in the carriers and non-carriers of the CYP2C19 reduced-function allele among the patients with stable coronary artery disease. * $p < 0.05$ vs baseline in both groups.

let function tests measured according to 20 $\mu\text{mol/L}$ of ADP-PRmax, 20 $\mu\text{mol/L}$ of ADP-PR area, PRU, %inhibition and PRI was selected ($p=0.805$, $p=0.644$, $p=0.939$, $p=0.855$ and $p=0.756$, respectively, according to the Hosmer-Lemeshow test).

Discussion

The present study demonstrated that the on-clopidogrel PA level and PRI, but not blood coagulation or inflammatory biomarkers, are predictors of CYP2C19 reduced-function gene variants in stable CAD patients undergoing elective PCI on DAPT. Although carriers of the reduced-function CYP2C19 allele on DAPT exhibit diminished platelet inhibition and are at higher risk after ACS, it was not clear whether CYP2C19 gene variants influence platelet

function tests and blood biomarkers of coagulation activation and inflammation in stable CAD patients undergoing elective PCI. To the best of our knowledge, this is the first report to show a relationship between CYP2C19 reduced-function gene variants, platelet function tests and blood biomarkers for coagulation activation and inflammation in stable CAD patients undergoing elective PCI.

Recent studies^{14, 32)} have shown that, among the functional genetic variants of CYP genes, carriers of at least one CYP2C19 reduced-function allele have significantly lower levels of the active metabolites of clopidogrel, reduced platelet inhibition and a higher rate of major adverse cardiovascular events (MACE), including stent thrombosis, than noncarriers among ACS patients treated with clopidogrel for PCI. However, few patients develop MACE after PCI among

Table 3. Results of the simple regression analysis of carriers of the CYP2C19 reduced-function allele

Characteristics	Simple Regression analysis		
	OR	95% CI	<i>p</i> value
Age, (yrs) ^a	1.37	0.50-3.72	0.716
Female sex, n (%)	1.38	0.51-3.72	0.693
Body mass index (kg/m ²) ^a	0.47	0.17-1.29	0.217
Current Smokers, n (%)	0.52	0.19-1.42	0.304
Diabetes Mellitus, n (%)	0.89	0.38-2.12	0.973
Hypertension, n (%)	1.03	0.45-2.36	0.883
Dyslipidemia, n (%)	0.52	0.22-1.24	0.202
Hemoglobin, (mg/dL) ^b	1.71	0.65-4.51	0.392
Hematocrit, (%) ^b	1.57	0.61-4.08	0.485
Platelet count, (10 ³ /μL) ^b	0.86	0.34-2.20	0.945
Fibrinogen, (mg/dL) ^b	1.12	0.47-2.65	0.973
Fasting blood glucose, (mg/dL) ^a	0.98	0.43-2.22	0.872
Hemoglobin A1c, (%) ^a	0.79	0.27-2.28	0.869
eGFR, (mL/min/1.73 m ²) ^a	1.15	0.42-3.16	0.982
B-type natriuretic peptide, (pg/mL) ^b	0.98	0.43-2.22	0.872
Left ventricular ejection fraction, (%) ^a	0.83	0.35-1.98	0.850
ADP-PRmax (%) ^b	9.00	3.25-24.96	<0.001
ADP-PR area (AU*min) ^b	4.00	1.56-10.24	0.003
PRU ^a	4.71	1.80-12.33	0.001
%Inhibition (%) ^a	5.43	2.09-14.11	<0.001
PRI (%) ^a	5.43	2.09-14.11	<0.001
Platelet Adhesiveness (%) ^b	1.38	0.56-3.39	1.000
vWF (mU/mL) ^b	1.23	0.51-2.93	0.649
ADAMTS13 (mU/mL) ^b	2.44	0.96-6.20	0.057
sCD40L (pg/mL) ^b	0.65	0.27-1.57	0.340
sP-selectin (ng/mL) ^b	1.20	0.48-2.95	0.700
IL-6 (pg/mL) ^b	0.91	0.35-2.35	1.000
sICAM-1 (ng/mL) ^b	0.68	0.27-1.73	0.569
hs-CRP (mg/mL) ^b	0.65	0.29-1.97	0.588
hs-TnT (pg/mL) ^a	0.37	0.11-1.25	0.188

The data are presented as the mean ± SD or n (%).

^aData for these parameters were divided into two groups based on the following criteria: age >75 years, Body mass index >25 kg/m², Fasting blood glucose >126 mg/dL, Hemoglobin A1c >6.1% (JDS), eGFR <60 mL/min/1.73 m², Left ventricular ejection fraction >60%, PRU >230, % Inhibition <24%, PRI >50%, hs-TnT >40 ng/mL. ^bData for these parameters were divided into two groups based on the median value. The abbreviations are as in Tables 1 and 2.

patients with stable CAD. In this regard, subclinical intrastent thrombosis has been reported to be more common in CYP2C19*2 carriers than in noncarriers on follow-up OCT after DES implantation among stable CAD patients³³, although the same study reported a lower frequency of MACE in both the carriers and noncarriers³³. Based on these observations, the development of medical treatments that can be used to discriminate the risk of reduced-function CYP2C19 gene variants is needed, even for patients with stable CAD treated with DAPT.

Several blood biomarkers of platelet and coagula-

tion activation and markers of inflammation are thought to play important roles in the pathogenesis of atherothrombotic complications, such as acute or sub-acute stent thrombosis, in patients undergoing PCI. In regard to this point, it has been demonstrated that, in patients undergoing PCI, a high clopidogrel maintenance dose is associated with stronger platelet inhibition, an improvement of the endothelial function and a reduction of inflammation compared with the currently recommended 75-mg/day regimen³⁴ and that the 75-mg/day clopidogrel regimen differentially affects platelet aggregation and platelet-induced

Table 4. Results of the multiple regression analysis for predicting the presence of carriers of the CYP2C19 reduced-function allele

Variable	Multiple Regression analysis									
	ADP-PRmax selected		ADP-PR area selected		PRU selected		% Inhibition selected		PRI selected	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
ADP-PRmax (%) ^a	13.17 (3.62-47.93)	<0.001								
ADP-PR area (AU*min) ^a			4.84 (1.59-14.77)	0.006						
PRU ^a					4.85 (1.66-14.13)	0.004				
%Inhibition (%) ^a							6.31 (1.98-20.08)	0.002		
PRI (%) ^a									6.32 (2.19-18.24)	0.001
Platelet Adhesiveness (%)	2.37 (0.63-8.90)	0.210	1.64 (0.50-5.37)	0.415	1.38 (0.45-4.25)	0.571	1.98 (0.56-6.95)	0.289	1.48 (0.48-4.57)	0.501
VWF (mU/mL) ^a	1.75 (0.51-6.02)	0.373	1.05 (0.35-3.13)	0.927	0.823 (0.28-2.40)	0.721	0.97 (0.32-2.93)	0.963	1.20 (0.39-3.70)	0.754
ADAMTS13 (mU/mL) ^a	2.22 (0.75-6.61)	0.151	2.64 (0.96-7.27)	0.060	2.27 (0.83-6.16)	0.109	1.77 (0.63-4.96)	0.277	2.91 (1.02-8.26)	0.045
sCD40L (pg/mL) ^a	1.23 (0.37-4.06)	0.731	1.64 (0.56-4.82)	0.371	1.50 (0.51-4.40)	0.464	1.75 (0.58-5.30)	0.321	1.34 (0.42-4.27)	0.617
sP-selectin (ng/mL) ^a	1.78 (0.46-6.84)	0.402	1.10 (0.34-3.56)	0.875	0.907 (0.29-2.83)	0.866	0.78 (0.24-2.50)	0.670	0.94 (0.28-3.14)	0.914

^aData for these parameters were divided into two groups based on the same criteria as in Table 3. The abbreviations are as in Tables 1, 2 and 3.

inflammation in ACS patients within the first month of treatment³⁵). Because the frequency of CYP2C19 reduced-function gene variants is higher in Japanese individuals (65% of the total population) than in Westerners (approximately 30% of the total population), it is possible that these biomarkers are affected by CYP2C19 reduced-function alleles in the Japanese population. Based on these observations, we hypothesize that CYP2C19 reduced-function gene variants alter the levels of blood biomarkers of coagulation activation and inflammation in CAD patients undergoing PCI treated with DAPT. However, our results showed that there were no significant differences in the levels of such biomarkers or the serum hs-TnT levels at any time point between the carriers and non-carriers. In this regard, Husted *et al.*³⁶ reported that, in their study, inflammatory biomarkers, such as CRP, IL-6, myeloperoxidase and sCD40L, were not significantly different between treatment groups of clopidogrel and the potent P2Y₁₂ receptor inhibitor, ticagre-

lor, at baseline, discharge and four weeks. The CURE study also reported similar findings: that clopidogrel did not suppress biomarkers of coagulation activation in aspirin-treated patients with non-ST elevation ACS³⁷), although the authors did not discuss the relationship between CYP2C19 reduced-function gene variants and biomarkers of platelets and coagulation activation. A potential explanation for the present finding is patient selection; the enrolled patients were low-risk stable CAD patients with less marked platelet and coagulation activation and a lower inflammatory status, thereby limiting the statistical power of this study to detect differences in these markers between the carriers and noncarriers. However, the baseline characteristics and rates of clinical events were similar, while the on-clopidogrel PA levels measured using LTA, PRU and %inhibition were significantly different between the two groups, and the study population size was determined using a power analysis. Therefore, the lack of statistical power is unlikely to account for

the present findings.

In the present study, the on-clopidogrel PA level and PRI were found to be factors associated with the presence of the CYP2C19 reduced-function allele in patients with stable CAD undergoing elective PCI. LTA is considered to be a standard test that includes measurements of the on-clopidogrel PA levels in CAD patients receiving dual antiplatelet therapy^{11, 15, 38}. However, LTA has certain drawbacks in measuring platelet aggregation, such as the long sample processing time and need for expert personnel. The PRI (the VASP phosphorylation state of whole blood), which is measured using a standardized flow cytometric assay, is also useful for assessing on-clopidogrel platelet reactivity, although it is not a rapid assay system. On the other hand, the VerifyNow® P2Y12 system is an easy-to-use point-of-care system designed to measure platelet reactivity to various antiplatelet agents¹⁸, allowing for real-time monitoring of the PA levels in CAD patients treated with clopidogrel. In our previous study, we demonstrated that the on-clopidogrel PA level determined by the PRU and %inhibition was significantly correlated with that measured using LTA, confirming the reliability of the VerifyNow® P2Y12 system for measuring the on-clopidogrel PA levels in CAD patients undergoing elective PCI. Therefore, the present results demonstrate the usefulness of the on-clopidogrel PA level measured according to the PRU and %inhibition for predicting the presence of the CYP2C19 reduced-function allele in stable CAD patients undergoing elective PCI.

The present study found that carriers of the CYP2C19 reduced-function allele constituted 65% of the patients, compared with a noncarrier rate of 35%. In a recent study of healthy Westerners, the prevalence of carriers of the CYP2C19 reduced-function allele was 30% in the total study population¹⁴. However, adverse cardiovascular events, including stent thrombosis after primary or elective PCI, are lower in Japanese individuals than in Westerners³⁹⁻⁴¹, although the frequency of carriers of at least one CYP2C19 reduced-function allele is higher in Japanese patients than Western patients. The reason for the low risk of adverse cardiovascular events, including stent thrombosis, in Japan remains unknown; however, it is possible that other factors, such as the PCI procedure, the characteristics of the culprit coronary plaques or coronary risk factors, in addition to platelet aggregation, may influence the clinical outcomes of CAD patients treated with clopidogrel.

Conclusion

In summary, the present study demonstrated that carriers of the CYP2C19 reduced-function allele treated with DAPT have abnormal on-clopidogrel PA levels and PRI values, but not cardiac hs-TnT levels or biomarkers of coagulation activation or inflammation. These tests are potentially valuable for identifying such carriers and monitoring the efficacy of DAPT in Japanese patients undergoing elective PCI.

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Disclosures

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References

- 1) Fuster V, Badimon L, Badimon JJ, Chesebro J: The pathogenesis of coronary artery disease and acute coronary syndromes. *N Engl J Med*, 1992; 326: 242-250
- 2) Wiviott SD, Braunwald E, McCabe CH, Montalescot G, Ruzyllo W, Gottlieb S, Neumann FJ, Ardissino D, De Servi S, Murphy SA, Riesmeyer J, Weerakkody G, Gibson CM, Antman EM; TRITONTIMI 38 Investigators: Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med*, 2007; 357: 2001-2015
- 3) Wiviott SD, Braunwald E, McCabe CH, Horvath I, Keltai M, Herrman JP, Van de Werf F, Downey WE, Scirica BM, Murphy SA, Antman EM; TRITONTIMI 38 Investigators: Intensive oral antiplatelet therapy for reduction of ischaemic events including stent thrombosis in patients with acute coronary syndromes treated with percutaneous coronary intervention and stenting in the TRITONTIMI 38 trial: a subanalysis of a randomised trial. *Lancet*, 2008; 371: 1353-1363
- 4) Marcucci R, Gori AM, Panicia R, Giusti B, Valente S, Giglioli C, Buonamici P, Antonucci D, Abbate R, Genesini GF: Cardiovascular death and nonfatal myocardial

- infarction in acute coronary syndrome patients receiving coronary stenting are predicted by residual platelet reactivity to ADP detected by a point-of-care assay. *Circulation*, 2009; 119: 237-242
- 5) Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK, for the Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators: Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med*, 2001; 345: 494-502
 - 6) Mehta SR, Yusuf S, Peters RJ, Bertrand ME, Lewis BS, Natarajan MK, Malmberg K, Rupprecht H, Zhao F, Chrolavicius S, Copland I, Fox KA; Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial (CURE) Investigators: Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. *Lancet*, 2001; 358: 527-533
 - 7) Steinhubl SR, Berger PB, Mann JT 3rd, Fry ET, DeLago A, Wilmer C, Topol EJ; CREDO Investigators: Clopidogrel for the Reduction of Events During Observation. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. *JAMA*, 2002; 288: 2411-2420
 - 8) Chen ZM, Jiang LX, Chen YP, Xie JX, Pan HC, Peto R, Collins R, Liu LS; the COMMIT (CLOpidogrel and Metoprolol in Myocardial Infarction Trial) Collaborative Group: Addition of clopidogrel to aspirin in 45,852 patients with acute myocardial infarction: randomised placebo-controlled trial. *Lancet*, 2005; 366: 1607-1621
 - 9) Sabatine MS, Cannon CP, Gibson CM, López-Sendón JL, Montalescot G, Theroux P, Claeys MJ, Cools F, Hill KA, Skene AM, McCabe CH, Braunwald E; CLARITY-TIMI 28 Investigators: Addition of clopidogrel to aspirin and fibrinolytic therapy for myocardial infarction with ST-segment elevation. *N Engl J Med*, 2005; 352: 1179-1189
 - 10) Matetzky S, Shenkman B, Guetta V, Shechter M, Bienart R, Goldenberg I, Novikov I, Pres H, Savion N, Varon D, Hod H: Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. *Circulation*, 2004; 109: 3171-3175
 - 11) Hochholzer W, Trenk D, Bestehorn HP, Fischer B, Valina CM, Ferenc M, Gick M, Caputo A, Büttner HJ, Neumann FJ: Impact of the degree of peri-interventional platelet inhibition after loading with clopidogrel on early clinical outcome of elective coronary stent placement. *J Am Coll Cardiol*, 2006; 48: 1742-1750
 - 12) Gurbel PA, Bliden KP, Samara W, Yoho JA, Hayes K, Fisha MZ, Tantry US: Clopidogrel effect on platelet reactivity in patients with stent thrombosis: results of the CREST Study. *J Am Coll Cardiol*, 2005; 46: 1827-1832
 - 13) Trenk D, Hochholzer W, Fromm MF, Chialda LE, Pahl A, Valina CM, Stratz C, Schmiebusch P, Bestehorn HP, Büttner HJ, Neumann FJ: Cytochrome P450 2C19 681G> A polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare-metal stents. *J Am Coll Cardiol*, 2008; 51: 1925-1934
 - 14) Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, Walker JR, Antman EM, Macias W, Braunwald E, Sabatine MS: Cytochrome p-450 polymorphisms and response to clopidogrel. *N Engl J Med*, 2009; 360: 354-362
 - 15) Gurbel PA, Bliden KP, Guyer K, Cho PW, Zaman KA, Kreutz RP, Bassi AK, Tantry US: Platelet reactivity in patients and recurrent events post-stenting: results of the PREPARE POST-STENTING Study. *J Am Coll Cardiol*, 2005; 46: 1820-1826
 - 16) Breet NJ, van Werkum JW, Bouman HJ, Kelder JC, Ruven HJ, Bal ET, Deneer VH, Harmsze AM, van der Heyden JA, Rensing BJ, Suttorp MJ, Hackeng CM, ten Berg JM: Comparison of platelet function tests in predicting clinical outcome in patients undergoing coronary stent implantation. *JAMA*, 2010; 303: 754-762
 - 17) Ono T, Kaikita K, Hokimoto S, Iwashita S, Yamamoto K, Miyazaki Y, Horio E, Sato K, Tsujita K, Abe T, Deguchi M, Tayama S, Sumida H, Sugiyama S, Yamabe H, Nakamura S, Nakagawa K, Ogawa H: Determination of cut-off levels for on-clopidogrel platelet aggregation based on functional CYP2C19 gene variants in patients undergoing elective percutaneous coronary intervention. *Thromb Res*, 2011; 128: e130-136
 - 18) Malinin A, Pokov A, Spergling M, Defranco A, Schwartz K, Schwartz D, Mahmud E, Atar D, Serebruany V: Monitoring platelet inhibition after clopidogrel with the VerifyNow-P2Y12(R) rapid analyzer: the VERIfy Thrombosis risk ASsessment (VERITAS) study. *Thromb Res*, 2007; 119: 277-284
 - 19) Aleil B, Ravanat C, Cazenave JP, Rochoux G, Heitz A, Gachet C: Flow cytometric analysis of intraplatelet VASP phosphorylation for the detection of clopidogrel resistance in patients with ischemic cardiovascular diseases. *J Thromb Haemost*, 2005; 3: 85-92
 - 20) Schwarz UR, Geiger J, Walter U, Eigenthaler M: Flow cytometry analysis of intracellular VASP phosphorylation for the assessment of activating and inhibitory signal transduction pathways in human platelets-definition and detection of ticlopidine/clopidogrel effects. *Thromb Haemost*, 1999; 82: 1145-1152
 - 21) Kaneko M, Takafuta T, Cuyun-Lira O, Satoh K, Arai M, Yatomi Y, Ozaki Y: Evaluation of platelet function under high shear condition in the small-sized collagen bead column. *J Lab Clin Med*, 2005; 146: 64-75
 - 22) Kaikita K, Soejima K, Matsukawa M, Nakagaki T, Ogawa H: Reduced von Willebrand factor-cleaving protease (ADAMTS13) activity in acute myocardial infarction. *J Thromb Haemost*, 2006; 4: 2490-2493
 - 23) Lankeit M, Friesen D, Aschoff J, Dellas C, Hasenfuss G, Katus H, Konstantinides S, Giannitsis E: Highly sensitive troponin T assay in normotensive patients with acute pulmonary embolism. *Eur Heart J*, 2010; 31: 1836-1844
 - 24) Richards B, Skoletsky J, Shuber AP, Balfour R, Stern RC, Dorkin HL, Parad RB, Witt D, Klinger KW: Multiplex PCR amplification from the CFTR gene using DNA prepared from buccal brushes/swabs. *Hum Mol Genet*, 1993; 2: 159-163
 - 25) Kubota T, Chiba K, Ishizaki T: Genotyping of S-mephe-

- nytoin 4'-hydroxylation in an extended Japanese population. *Clin Pharmacol Ther*, 1996; 60: 661-666
- 26) Jinnai T, Horiuchi H, Makiyama T, Tazaki J, Tada T, Akao M, Ono K, Hoshino K, Naruse Y, Takahashi K, Watanabe H, Kita T, Kimura T: Impact of CYP2C19 polymorphisms on the antiplatelet effect of clopidogrel in an actual clinical setting in Japan. *Circ J*, 2009; 73: 1498-1503
 - 27) Angiolillo DJ, Bernardo E, Capodanno D, Vivas D, Sabaté M, Ferreiro JL, Ueno M, Jimenez-Quevedo P, Alfonso F, Bass TA, Macaya C, Fernandez-Ortiz A: Impact of chronic kidney disease on platelet function profiles in diabetes mellitus patients with coronary artery disease taking dual antiplatelet therapy. *J Am Coll Cardiol*, 2010; 55: 1139-1146
 - 28) Wiviott SD, Braunwald E, Angiolillo DJ, Meisel S, Dalby AJ, Verheugt FW, Goodman SG, Corbalan R, Purdy DA, Murphy SA, McCabe CH, Antman EM; TRITON-TIMI 38 Investigators: Greater clinical benefit of more intensive oral antiplatelet therapy with prasugrel in patients with diabetes mellitus in the trial to assess improvement in therapeutic outcomes by optimizing platelet inhibition with prasugrel-Thrombolysis in Myocardial Infarction 38. *Circulation*, 2008; 118: 1626-1636
 - 29) Berger JS, Bhatt DL, Steinhubl SR, Shao M, Steg PG, Montalescot G, Hacke W, Fox KA, Lincoff AM, Topol EJ, Berger PB; the CHARISMA Investigators: Smoking, clopidogrel, and mortality in patients with established cardiovascular disease. *Circulation*, 2009; 120: 2337-2344
 - 30) Gremmel T, Steiner S, Seidinger D, Koppensteiner R, Panzer S, Kopp CW: Adenosine diphosphate-inducible platelet reactivity shows a pronounced age dependency in the initial phase of antiplatelet therapy with clopidogrel. *J Thromb Haemost*, 2010; 8: 37-42
 - 31) Ho PM, Maddox TM, Wang L, Fihn SD, Jesse RL, Peterson ED, Rumsfeld JS: Risk of adverse outcomes associated with concomitant use of clopidogrel and proton pump inhibitors following acute coronary syndrome. *JAMA*, 2009; 301: 937-944
 - 32) Mega JL, Simon T, Collet JP, Anderson JL, Antman EM, Bliden K, Cannon CP, Danchin N, Giusti B, Gurbel P, Horne BD, Hulot JS, Kastrati A, Montalescot G, Neumann FJ, Shen L, Sibbing D, Steg PG, Trenk D, Wiviott SD, Sabatine MS: Reduced-function CYP2C19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for PCI: a meta-analysis. *JAMA*, 2010; 304: 1821-1830
 - 33) Sawada T, Shinke T, Shite J, Honjo T, Haraguchi Y, Nishio R, Shinohara M, Toh R, Ishida T, Kawamori H, Kozuki A, Inoue T, Hariki H, Hirata K: Impact of cytochrome P450 2C19*2 polymorphism on intra-stent thrombus after drug-eluting stent implantation in Japanese patients receiving clopidogrel. *Circ J*, 2010; 75: 99-105
 - 34) Patti G, Grieco D, Dicuonzo G, Pasceri V, Nusca A, Di Sciascio G: High versus standard clopidogrel maintenance dose after percutaneous coronary intervention and effects on platelet inhibition, endothelial function, and inflammation results of the ARMYDA-150 mg (antiplatelet therapy for reduction of myocardial damage during angioplasty) randomized study. *J Am Coll Cardiol*, 2011; 57: 771-778
 - 35) Kalantzi KI, Dimitriou AA, Milionis HJ, Goudevenos IA, Tselepis AD: Clopidogrel differentially affects platelet-mediated thrombosis and inflammatory response in patients with acute coronary syndromes. *J Thromb Haemost*, 2011; 9: 875-878
 - 36) Husted S, Storey RF, Harrington RA, Emanuelsson H, Cannon CP: Changes in inflammatory biomarkers in patients treated with ticagrelor or clopidogrel. *Clin Cardiol*, 2010; 33: 206-212
 - 37) Eikelboom JW, Weitz JI, Budaj A, Zhao F, Copland I, Maciejewski P, Johnston M, Yusuf S: Clopidogrel does not suppress blood markers of coagulation activation in aspirin-treated patients with non-ST-elevation acute coronary syndromes. *Eur Heart J*, 2002; 23: 1771-1779
 - 38) Geisler T, Langer H, Wydymus M, Göhring K, Zürn C, Bigalke B, Stellos K, May AE, Gawaz M: Low response to clopidogrel is associated with cardiovascular outcome after coronary stent implantation. *Eur Heart J*, 2006; 27: 2420-2425
 - 39) Daemen J, Wenaweser P, Tsuchida K, Abrecht L, Vaina S, Morger C, Kukreja N, Juni P, Sianos G, Hellige G, van Domburg RT, Hess OM, Boersma E, Meier B, Windecker S, Serruys PW: Early and late coronary stent thrombosis of sirolimus-eluting and paclitaxel-eluting stents in routine clinical practice: data from a large two-institutional cohort study. *Lancet*, 2007; 369: 667-678
 - 40) Aoki J, Lansky AJ, Mehran R, Moses J, Bertrand ME, McLaurin BT, Cox DA, Lincoff AM, Ohman EM, White HD, Parise H, Leon MB, Stone GW: Early stent thrombosis in patients with acute coronary syndromes treated with drug-eluting and bare metal stents: the Acute Catheterization and Urgent Intervention Triage Strategy trial. *Circulation*, 2009; 119: 687-698
 - 41) Kimura T, Morimoto T, Nakagawa Y, Tamura T, Kadota K, Yasumoto H, Nishikawa H, Hiasa Y, Muramatsu T, Meguro T, Inoue N, Honda H, Hayashi Y, Miyazaki S, Oshima S, Honda T, Shiode N, Namura M, Sone T, Nobuyoshi M, Kita T, Mitsudo K: Antiplatelet therapy and stent thrombosis after sirolimus-eluting stent implantation. *Circulation*, 2009; 119: 987-995