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Regular Article

# Chronic kidney disease status modifies the association of CYP2C19 polymorphism in predicting clinical outcomes following coronary stent implantation

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ABSTRACT

*Introduction:* There is some controversy regarding the effect of CYP2C19 polymorphism on clinical outcome in patients with dual antiplatelet therapy. Chronic kidney disease (CKD) is associated with increased risk of cardiovascular event, but the association between the possession of CYP2C19 loss-of-function (LOF) alleles and clinical outcome according to the presence of CKD is poorly understood. The aim of this study was to investigate whether CKD status modifies the association of CYP2C19 polymorphism in predicting outcomes in a prospective cohort study.
*Material and Methods:* We enrolled 331 patients following coronary stent implantation. Patients were divided into two groups: CKD (n = 154) and non-CKD (n = 177). Platelet reactivity and CYP2C19 polymorphism were examined. The subjects were further divided into two groups according to the possession of CYP2C19 LOF alleles: carriers and non-carriers. Patients were followed up and clinical events were evaluated according to CKD and carrier status.
*Results:* The proportion of high platelet reactivity was significantly higher in carriers than in non-carriers in both CKD (42.4% versus 21.7%; $P = 0.016$) and non-CKD groups (34.3% versus 3.7%; $P < 0.001$). In the non-CKD group alone, the incidence of cardiovascular events was significantly higher in carriers than in non-carriers (13.7% versus 1.7%; $P = 0.013$). Kaplan-Meier analysis demonstrated a significantly higher probability of cardiovascular events in carriers than in non-carriers in the non-CKD group (log-rank test: $P = 0.013$) and there was no significant difference in the CKD group (log-rank test: $P = 0.591$). Multivariate analysis identified carriers as an independent predictor of cardiovascular events only in the non-CKD group alone (hazard ratio: 8.048; 95% confidence interval: 1.066 to 60.757; $P = 0.043$).
*Conclusions:* CYP2C19 polymorphism significantly correlates with clinical outcome in non-CKD patients, and CKD status modifies the association of CYP2C19 polymorphism in predicting clinical outcomes following coronary stent implantation.



## Introduction

Dual antiplatelet therapy (DAPT) is currently recommended for the prevention of adverse cardiovascular events in patients undergoing percutaneous coronary intervention (PCI) [1–3]. Clopidogrel is the mainstay drug for DAPT; however, in some patients, an adequate antiplatelet effect is not achieved, and atherothrombotic events including stent thrombosis are not completely prevented during DAPT including low-dose aspirin and clopidogrel [4,5]. The antiplatelet efficacy of clopidogrel varies widely and high on-clopidogrel platelet reactivity is considered an independent risk factor for cardiovascular events in patients treated with stent implantation [6–8]. Thus, more intense antiplatelet agents, such as prasugrel, ticagrelor and GPIIbIIIa inhibitor, have been developed and used in Western countries, although they remain uncommon in Japan.

The mechanisms leading to high residual platelet reactivity are associated with several demographic and clinical characteristics, such as age, renal failure, obesity, diabetes mellitus, high plasma fibrinogen, genetic polymorphism and lack of adherence [9–12]. Of the genetic factors, cytochrome P450 (CYP) polymorphism correlates with diminished antiplatelet efficacy of clopidogrel and high risk for adverse cardiovascular events following stent implantation [8–10,13,14]. The incidence of the CYP2C19 loss-of-function (LOF) genotype is higher in the Japanese population than in Caucasians [15], and we have demonstrated the association of CYP2C19 genotype with high residual platelet reactivity and increased risk of cardiovascular events in Japanese coronary artery disease patients treated with stent placement [8,16]. Chronic

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kidney disease (CKD) is thought to be associated with cardiovascular events, increased platelet activation and reduced platelet inhibition by DAPT [17]. At present, the association of the possession of CYP2C19 LOF alleles with clinical outcome according to the presence of CKD is poorly understood. Given that residual platelet reactivity and clinical outcome depend on the presence of CYP2C19 LOF alleles and CKD, it is important to determine the effect of CYP2C19 LOF alleles on clinical outcome according to the presence or absence of CKD. The aim of the present study was thus to investigate whether CKD status modifies the association of CYP2C19 polymorphism in predicting outcomes in Japanese patients undergoing coronary stent implantation.

## Material and Methods

### *Study population*

A total of 556 consecutive patients who underwent PCI from January 2009 to November 2012 in our hospital were eligible for this study. We excluded patients who admitted for acute coronary syndrome, and patients who had been treated with steroids, thrombolytic agents, sarpogrelate or cilostazol, and patients with deep vein thrombosis, arterial fibrillation, collagen disease, liver dysfunction and malignant diseases. Thus, a total of 331 patients were enrolled in this study. CKD was defined as estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m [2], and patients were divided into two groups: CKD (n = 154) and non-CKD (n = 177). All patients underwent cardiac catheterization and PCI during hospitalization, and they received DAPT with maintenance doses of 100 mg/day aspirin and 75 mg/day clopidogrel after a loading dose of 300 mg of clopidogrel. This is a prospective single-center study, with a mean follow-up of 890 days. The study protocol was approved by the ethics committee of the institution and written informed consent was obtained from each patient or their family.

Hypertension was defined as blood pressure of 140/90 mmHg or higher, or the use of antihypertensive agents, and dyslipidemia as low-density lipoprotein >140 mg/dl, high-density lipoprotein <40 mg/dl, or triglyceride >150 mg/dl. Diabetes was defined as a result on the 2-hour glucose tolerance test of at least 200 mg/dl, a fasting glucose level of ≥126 mg/dl (≥7.0 mmol/l), HbA1c ≥6.5%, physician-diagnosed diabetes and/or use of diabetic medication.

### *Genotyping*

Genomic DNA was extracted from whole blood using the DNA Extractor WB kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan) using the modified protocol described by Richards et al. [18] Polymerase chain reaction (PCR) restriction fragment length polymorphism (RELP) for CYP2C19*2 (681G > A) and CYP2C19*3 (636G > A) was performed as described previously [19,20]. CYP2C19*2 and *3 are considered to account for >99% of alleles generating the null-activity enzyme protein in the Japanese population [19]. Therefore, the subjects were divided according to the CYP2C19 genotypes into two groups: carriers with at least one CYP2C19 LOF allele (*1/*2, *1/*3, *2/*2, *3/*3 or *2/*3) and non-carriers (*1/*1).

### *Measurement of platelet reactivity*

Platelet reactivity was measured the day after clopidogrel loading and administration of the maintenance dose for elective PCI. GPIIbIIIa inhibitor, prasugrel, and ticagrelor were not available under the Japanese health care system. As reported previously [8], aggregation in platelet-rich plasma induced by 20 μmol/L adenosine diphosphate (ADP; Chrono-Log) platelet reactivity was measured using a light transmission aggregometer (MCM HEMA TRACER 313; PAM12C, LMS Inc., Japan). Residual platelet reactivity was defined as the area under the platelet aggregation curve, which was used to express the aggregation response over the measured time (aggregation units*min; AU*min). The area under the aggregation curve (AU*min) is probably more sensitive and precise than maximal platelet aggregation calculated from the percentage of inhibition [8,21]. Moreover, we previously reported a significant positive correlation between residual platelet aggregation measured by 20 μmol/L ADP-induced platelet reactivity maximum aggregation and 20 μmol/L ADP-induced platelet reactivity area. [22] Thus, we used the area under the aggregation curve as a measure of on-treatment platelet reactivity during antiplatelet therapy. We defined high platelet reactivity as above 5000 AU*min. The threshold for high platelet reactivity at this study was different from previous studies [23]. The incidence of cardiovascular events after acute coronary syndrome or PCI is lower in Japanese patients compared with Caucasians, so it is difficult to determine the cut-off value of high platelet reactivity for cardiovascular events following coronary stent implantation. In our previous study [22], Japanese cut-off levels of platelet reactivity by VerifyNow P2Y system that allowed discrimination of carriers of at least one CYP2C19 loss-of-function allele from non-carriers were relatively higher than previous studies in Western countries (cut-off P2Y12 reaction units levels of 256 versus 230-240), and in our another study, CYP2C19 poor metabolizer platelet reactivity was 5088 ± 1080 AU*min by light transmission aggregometer [8]. Based on this background, we defined high platelet reactivity as above 5000 AU*min in this study.

### *Clinical outcomes*

The endpoint was a composite of cardiovascular death, nonfatal myocardial infarction, stroke, unstable angina, revascularization or intra-procedural thrombotic events (IPTE). Patients were followed up every month after discharge in the outpatient department and we performed follow-up angiography at 6 to 9 months after the procedure. Then, the patients were followed every 6 months after the re-study and contacted by phone (or their families were contacted) in the absence of hospital visits. Cardiovascular death was defined as death due to myocardial infarction, congestive heart failure or documented sudden cardiac death. We used the universal definition of myocardial infarction in this study [24]. The diagnosis of stroke was based on clinical and radiological evidence of stroke. Revascularization therapy based only on angiographic data, including PCI-mediated restenosis, was not counted as a cardiovascular event. Revascularization was defined as revascularization therapy for ischemic heart disease due to new lesions. An IPTE was defined as the development of new or increasing thrombus, abrupt vessel closure, no reflow or slow reflow, or distal embolization occurring at any time during the procedure [25,26]. For subjects experiencing more than two acute events, only the first event was considered in the analysis.

### *Statistical analysis*

Continuous variables are expressed as mean ± SD, and were compared using unpaired t-test or Mann-Whitney, as appropriate. Categorical variables are expressed as numbers or percentages, and were compared using chi-square test or Fisher's exact test. The cumulative event-free probability was analyzed from the time of stent implantation to the first event according to the Kaplan-Meier method, and between-group differences were evaluated by the log-rank test. Univariate analysis was performed using clinical variables that are considered to be associated with cardiovascular events (CYP2C19 LOF carrier status, sex, age, hypertension, dyslipidemia, diabetes, current smoking, left ventricular ejection fraction, high platelet reactivity, previous myocardial infarction, previous stroke, peripheral artery disease and usage of only drug-eluting stent). Factors with a P value <1.0 were subsequently entered into multivariate analysis. Cox proportional hazard models were used to calculate hazard ratios (HRs) and to test for the interaction between CKD and the possession of CYP2C19 LOF alleles. The results of this analysis are expressed as HRs for comparison of risk with 95%