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# Concomitant Use of Proton-Pump Inhibitors and Clopidogrel Increases the Risk of Adverse Outcomes in Patients With Ischemic Stroke Carrying Reduced-Function *CYP2C19**2*

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## Abstract

**Objective:** Conflicting data exist as to whether proton-pump inhibitors (PPIs) diminish the efficacy of clopidogrel. The aim of this study was to investigate the association between cytochrome P450 (*CYP*) genetic variants and clinical adverse outcomes of concomitant use of PPIs and clopidogrel by patients. **Methods:** We consecutively enrolled 523 patients with ischemic stroke receiving clopidogrel. Platelet aggregation was measured before and after 7 to 10 days of clopidogrel treatment. Single-nucleotide polymorphisms of *CYP3A4*, *CYP3A5*, *CYP2C19**2, and *CYP2C19***3* were examined using mass spectrometry. The primary outcome was a composite of recurrent ischemic stroke (RIS), myocardial infarction (MI), and vascular death that occurred during the 1-year follow-up period. The safety outcome was hemorrhagic episodes that occurred during the 1-year follow-up period. **Results:** This study comprised a total of 523 patients with IS, 96.3% (155/161) patients treated with PPIs and 95.9% (347/362) in patients treated without PPIs completed 1-year follow-up. The primary outcome was observed in 69 (13.7%) patients (56 RIS, 7 MI, and 6 died). There was no significant difference in the frequencies of primary outcome and safety outcome between patients treated with or without PPIs. The frequency of primary outcome was significantly higher in patients carrying *CYP2C19**2 AG/AA genotype receiving PPIs compared with the same genotype in those not receiving PPIs. The PPIs used in patients carrying *CYP2C19**2 AG/AA was independently associated with the primary outcome after adjusting for other risk factors. **Conclusions:** The concomitant use of PPIs and clopidogrel may be associated with an increased risk of RIS, MI, or vascular death in patients with IS carrying reduced-function *CYP2C19**2.

## Keywords

clopidogrel, proton-pump inhibitor, cytochrome P450 enzymes, single-nucleotide polymorphism, ischemic stroke, cohort study

## Introduction

Stroke is a worldwide health problem and is 1 of the leading causes of death among the elderly individuals.[1] Patients with ischemic stroke (IS) are also at a high risk of developing a recurrent ischemic stroke (RIS). Antiplatelet medications, such as aspirin and clopidogrel, have accordingly been proven to be beneficial in reducing the recurrent thrombotic events in patients with IS.[2,3] Thus, antiplatelet therapy using aspirin or clopidogrel is highly recommended for these patients.[4,5] A previous study demonstrated that clopidogrel was shown to be superior to aspirin to reduce the IS risk.[6]

Clopidogrel is a prodrug that requires biotransformation into an active metabolite by cytochrome P-450 (CYP) enzymes which can irreversibly inhibit the platelet P2Y12 adenosine 5′-diphosphate (ADP) receptor.[7] The CYP enzymes including CYP2C9, CYP2C19, and CYP3A4/5 are involved in the metabolism of clopidogrel.[8] Proton-pump inhibitors (PPIs) are often prescribed in combination with clopidogrel to help reduce the risk of gastrointestinal bleeding, a strategy that is endorsed by the existing consensus guidelines.[9] However, several studies

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have raised concern that PPIs might diminish the antiplatelet effect[10,11] and the clinical effectiveness[12,13] of clopidogrel, possibly through inhibition of the hepatic CYP isoenzymes and, therefore, the conversion of clopidogrel into its active metabolite. In recent years, a number of other observational studies, however, did not show an interaction between clopidogrel and PPIs.[14,15] Given the conflicting data regarding a possible interaction between clopidogrel and PPIs, the optimal care of patients who require concomitant therapy with clopidogrel and PPIs remains uncertain.

The activities of CYP enzymes are affected by *CYP* gene single-nucleotide polymorphisms (SNPs). Genetic polymorphisms of CYP isoenzymes have been identified which could affect the response to clopidogrel.[16-18] Loss of function polymorphisms in the gene encoding for CYP2C19 and CYP3A5 are associated with lower level of the active metabolite of clopidogrel, diminished platelet inhibition during clopidogrel treatment, and an increased risk of cardiovascular events.[17,18] However, it is unclear whether *CYP* genetic variants increase the likelihood of drug interactions mediated by CYP. A very limited number of studies have investigated whether polymorphism of *CYP* genes affects the pharmacodynamic effect and clinical efficacy of clopidogrel in patients with IS who use concomitant clopidogrel and PPIs.

In this study, we assessed the association between concomitant use of PPIs with clopidogrel and adverse outcomes during a 1-year follow-up of patients with acute IS. We also examined whether reduced-function *CYP* alleles were associated with a lower inhibition of platelet aggregation and a higher risk of adverse outcomes in patients with IS with concomitant use of PPIs and clopidogrel.

## Materials and Methods

### Study Population

The protocol for this prospective cohort study has been reviewed and approved by the ethics committees of the People's Hospital of Deyang City and the Third Affiliated Hospital of Wenzhou Medical College. Written informed consent was obtained from each participant before being enrolled in this study. The study was registered at http://www.chictr.org/with theuniqueidentifierofChiCTR-OCH-14004724.

We consecutively enrolled 523 patients who suffered their first IS, admitted them to the 2 participating hospitals within 7 days of the stroke onset, and treated them with clopidogrel (this medication only or combined with aspirin) between June 2014 and May 2015. The diagnosis of IS was confirmed by brain magnetic resonance imaging. All patients were subjected to computed tomographic angiography or magnetic resonance angiography of the brain as well as color duplex ultrasound investigation of the carotid arteries. Common electrocardiogram (ECG), 24-hour Holter ECG, and echocardiogram were performed to reveal any possible cardioembolic stroke. The inclusion criteria were (1) age $\geq$40 years, (2) no history of clopidogrel treatment for at least 14 days before admission, (3) patients with IS related to atherothrombotic or small artery disease according to the Trial of ORG 10172 in the Acute Stroke Treatment (TOAST) classification system,[19] (4) National Institutes of Health Stroke Scale (NIHSS) score <15, and (5) consent to participate in this study. Exclusion criteria were (1) allergy to clopidogrel; (2) cerebral embolism and other determined etiology or undetermined etiology IS; (3) taking other nonsteroidal anti-inflammatory drugs except aspirin, or anticoagulants with warfarin or heparin within 2 weeks; (4) platelet count $<100 \times 10^9$/L or $>450 \times 10^9$/L; (5) any major surgical procedure or severe trauma within 1 week prior to enrollment; (6) fever, hypoxia, or any relevant hemodynamic compromise on admission; (7) myelodysplastic syndrome or other blood diseases; and (8) a history of carotid endoartectomy or carotid stent therapy or carotid endoartectomy or carotid stent therapy during the follow-up period.

All patients received standard therapies based on the guideline recommendation,[4] including 75 mg clopidogrel once daily (Sanofi Co Ltd, Beijing, China), or clopidogrel (75 mg once daily) plus aspirin (200 mg, once daily; Bayer Healthcare Co Ltd, Beijing, China) for 2 weeks in patients with minor stroke whose NIHSS score was $\leq$3 or symptomatic carotid or intracranial artery stenosis, followed by clopidogrel (75 mg once daily). The decision to cotreat with PPI was at the discretion of the treating physician in this study.

Demographic information and information regarding vascular risk factors including body mass index, body weight, current smoker, diabetes mellitus, and hypertension were collected. Fasting blood samples were collected for the analysis of blood sugar, total plasma cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol, platelet aggregation test, and genotyping.

### Assessment of Clinical Outcomes

The primary outcome was a composite of RIS, myocardial infarction (MI), and death during the first 1 year after treatment. The RIS was defined as a new focal neurologic deficit of vascular origin lasting at least 24 hours, diffusion weighted imaging (DWI)-positive lesion(s) which corresponded to their clinical symptom(s) and proven to be nonhemorrhagic. Myocardial infarction was defined as the presence of at least 2 of these criteria: prolonged angina >30 min; total creatinine kinase isoenzyme elevation more than twice the upper limit of normal; and electrocardiographic evidence of infarction. Death was defined as vascular mortality due to MI, IS, and any other vascular causes. The safety end points included hemorrhagic episodes that occurred within a 1-year treatment. Hemorrhagic episodes were defined as the presence of any of the following: (1) symptomatic or asymptomatic hemorrhagic transformation, (2) symptomatic or asymptomatic intracerebral hemorrhage, and (3) extracranial hemorrhages (eg, gastrointestinal bleeding, hematoma, hematuria, and skin or mucosal bleeding). Serious hemorrhage was defined as any symptomatic intracranial hemorrhage or any hemorrhage requiring blood transfusion.

All patients were followed up by telephonic interview every 2 months. All data were collected on case report forms. All study end points were confirmed on the basis of source documentation and adjudicated by an independent clinical events committee whose members were unaware of patients' data, such as deoxyribonucleic acid genotyping data and use of PPIs. For those patients who reached at least 1 of the primary end points, a medical chart review was initiated to determine whether the event met the definitions described above. After discharge, scheduled follow-up telephone calls were made every month to encourage compliance, to answer any queries, and to document any side effects.

### Genotyping

The 4 variants of *CYP* gene, namely, *CYP3A4* (rs2242480), *CYP3A5* (rs776746), *CYP2C19*2* (rs4244285), and *CYP2C19*3* (rs4986893), were selected from the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/SNP) according to the following criteria: (1) SNPs that have been assessed in previous studies,[16-18] (2) SNPs with minor allele frequency >.05, and (3) SNPs leading to amino acid changes.

Whole blood (3 mL) was drawn from an arm vein into a sterile tube containing ethylenediaminetetraacetic acid and stored at −80°C until genotype analysis was performed. Genotypes of the 4 variants were examined using a matrix-assisted laser desorption/ionization time of flight mass spectrometry method according to our previous study.[16,20] In brief, each SNP was amplified using 2 specific polymerase chain reaction primers and 1 extension primer. Genotype call was performed in real time with Mass ARRAY RT software, version 3.0.0.4 and analyzed using Mass ARRAY Typer software, version 3.4 (Sequenom Inc, San Diego, California).

Each allele of these *CYP* SNPs was classified by its known effect on enzymatic function according to the literature and with the use of established common-consensus star allele nomenclature.[16,21,22] For each gene, patients were dichotomized a priori into 2 groups based on whether they possessed at least 1 allele with a significantly reduced function.

### Adenosine 5′-Diphosphate-Induced Platelet Aggregation Test

Before and after 7 to 10 days of treatment with clopidogrel, 5 mL fasting venous blood was collected for ADP-induced platelet aggregation test. The exact procedures and the consistency test were demonstrated in our previous research.[16,23] The platelet aggregation test was performed using ADP (Helena Laboratories, Beaumont, Texas) at 10.0 μM and measured by the light transmittance aggregometry with a BioData PAPS-4 platelet aggregometer (Helena Laboratories). Platelet aggregation rate was recorded as change in light transmission.

### Statistical Analysis

We calculated that a sample of 500 patients would provide 80% power to detect a relative risk increment of 10% in the percentage of primary outcome in the patients receiving PPI treatment, with a 2-sided type I error of .05, assuming a primary outcome rate of 8% in the patients not receiving PPI.

All statistical analyses were performed using SPSS 16.0 (SPSS Inc, Chicago, Illinois). The $\chi^2$ test was used to analyze the deviation of Hardy-Weinberg equilibrium and to compare genotype frequencies. Continuous variables were compared using Student's $t$ test. Discrete variables were compared using $\chi^2$ tests or Fisher's exact test if expected cell frequencies were small. Cox proportional hazard regression analysis was performed to adjust some risk factors to assess the independent contribution of *CYP* SNPs and PPIs to the risk of primary outcome during the 1-year follow-up. The values of hazard ratio with 95% confidence interval were reported. All tests were 2 sided, and a $P$ value of .05 was considered statistically significant.

## Results

### Baseline Characteristics of Study Patients

A total of 523 patients with IS enrolled in the present study. Among them, 161 (30.8%) were recorded to be taking PPI and clopidogrel concomitantly for at least 2 weeks. Of the 161 patients taking PPI, 72 were recorded to be on lansoprazole, 69 on pantoprazole, and 20 on omeprazole; 96.3% (155/161) in patients treated with PPIs and 95.9% (347/362) in patients treated without PPIs completed 1-year follow-up. In all, 10 (1.9%) patients lost to follow-up, 11 (2.1%) patients discontinued the study medication because of patients' choice before the end of the study.

### Clinical Outcomes

The primary outcome was observed in 69 (13.7%) patients (56 RIS, 7 MI, and 6 died) during the 1-year follow-up. The patients with the primary outcome were older, more likely to have diabetes or hypertension, and have higher LDL-C level and fasting plasma glucose level compared with the patients without the primary outcome (Table 1).

### Association of PPIs With Clinical Outcomes

The prevalence of primary outcome was 16.1% (25/155) in PPI treatment group, and 12.7% (44/347) in without PPI treatment group. However, there was no significant difference in frequencies of primary outcome and safety end points between the 2 groups (all $P$s > .05; Table 2).

### The Influence of PPIs on Platelet Aggregation

Before treatment, platelet aggregation was similar between patients receiving or not receiving PPIs. After 7 to 10 days of treatment with clopidogrel, platelet aggregation reduced

**Table 1.** Baseline Characteristics at Enrollment in Patients With or Without Primary Outcome.

| Variables | Patients With Primary Outcome (n = 69) | Patients Without Primary Outcome (n = 433) | *P* Value |
|---|---|---|---|
| Age | 70.97 ± 13.24 | 67.23 ± 12.37 | .024 |
| Males, n (%) | 44 (63.76) | 279 (64.44) | .86 |
| Diabetes, n (%) | 43 (62.32) | 190 (43.88) | .02 |
| Hypertension, n (%) | 64 (92.75) | 347 (80.14) | .01 |
| Previous MI, n (%) | 2 (2.89) | 5 (1.15) | .88 |
| Body mass index, kg/m$^2$ | 24.74 ± 3.87 | 24.28 ± 4.68 | .34 |
| Current smoker, n (%) | 28 (40.58) | 178 (41.11) | .94 |
| Alcohol, n (%) | 30 (43.48) | 199 (45.96) | .86 |
| TG, mmol/L | 1.72 ± 0.58 | 1.69 ± 0.53 | .82 |
| TC, mmol/L | 5.64 ± 1.62 | 5.35 ± 1.16 | .16 |
| LDL-C, mmol/L | 2.96 ± 0.98 | 2.61 ± 0.89 | .006 |
| HDL-C, mmol/L | 1.18 ± 0.45 | 1.21 ± 0.54 | .84 |
| Fasting glucose, mmol/L | 7.64 ± 2.02 | 6.83 ± 2.21 | .003 |
| NIHSS score at enrollment | 5.82 ± 2.13 | 5.67 ±1.89 | .65 |
| Stroke subtype | | | |
| Atherothrombotic, n (%) | 46 (66.67) | 273 (63.05) | .57 |
| Small artery disease, n (%) | 23 (33.33) | 160 (36.95) | .57 |
| Previous treatment, n (%) | | | |
| Antihypertensive drugs | 28 (40.58) | 191 (44.11) | .61 |
| Hypoglycemic drugs | 25 (36.23) | 153 (35.33) | .96 |
| Statins | 10 (14.49) | 78 (18.01) | .47 |
| Aspirin | 17 (24.64) | 123 (28.41) | .52 |
| Current treatment, n (%) | | | |
| Antihypertensive drugs | 58 (84.06) | 368 (84.99) | .84 |
| Hypoglycemic drugs | 44 (63.77) | 212 (48.96) | .03 |
| Statins | 68 (98.55) | 426 (98.38) | .99 |
| Clopidogrel plus aspirin | 41 (59.42) | 259 (59.82) | .97 |
| Thrombolysis | 2 (2.89) | 14 (3.23) | .96 |
| PPIs | 25 (36.23) | 130 (30.02) | .29 |

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NIHSS, National Institutes of Health Stroke Scale; PPIs, proton-pump inhibitors; TC, total cholesterol; TG, triglycerides.

**Table 2.** The Influence of PPI on Clinical Outcomes.

| Outcomes | With PPI Treatment (n = 155) | Without PPI Treatment (n = 347) | *P* Value |
|---|---|---|---|
| Primary outcomes, n (%) | 25 (16.1) | 44 (12.7) | .29 |
| RIS, n (%) | 19 (12.3) | 37 (10.7) | .63 |
| MI, n (%) | 3 (1.9) | 4 (1.2) | .48 |
| Death, n (%) | 3 (1.9) | 3 (0.9) | .32 |
| Safety end points | 6 (3.8) | 12 (3.4) | .77 |
| Asymptomatic HT, n (%) | 2 (1.3) | 5 (1.4) | .89 |
| Asymptomatic ICH, n (%) | 1 (0.6) | 1 (0.3) | .59 |
| Extracranial bleeding, n (%) | 3 (1.9) | 6 (1.7) | .92 |

Abbreviations: HT, hemorrhagic transformation; ICH, intracerebral hemorrhage; MI, myocardial infarction; PPI, proton-pump inhibitors; RIS, recurrent ischemic stroke.

**Table 3.** The Influence of Different PPI on ADP-Induced Platelet Aggregation.

| Treatment | Platelet Aggregation (%) | | Inhibition of Platelet Aggregation (%) |
|---|---|---|---|
| | Before Treatment | After 7-10 Day Treatment | |
| Without PPIs treatment (n = 362) | 73.84 ± 20.24 | 33.76 ± 12.25[a] | 42.32 ± 12.36 |
| With PPIs treatment (n = 161) | 72.76 ± 18.47 | 34.28 ± 11.64[a] | 41.26 ± 13.02 |
| Lansoprazole (n = 72) | 73.16 ± 19.38 | 34.26 ± 11.43[a] | 40.43 ± 11.87 |
| Pantoprazole (n = 69) | 72.35 ± 17.88 | 33.13 ± 11.08[a] | 41.56 ± 12.14 |
| Omeprazole (n = 20) | 72.67 ± 19.62 | 34.03 ± 10.72[a] | 40.09 ± 11.34 |

Abbreviations: ADP, adenosine 5′-diphosphate; PPI, proton-pump inhibitor.
[a]$P < .001$, compared with before treatment.

significantly, regardless of treatment with or without PPIs ($P < .001$). However, the inhibition of platelet aggregation did not substantially differ between patients with or without PPI treatment or among patients receiving different PPI treatment (Table 3).

## *The Influence of CYP Genetic Variants on Primary Outcome and Platelet Aggregation*

The genotype distributions of the 4 variants examined in this study were in Hardy-Weinberg equilibrium ($P > .05$). The frequency of primary outcome was significantly higher in patients carrying *CYP2C19*2* AG/AA genotype and receiving PPI treatment compared with same genotype in those not receiving PPI treatment ($P = .04$; Table 4).

There were no significant differences in the activity of pretreatment platelet aggregation among the genotypes of the 4 variants. After 7 to 10 days of treatment with clopidogrel, regardless of cotreated with PPIs or not, the inhibition of platelet aggregation was significantly lower in patients with *CYP2C19*2* AG/AA genotype (reduced function) than with GG genotype, and *CYP3A5* GG/AG genotype (reduced function) than AA genotype ($P < .001$; Table 5). For patients carrying the same genotype of *CYP2C19*2* AG/AA, the inhibition of platelet aggregation was significantly lower in patients receiving PPI treatment than those not receiving PPI treatment ($P < .001$; Table 5). This indicated that *CYP2C19*2* AG/AA genotype had an effect on platelet aggregation in patients receiving PPI treatment.

## *Cox Regression Analysis of Risk Factors for Primary Outcome*

Cox proportional hazard regression analysis was used to describe the risks for primary outcome. The variables entered into the model were age, diabetes, hypertension, LDL-C,

**Table 4.** The Influence of *CYP* Genetic Variants on Primary Outcome.

| | With PPIs | | Without PPIs | | |
|---|---|---|---|---|---|
| Genotypes | N | N With Primary Outcome (%) | N | N With Primary Outcome (%) | *P* Value[a] |
| *CYP2C19*2* (rs4244285) | | | | | |
| GG | 70 | 6 (8.6) | 151 | 19 (12.6) | .41 |
| AG+AA | 85 | 19 (22.4) | 196 | 25 (12.8) | .04 |
| *CYP3A5* (rs776746) | | | | | |
| AA | 20 | 2 (10.0) | 48 | 4 (8.3) | .94 |
| GG+AG | 135 | 23 (17.0) | 299 | 40 (13.4) | .27 |
| *CYP3A4* (rs2242480) | | | | | |
| CC | 83 | 13 (15.6) | 189 | 23 (12.2) | .45 |
| TT+CT | 72 | 12 (16.7) | 158 | 21 (13.3) | .49 |
| *CYP2C19*3* (rs4986893) | | | | | |
| GG | 139 | 22 (15.9) | 313 | 38 (12.1) | .27 |
| AG | 16 | 3 (18.8) | 34 | 6 (17.6) | .98 |

Abbreviations: PPIs, proton-pump inhibitors; *CYP,* cytochrome P-450.
[a]Compared with same genotype between 2 groups.

**Table 5.** The Influence of *CYP* Gene Variants on ADP-Induced Platelet Aggregation in Patients Treated With or Without PPI.

| | With PPI Treatment | | Without PPI Treatment | | |
|---|---|---|---|---|---|
| Genotypes | n | Inhibition of Platelet Aggregation (%) | n | Inhibition of Platelet Aggregation (%) | *P* Value[a] |
| *CYP3A4* (rs2242480) | | | | | |
| CC | 83 | 43.14 ± 11.65 | 189 | 43.97 ± 14.23 | .67 |
| TT+CT | 72 | 42.68 ± 11.86 | 158 | 43.54 ± 12.78 | .72 |
| *CYP3A5* (rs776746) | | | | | |
| AA | 20 | 42.94 ± 13.14 | 48 | 43.78 ± 13.08 | .68 |
| GG+AG | 135 | 33.85 ± 11.32[b] | 299 | 35.34 ± 12.18[b] | .24 |
| *CYP2C19*2* (rs4244285) | | | | | |
| GG | 70 | 43.85 ± 12.57 | 151 | 44.32 ± 13.87 | .89 |
| AG+AA | 85 | 31.24 ± 10.22[b] | 196 | 37.99 ± 10.11[b] | <.001 |
| *CYP2C19*3* (rs4986893) | | | | | |
| GG | 139 | 42.96 ± 12.72 | 313 | 42.76 ± 13.11 | .97 |
| AG | 16 | 41.42 ± 11.76 | 34 | 43.21 ± 14.23 | .36 |

Abbreviations: ADP, adenosine 5′-diphosphate; CYP, cytochrome P-450; PPI, proton-pump inhibitor.
[a]Compared with same genotype between 2 treatment groups.
[b]$P < .001$, compared with another genotype in the same treatment group.

fasting glucose fasting glucose, PPI use, *CYP2C19*2* AG/AA, and *CYP3A5* GG/AG. The results showed that PPIs use in patients carrying *CYP2C19*2* AG/AA were the independent risk factors for primary outcome after adjusting for other risk factors (Table 6).

## Discussion

Our data showed that the primary outcome was observed in 69 patients. The prevalence of primary outcome was 16.1% in PPI treatment group, and 12.7% in without PPI treatment group.

**Table 6.** Cox Regression Analysis of Risk Factors for Adverse Outcome.

| Variables | HR | 95% CI | *P* Value |
|---|---|---|---|
| Age | 0.69 | 0.72-1.72 | .32 |
| Hypertension | 1.24 | 0.94-3.26 | .08 |
| Diabetes | 1.92 | 1.16-3.98 | .02 |
| High LDL-C | 0.93 | 0.78-1.87 | .48 |
| High fasting glucose | 0.82 | 0.64-1.58 | .65 |
| PPIs | 0.91 | 0.83-1.96 | .45 |
| *CYP3A5* GG/AG | 1.01 | 0.94-2.68 | .12 |
| *CYP2C19*2* AG/AA | 2.04 | 1.24-6.38 | <.01 |
| *CYP2C19*2* AG/AA+PPIs | 2.86 | 2.03-9.485 | <.01 |

Abbreviations: CI, confidence intervals; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol; PPI, proton-pump inhibitor.

There was no significant difference in the frequencies of primary outcome and safety end points between patients treated with or without PPIs. The noteworthy finding in the present study was that PPI use in patients carrying *CYP2C19*2* AG/AA had an effect on platelet aggregation and were independently associated with the risk for adverse outcomes.

The PPIs are often administered to patients in combination with clopidogrel to help reduce the risk of bleeding after IS, acute coronary syndrome, or percutaneous coronary intervention. However, some studies have explored the possibility that some PPIs may interfere with the effect of clopidogrel by inhibiting CYP2C19, hindering enzymatic conversion to its active metabolite. Several in vitro studies demonstrate that PPIs can attenuate the antiplatelet effect of clopidogrel,[10,11] but the clinical significance of this drug interaction is disputed. Analyses from 3 large population-based studies have found that use of PPIs is associated with an increased risk of adverse events in patients treated with clopidogrel.[12,13,24] In response to these findings, both the European Medicines Agency and the US Food and Drug Administration released statements or communications warning of a potential interaction between PPIs and clopidogrel and discouraging their combined use in the absence of a strong indication. However, a number of recent studies showed that concomitant use of PPIs with clopidogrel was not associated with reduced antiplatelet efficacy of clopidogrel or increased risk of IS or cardiovascular events.[14,15,25,26] The prematurely terminated Clopidogrel and the Optimization of Gastrointestinal Events Trial showed that there was no significant difference between fixed-dose combination, clopidogrel + omeprazole versus clopidogrel alone ($P = .43$).[27] The reason for conflicting data among different studies is unclear. Without a randomized trial, concern exists that the higher cardiovascular event rates in patients prescribed a PPI in population-based studies might partly be explained by differences in baseline comorbidities. By contrast, less heterogeneity might exist in a trial population, which could help to reduce the probability of confounding by indication.

To date, few studies investigate whether *CYP* gene variants affect the pharmacodynamic effect or clinical efficacy of

clopidogrel in patients with IS who use concomitant therapy with clopidogrel and PPIs. The noteworthy finding in this study was that PPI use in patients with IS carrying *CYP2C19*2* AG/AA had an effect on platelet aggregation and increased risk of adverse events. Some previous studies indicated that many PPIs could inhibit CYP isoenzymes, including CYP2C19 and CYP3A5,[28] and therefore the concomitant use of a PPI could impede or prevent the metabolism of clopidogrel to their active metabolites through competing for the same enzyme. Our previous studies[16,29] and some other studies[17,18] showed that genetic polymorphism of the *CYP2C19*2* allele is independently associated with an increased risk of adverse outcomes for patients treated with clopidogrel, supporting the concept that diminished CYP2C19 activity might increase the risk of adverse events in clopidogrel-treated patients. With regard to the effect of, *CYP2C19* loss-of-function alleles had an effect on platelet aggregation, and were independently associatied with the risk for adverse outcomes. Tsantes et al[30] showed that PPI coadministration did not influence clopidogrel's antiplatelet effect on laboratory testing in patients with coronary artery disease (CAD). However, the patients who carry *CYP2C19*2* genotype had significantly higher residual platelet reactivity. Simon et al[31] found that PPI use and *CYP2C19* loss of function alleles are associated with reduced responsiveness to standard clopidogrel doses and increased cardiovascular events in patients with CAD. A systematic meta-analysis also showed that CAD patients with loss of function *CYP2C19*2* allele and who are users of clopidogrel together with PPIs increased the risk for ischemic outcomes.[32] This study was the first to show that PPI use in patients carrying *CYP2C19*2* AG/AA was independently associated with the risk of adverse outcomes in patients with IS taking clopidogrel.

Although our results indicated that IS patients carrying *CYP2C19*2* AG/AA should avoid concomitant therapy with PPIs and standard dose of clopidogrel, Moceri et al[33] demonstrated that doubling the dose of clopidogrel (150 mg) could restore the loss of antiplatelet effect induced by PPIs. Therefore, the clopidogrel/PPIs interaction can be diminished by increasing the dose of clopidogrel to 150 mg. The plasma half-lives of both clopidogrel and most PPIs are short. It has been suggested that staggering administration of clopidogrel and PPIs may overcome this pharmacodynamic interaction.[34] However, Ferreiro et al[35] reported that even a 12-hour separation of dosing could not prevent drug interactions between omeprazole and clopidogrel. Further well-designed studies are necessary to confirm whether the dose of clopidogrel or different timing of taking PPIs and clopidogrel could prevent drug interactions between clopidogrel and PPIs.

Our study has important strengths. To the best of our knowledge, the present study is the first to identify reduced function *CYP* alleles were associated with a lower inhibition of platelet aggregation and a higher risk of adverse outcomes in patients with IS who use concomitant therapy with clopidogrel and PPIs. Our study helps to better understand the relationship of the reduced function *CYP* alleles, clopidogrel responsiveness, and IS risk. The findings of this study will lead to further research to better understand the research question of interest.

Our study also has limitations. First, due to the limited sample size and 2-center design, the results of this study may not represent the full spectrum of the Chinese population. The findings must be validated in larger, multicenter studies. Second, the use of PPIs was not randomized and was left to the physician's discretion. Although we did extensive multivariable adjustment for potential confounders, the possibility of residual confounding remains. Thus, our findings should be identified by randomized study designed in future. Third, PPIs could be discontinued during the course of follow-up, and compliance cannot be ensured for individual patient. Fourth, our results showed that concomitant use of PPIs and standard dose of clopidogrel is associated with an increased risk of adverse outcomes in patients with IS carrying reduced function *CYP2C19*2*. However, we did not assess the effects of clopidogrel dose or different timing of taking PPIs and clopidogrel on platelet aggregation and adverse outcomes. Finally, although we genotyped multiple known functional variants in the relevant *CYP* genes in this cohort of patients, some rare functional variants may have been left undetected in this population. Furthermore, genetic factors for clopidogrel responsiveness may be very complex; it may be that genetic variants at individual loci only contribute to clopidogrel resistance by interaction with other gene variants.[29,30] Thus, the investigation of multiple gene–gene interactions is necessary to better understand the genetic basis of clopidogrel responsiveness and PPI user in patients with IS.

In summary, we found that among patients treated with clopidogrel after IS, the concomitant use of PPIs is not associated with an increased risk of adverse outcomes of RIS, MI, and vascular death. However, concomitant use of PPIs and clopidogrel in patients carrying *CYP2C19*2* AG/AA had an effect on platelet aggregation and was independently associated with the risk for adverse outcomes.

## Authors' Note

Clinical Trial Registration information http://www.chictr.org/. Unique Identifier: ChiCTR-OCH-14004724.

## Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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