CYP2C19 Polymorphisms and Antiplatelet Effects of Clopidogrel in Acute Ischemic Stroke in China

Dong-mei Jia, MD*; Zhi-bin Chen, MD*; Mei-juan Zhang, MD, PhD*; Wen-jie Yang, MD; Jia-li Jin, MD; Yong-quan Xia, MS; Chun-lei Zhang, MS; Yuan Shao, MD; Cong Chen, MD; Yun Xu, MD, PhD

Background and Purpose—Little research regarding genotypes and clopidogrel response related to acute ischemic stroke has been published. This study was conducted to investigate whether the polymorphisms of receptors or enzymes involved in the metabolic process of clopidogrel affect clopidogrel response and prognosis related to acute stroke.

Methods—A total of 259 patients with acute ischemic stroke were enrolled in this study; all received follow-up evaluations 3 and 6 months after clopidogrel treatment. CYP2C19, CYP3A4, and P2Y12 were screened. The adenosine diphosphate-induced platelet aggregation test, the National Institutes of Health Stroke Scale (NIHSS), and the modified Rankin Scale (mRS) were used, and blood vascular events were evaluated.

Results—The difference before and after clopidogrel treatment on adenosine diphosphate-induced platelet aggregation was significantly smaller in patients carrying 1 or 2 CYP2C19 loss-of-function alleles (*2, *3) compared with patients carrying none. Patients with none had better outcomes than patients with CYP2C19 loss-of-function alleles, as demonstrated by NIHSS and mRS scores at 3 and 6 months after treatment. Regression analysis showed that CYP2C19 was an independent predictor of clopidogrel resistance.

Conclusions—CYP2C19 genotypes had significant impact on clopidogrel response and prognosis of patients with stroke.


Key Words: clopidogrel resistance ■ CYP2C19 genotype ■ ischemic stroke ■ stroke outcome

Clopidogrel treatments are used to inhibit platelet aggregation and reduce the risk of recurrent stroke at acute stages.1 Multiple G-protein–coupled receptors or enzymes are involved in the clopidogrel metabolic process.2,3 Considerable data have shown that genetic variants such as CYP2C19 (extensive metabolizers [EM], intermediate metabolizers [IM], poor metabolizers [PM]), CYP3A4, and P2Y12 polymorphisms affect the antiplatelet function of clopidogrel.4 This study was conducted to evaluate whether patients with stroke with different genotypes may have different sensitivities to clopidogrel and different prognoses.

Materials and Methods

This study was approved by the ethics committee of the Affiliated Drum Tower Hospital of Nanjing University. Between August 2011 and July 2012, a total of 259 patients were enrolled in this study based on the following inclusion criteria: (1) clinical diagnosis of acute atherosclerotic cerebral infarction and (2) aged 45 to 80 years. Exclusion criteria were the following: (1) exposure to thienopyridine or glycoprotein IIb/IIIa inhibitor within 1 week, (2) cerebral embolism and small vessel disease, and (3) intracranial hemorrhage after cerebral infarction.

The participants were given ozagrelum injection 80 mg/d for 7 days and 75 mg clopidogrel once daily for ≥3 months after stroke onset. Whole blood (5 mL) was obtained for genotyping and adenosine diphosphate-induced platelet aggregation testing. The polymorphisms of 5 gene loci within 3 genotypes, including CYP2C19 (EM, IM, PM),5–7 CYP3A4 (894C>T), and P2Y12 (34C>T, 52G>T), were screened for mutations. The neurological function of each patient was assessed using the NIHSS and the mRS. The assessments were conducted by attending neurologists at baseline, 7 days, and at 3 and 6 months.

All data were analyzed using SPASS version 16.0 (Chicago, IL) statistical analysis software. The effect of the studied genotypes on clopidogrel response was evaluated by 1-way ANOVA. The χ2 test was used to compare changes in mRS score among the 3 CYP2C19 genotypic groups at baseline and at 3-month and 6-month follow-up.
Table 1. The Effect of CYP2C19 SNPs on Clopidogrel Response

<table>
<thead>
<tr>
<th></th>
<th>EM (R/N)</th>
<th>IM (R/N)</th>
<th>PM (R/N)</th>
<th>( \chi^2 )</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.011</td>
<td>0.989–1.034</td>
<td>0.332</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C19</td>
<td>1.633</td>
<td>1.133–2.397</td>
<td>0.012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P2Y12 G&gt;52T</td>
<td>0.648</td>
<td>0.389–1.080</td>
<td>0.096</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N indicates no resistance; R, resistance; and SNPs, single nucleotide polymorphisms.

Table 2. Significant Independent Predictors of Clopidogrel Resistance by Binary Logistic Regression Analysis

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>1.258</td>
<td>0.721–2.197</td>
<td>0.419</td>
</tr>
<tr>
<td>Age</td>
<td>1.011</td>
<td>0.989–1.034</td>
<td>0.332</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>1.633</td>
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<td>0.096</td>
</tr>
</tbody>
</table>

Significant independent predictors of clopidogrel resistance were detected by binary logistic regression analysis.

Results

Baseline characteristics of the study population are demonstrated as follows (Table I in the online-only Data Supplement): The average age was 66.5±11.8 years, and the mean systolic blood pressure was 156.0±25.9 mm Hg. Of the 259 patients, 157 patients received atorvastatin treatment, 186 were the occlusion of anterior circulation, 68 had hyperlipidemia, and 81 patients had diabetes mellitus (China Clinical Trials number, ChiCTR-OCH-12002681).

Clopidogrel response was assessed by the change in adenosine diphosphate-induced platelet aggregation before and after 7-day treatment. Data demonstrated that clopidogrel response in both the IM and the PM group were significantly lower than that in the EM group (P=0.020 and 0.016, respectively). Similarly, clopidogrel response in P2Y12 52G>T subjects with TT alleles was significantly greater than that in P2Y12 52G>T with GG and GT genotypic subjects (*P<0.05 versus GG group, #P<0.05 versus GT group). However, both of the selected single nucleotide polymorphisms in CYP3A4 and P2Y12 34C>T had no influence on adenosine diphosphate-induced platelet aggregation (Figure I in the online-only Data Supplement). Furthermore, within 7 days after treatment, a reduction of <10% adenosine diphosphate-induced platelet aggregation was observed in 37.4%, 52.9%, and 58.5% of EM, IM, and PM patients, respectively (Table 1).

A univariate regression analysis was then used to evaluate clinical characteristics that may be related to clopidogrel resistance (Table II in the online-only Data Supplement). CYP2C19 and P2Y12 52G>T genotypes were correlated with increased risk of clopidogrel resistance. A multivariate logistic regression analysis was used to study the factors with P value <0.2. It was found that only CYP2C19 genotype had a significant impact on clopidogrel resistance (Table 2).

The correlation between CYP2C19 genotype and neurological function of patients who were treated with clopidogrel was detected by comparing NIHSS and mRS scores among 3 CYP2C19 genotypic groups at different points. A good outcome was defined as mRS ≤2 points, whereas >2 points was considered as poor outcome. As shown in Table 3, at 3 and 6 months after stroke, 86.9% and 86% of patients scored good in the EM group, respectively, whereas 73.9% and 77.8% of patients scored good in the IM group, and 73.2% and 65.6% of patients scored good in the PM group, respectively. Furthermore, at 6 months, the rate of stroke recurrence was 1.0% (1 of 99 patients) in EM group, 3.4% (4 of 119 patients) in IM group, and 2.4% (1 of 41 patients) in PM group, respectively.

Discussion

It was found that CYP2C19 genotypes had significant impact on clopidogrel response and the prognosis of patients with stroke in the studied population. Previous studies, conducted in different populations, have also reported that carriers with a1 variant of CYP2C19 alleles (2*, 3*) had significantly lower levels of the active metabolite of clopidogrel. This is attributable to decreased formation of the active metabolite. More specific data demonstrated that the presence of a CYP2C19 reduced–function allele is associated with active metabolite and platelet inhibition levels 25% to 33% less often than observed in noncarriers.

Notably, some studies suggest that the relationship between CYP2C19*2 polymorphism and clopidogrel effects is time dependent. It is reported that carriers of the reduced-function CYP2C19 allele variants showed a higher rate of cardiovascular death, myocardial infarction, or stroke, mainly within the first 30 days after start of treatment for acute coronary syndromes. Also, in another investigation, it was found that, only in the first month, carriers of loss-of-function alleles consistently display higher anticlopidogrel platelet reactivity (an independent predictor of poor prognosis) than other patients.

Limitations of the current study include the following points: (1) the plasma levels of clopidogrel and its active metabolite in each genotype were not detected; (2) an analysis of study-related genotypes and whether these genes correlated with each other and covalently contributed to clopidogrel resistance was not conducted; and (3) the recurrence rate of stroke cannot be a valid statistical analysis because of shorter follow-up. Further studies are needed.

Conclusions

We conclude that CYP2C19 had substantial impact on clopidogrel resistance and the prognosis of patients with stroke.
Acknowledgments
We thank Dr Peterson and Dr Adams for revising the article.

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Disclosures
None.

References
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SUPPLEMENTAL MATERIAL:

Supplemental methods:

Patients and Study Protocol

This prospective study was approved by the ethics committee of the Affiliated Drum Tower Hospital of Nanjing University and all data were obtained from the hospital. Between August 2011 and July 2012, 263 hospitalized patients were enrolled in this study based on the following inclusion criteria: 1) clinical diagnosis of acute ischemic stroke and 2) aged 45 to 80 years. The following exclusion criteria were also applied: 1) exposure to thienopyridine or GP IIb/IIIa inhibitor within one week, 2) cerebral embolism and 3) intracranial hemorrhage after cerebral infarction. Written informed consent was obtained from each patient prior to their participation in the study. The participants were given a standard dose of 75 mg clopidogrel once daily for at least 3 months.

Blood Sample

Whole blood (5 mL) was obtained from the forearm vein for genotyping and ADP-induced platelet aggression testing. All samples were stored in sterile tubes containing EDTA before administration of clopidogrel. Blood samples were also drawn for another ADP-induced platelet aggression test at 7 days after treatment. Samples were processed within 1 h after blood drawing. Routine laboratory detections were measured on admission.

CYP2C19, CYP3A4, P2Y12 Genotyping

For each sample, the polymorphisms of 5 gene loci within 3 genotypes, including CYP2C19 (636G>A, 681G>A), CYP3A4 (894C>T), P2Y12 (34C>T, 52G>T), were screened for mutations. CYP2C19*2 and CYP2C19*3 mutant alleles were detected using a commercially available validated genotyping microarray (Baio Technology Co, Ltd, Shanghai, China). CYP3A4 (894C>T) and P2Y12 (34C>T, 52G>T) were screened by gene sequence assay (ABI 3730XL). The primers of CYP3A4 and P2Y12
were as follows:

CYP3A4: Forward: GGAATGGAAAAGACTGCTGTAGG
      Reverse: CATGATAGGTGACAGAGATATGCT.

P2Y12: Forward: TATTTTGGGATTCTAGTTTTC
       Reverse: GTGGCTCTTCTGGTAGCGATC

**ADP-Induced Platelet Aggression Test**

Aggregation studies were performed using a turbidimetric method. Platelet-rich plasma, with the platelet count adjusted to 250×10^3/mm^3, was obtained by centrifugation of blood samples at 160 g for 3 min at room temperature. Final concentrations of 5 umol/L ADP were added to 200 uL PRP.

Platelets aggregate when the agonist is also added, thereby leading to an increase in light transmission, which was recorded for 5 minutes. The extent of aggregation was defined as the maximum percent change in light transmission from baseline, using platelet-poor plasma as reference (arbitrarily 100%). The change of values in the ADP-induced platelet aggression test, between pre-administration and 7 days after clopidogrel treatment, reflect the sensitivity of patients to clopidogrel.

**Measurement of Stroke Outcome**

The neurological function of each patient at admission was assessed by the National Institute of Health Stroke Scale (NIHSS) and the modified Rankin Scale (mRS). The scales were assessed by an attending neurologist at baseline and 7 days. NIHSS is a 42-point scale quantifying neurologic impairment in 11 categories, with higher values reflecting more severe neurological deficit. The mRS is a simplified overall assessment of function in which a score of 0 indicates the absence of symptoms, 5 indicates severe disability, and 6 indicates death. All patients were asked to return to the hospital and were re-evaluated with NIHSS and mRS scores routinely at 3 and 6 months after discharge. The clinical outcomes were evaluated by mRS scores between baseline and follow-up.
**Statistical Analysis**

All data were calculated using SPSS version 17.0. The significance of inter-group differences was assessed using the chi-square test for categorical variables and the univariate logistic regression analysis for continuous variables. The effect of the studied genotypes on clopidogrel response (assessed by 5 umol/L ADP–induced platelet aggregation) across time was evaluated by using one-way ANOVA. The chi-square test was used to compare changes in mRS score among the three CYP2C19 genotypic groups at baseline, 3-month and 6-month follow-up. Significant independent predictors of clopidogrel resistance were detected by using logistic regression analysis.

**Supplemental data:**

Supplemental Table 1. Baseline Characteristics of the Patients.

<table>
<thead>
<tr>
<th>Total cases, n</th>
<th>259</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66.3 ±11.8</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>167 (64.5)</td>
</tr>
<tr>
<td>SBP(mmHg)</td>
<td>153.8±25.3</td>
</tr>
<tr>
<td>DBP(mmHg)</td>
<td>85.9±14.1</td>
</tr>
<tr>
<td>Anterior circulation infarct, n (%)</td>
<td>186 (71.8)</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>68 (26.3)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>81 (37.3)</td>
</tr>
<tr>
<td>Statins, n (%)</td>
<td></td>
</tr>
<tr>
<td>Atorvastatin(20mg/d)</td>
<td>157 (60.6)</td>
</tr>
<tr>
<td>Rosuvastatin(10mg/d)</td>
<td>76(29.3)</td>
</tr>
</tbody>
</table>
Simvastatin (40mg/d)  26 (10.1)

Antihypertensive agent, n (%)  
- CCB  98 (37.8)
- Others  40 (15.5)
- No use  121 (46.7)

SBP=systolic blood pressure; DBP=diastolic blood pressure; CCB= Calcium channel blocker.
Supplemental Table 2. Predictors with P Value < 0.2 by Univariate Analysis Entered in Multivariate Models for Clopidogrel Resistance.

<table>
<thead>
<tr>
<th>Predictors</th>
<th>OR</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>1.456</td>
<td>0.850-2.492</td>
<td>0.171</td>
</tr>
<tr>
<td>Age</td>
<td>1.016</td>
<td>0.994-1.038</td>
<td>0.157</td>
</tr>
<tr>
<td>ICA/VBA</td>
<td>0.728</td>
<td>0.418-1.266</td>
<td>0.260</td>
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<tr>
<td>SBP</td>
<td>0.996</td>
<td>0.986-1.006</td>
<td>0.420</td>
</tr>
<tr>
<td>DBP</td>
<td>0.995</td>
<td>0.977-1.013</td>
<td>0.558</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>0.983</td>
<td>0.554-1.744</td>
<td>0.952</td>
</tr>
<tr>
<td>2-DM</td>
<td>0.855</td>
<td>0.494-1.479</td>
<td>0.575</td>
</tr>
<tr>
<td>Statins</td>
<td>1.055</td>
<td>0.723-1.539</td>
<td>0.782</td>
</tr>
<tr>
<td>Antihypertensive agent</td>
<td>1.072</td>
<td>0.814-1.412</td>
<td>0.620</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>1.724</td>
<td>1.180-2.520</td>
<td>0.005</td>
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<tr>
<td>P2Y12 C&gt;34T</td>
<td>0.886</td>
<td>0.571-1.377</td>
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<td>P2Y12 G&gt;52T</td>
<td>0.592</td>
<td>0.359-0.974</td>
<td>0.039</td>
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<tr>
<td>CYP3A4</td>
<td>1.197</td>
<td>0.709-2.024</td>
<td>0.501</td>
</tr>
</tbody>
</table>

CI= confidence interval; ICA= internal carotid artery; VBA= vertebral basilar artery; SBP=systolic blood pressure; DBP=diastolic blood pressure; CCB= Calcium channel blocker.
Supplemental Table 3. The Effect of antiplatelet effects on Clinical Outcome in Stroke Patients.

<table>
<thead>
<tr>
<th>Time</th>
<th>R (good/poor)</th>
<th>N (good/poor)</th>
<th>$\chi^2$</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>75/49 (60.5%)</td>
<td>83/52 (61.5%)</td>
<td>0.027</td>
<td>0.869</td>
</tr>
<tr>
<td>7d</td>
<td>89/35 (71.8%)</td>
<td>106/29 (78.5%)</td>
<td>1.580</td>
<td>0.209</td>
</tr>
<tr>
<td>3m</td>
<td>91/33 (73.4%)</td>
<td>113/22 (83.7%)</td>
<td>4.113</td>
<td>0.043</td>
</tr>
<tr>
<td>6m</td>
<td>75/26 (74.3%)</td>
<td>89/16 (84.8%)</td>
<td>3.500</td>
<td>0.061</td>
</tr>
</tbody>
</table>

R=resistance, N=no resistance
Figure legends:

Supplemental Figure 1. The Effects of CYP2C19, CYP3A4, P2Y12 SNPs on the Antiplatelet Effect of Clopidogrel. The ADP difference was evaluated 7 days after clopidogrel administration. A) for CYP2C19 genotype, ADP difference for both the IM and PM groups are significantly lower than in the EM group (*p<0.05 vs EM group). B) for CYP3A4 genotype, there is no significant difference between groups. C) for P2Y12 34C>T genotype, there is also no significant difference between groups. D) for P2Y12 52G>T genotype, ADP difference for TT groups are significantly higher than in the GG and GT groups (*p<0.05 vs GG group, #p<0.05 vs GG group). Data are mean ±SE. EM, extensive metabolizers (*1/*1); IM, intermediate metabolizers (*1/*2 and *1/*3); PM, poor metabolizers (*2/*2 and *2/*3).
supplemental fig.1

A. CYP2C19

- ADP difference (%)
  - EM (n=99)
  - IM (n=119)
  - PM (n=41)

B. CYP3A4

- ADP difference (%)
  - CC (n=196)
  - CT (n=57)
  - TT (n=6)

C. P2Y12 34C>T

- ADP difference (%)
  - CC (n=154)
  - CT (n=95)
  - TT (n=10)

D. P2Y12 34C>T

- ADP difference (%)
  - GG (n=186)
  - GT (n=68)
  - TT (n=5)