Relationship between cytochrome P450 2C19*17 genotype distribution, platelet aggregation and bleeding risk in patients with blood stasis syndrome of coronary artery disease treated with clopidogrel

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OBJECTIVE: To assess the impact of cytochrome P450 (CYP) 2C19*17 allelic variant on platelet aggregation and bleeding risk in Chinese patients with blood stasis syndrome undergoing percutaneous coronary intervention (PCI) and treated with clopidogrel.

METHODS: A total of 520 patients with blood stasis syndrome undergoing PCI after pretreatment with 300 mg clopidogrel and aspirin were studied from July 2009 to April 2011 in Fujian Provincial Institute of Cardiovascular Diseases. CYP2C19*17 genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism. Platelet aggregation induced by 5 μmol/L of adenosine diphosphate (ADP) was analyzed with platelet-rich plasma and platelet-poor plasma by turbidimetry method before and after 10 d of treatment with clopidogrel.

RESULTS: Bleeding events were observed in 5.96% of patients after thrombolysis for myocardial infarction, and the ratio of patients with CYP2C19*17 allele was 7.98%. The bleeding rate in patients carrying CYP2C19*17 allele, heterozygous (wt/*17) and homozygous (*17/*17), was higher than that in patients with wild-type homozygotes (wt/wt) (P<0.01). At baseline, ADP-induced light transmission at maximal aggregation, 5-min aggregation and disaggregation showed no significant difference among patients with the three different CYP2C19*17 genotypes. However, after 10-day administration of clopidogrel, values of ADP-induced platelet aggregation in *17/*17 and wt/*17 carriers were significantly decreased compared with the wild-type homozygotes (P<0.05, P<0.01); the inhibition rate of platelet aggregation was higher in patients carrying *17/*17 and wt/*17 than those only carrying wt/wt, and the same result was found in disaggregation of platelet after 10-day treatment (P<0.05, P<0.01). Patients with wt/*17 and *17/*17 allele of CYP2C19 showed a higher risk of bleeding than those with wild-type allele (P<0.01), and the occurrence of bleeding was highest in patients with CYP2C19*17

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homozygotes.

**CONCLUSION**: CYP2C19*17 allele is associated with enhanced response to clopidogrel and an increased risk of bleeding in patients with blood stasis syndrome of coronary artery disease treated by clopidogrel.

**KEYWORDS**: cytochrome P-450 enzyme system; bleeding; platelet aggregation inhibitors; blood stasis

Coronary heart disease (CHD) is a serious threat to human health and has increasingly become a leading cause of death in patients. CHD is caused by atheromatous plaque and could result in coronary stenosis, vasospasm and thrombus. The heart fails to get enough blood, and consequently, a series of clinical myocardial ischemic symptoms appears[1]. Though there is no direct translation of the term of CHD in traditional Chinese medicine, numerous descriptions of similar symptoms were recorded in traditional Chinese medicine classics such as the terms of sudden chest pain, chest congestion pain and precordial pain. Blood stasis syndrome (BSS) is the most common syndrome in CHD. Xu et al[2] pointed out that clinical symptoms of BSS could be used to assess the severity of CHD. As a basic pathogenesis and syndrome, BSS pervades the whole process of CHD. Patients with BSS usually have platelet activation. Consequently, antiplatelet therapy has become an essential approach to prevent CHD. Currently, percutaneous coronary intervention (PCI) provides a new choice for patients with CHD. Besides, dual antiplatelet therapy with aspirin and clopidogrel is routinely administered to prevent thrombotic events after the coronary stent placement. However, this therapy significantly contributes to the occurrence of bleeding risk. According to a research conducted by Chen et al[3], aspirin alone may cause a higher risk of upper gastrointestinal hemorrhage. Moreover, clopidogrel is related to bleeding events caused by a newly discovered allelic variant. And further research conducted by Sim et al[4] found a novel allelic variant, cytochrome P450 (CYP) 2C19*17, that may result in an increased enzyme function of CYP2C19 due to the mutation (806C>T) in the 5-flanking region of the gene, which may cause an increased transcription of CYP2C19. Such increased transcriptional activity of CYP2C19 may confer an extensive metabolization of CYP2C19 substrates, which may lead to an enhanced response to clopidogrel and an increased risk of bleeding.

The aim of this study was to assess the impact of CYP2C19*17 allelic variant on platelet aggregation and the risk of bleeding in clopidogrel-treated Chinese patients with BSS and coronary stent placement.

1. **Materials and methods**

1.1 **Study population**

1.1.1 **Diagnostic criteria** World Health Organization defined myocardial infarction (MI) as follows[5]: (1) electrocardiogram (ECG) showing unequivocal pathological Q waves and ST segment elevation or depression in serial recordings; (2) a history of typical or atypical angina pectoris, together with equivocal changes on ECG and elevated enzymes; (3) a history of typical angina pectoris and elevated enzymes with no changes on ECG or not available; (4) fatal cases, whether sudden or not, with naked-eye appearances of fresh MI or recent coronary occlusion at necropsy (antemortem thrombus, haemorrhage into an atheromatous plaque or embolism). Patients meeting any of the above criteria were included.

1.1.2 **Diagnostic criteria of traditional Chinese medicine** BSS was diagnosed according to the proceedings of the Second National Academic Conference on Blood Stasis Syndrome Research[6].

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The diagnostic criteria were as follows: the main symptom includes chest pain such as angina and stabbing pain, particularly at night; secondary symptoms include blue or purple lips and face, sublingual collateral vessels with petechiae or ecchymosis, and a rough pulse. Patients with the main symptom or at least two secondary symptoms can be diagnosed.

1.1.3 Inclusion criteria From July 2009 to April 2011 in Fujian Provincial Institute of Cardiovascular Diseases, 520 patients with BSS who were going to have stent placement were enrolled in this study. A total of 311 males and 209 females were included in this prospective trial, and the mean age was $(61.5 \pm 10.2)$ years. Patients were eligible after successful PCI. The successful criteria for PCI included relieved or eradicated clinical symptoms of MI, the degree of coronary artery stenosis $<20\%$, thrombolysis in myocardial infarction (TIMI) grade 3 flow, no severe complications such as acute myocardial infarction (AMI), coronary bypass grafting or death.

1.1.4 Exclusion criteria Major PCI exclusion criteria included hemodynamic instability, active bleeding and bleeding diatheses, oral anticoagulation therapy with warfarin, use of periprocedural glycoprotein IIb/IIIa inhibitors, contraindication to antiplatelet therapy, left ventricular ejection fraction $<30\%$, leukocyte count $<3000/\text{mm}^3$, platelet count $<100 \times 10^9/\text{L}$, aspartate aminotransferase or alanine aminotransferase levels three times above the normal, serum creatinine level $>25 \text{mg}/\text{L}$, stroke attack within three months, noncardiac disease with a life expectancy less than one year or inability to follow the protocol.

The study protocol was approved by the Institutional Review Board of Provincial Clinical College of Fujian Medical University, and the patients were all provided with written informed consent for participation. The study was performed in the Clinical Pharmacological Base of Fujian Provincial Hospital.

1.2 Study methods
2.1.1 Medical history taking All patients were asked in detail for their medical history. All data were input into the computer database for storage.

1.2.2 Study design CYP2C19*17 genotyping and adenosine diphosphate (ADP)-induced platelet aggregation were completed in the Fujian Provincial Key Laboratory of Cardiovascular Diseases and Fujian Provincial Clinical Laboratory, respectively. We used prospective study method and employed polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach to analyze the relationships between CYP2C19*17 genotype and platelet aggregation along with bleeding events in clopidogrel-treated patients. First, 2 mL serum specimen for genomic DNA extraction and genotyping was taken from each patient by using disposable injector containing the anticoagulant of ethylene diamine tetraacetate acid. Genomic DNA was extracted from 200 $\mu\text{L}$ blood with commercially available kits (Takara Biotechnology, Dalian, China) according to the manufacturer’s instructions. CYP2C19*17 genotypes were determined with PCR-RFLP by using a PCR amplifier (Multigene Gradient, LABNET, USA). Primers 5'-GCCCTTAGCACCACCATCTCTC-3' and 5'-TTAACACCCATAAAAACACCG-3' were used to amplify the sequence of approximately 470 base pairs (bp) of the CYP2C19 gene containing single nucleotide polymorphism $-806\text{C}>\text{T}$ (rs12248560) in the 5'-flanking region of the gene. Followed by RFLP analysis, the PCR product (10 $\mu\text{L}$) of the 470 bp fragment containing the single-nucleotide polymorphism at position $-806\text{C}>\text{T}$ was digested at 37 $^\circ\text{C}$ with 3 U of Lwe I (Fermentas, MBI, USA) for 3 h. The enzymatic reaction was stopped by incubation for 20 min at 65 $^\circ\text{C}$, and the fragments were separated on a 3% agarose gel by using a electrophoresis apparatus (Tanon, Shanghai, China). Presence of $-806\text{T}$ (variant allele) yielded three fragments of 217, 142, and 113 bp, whereas presence of $-806\text{C}$ (wild type) yielded three fragments of 183, 142, and 113 bp after electrophoresis. To control the correct sample handling, genotypes were directly observed with ImageMaster VDS-CL (Amersham Pharmacia Co., Sweden) and were confirmed with ABI 3700 Automated DNA Sequencer. Then, ADP-induced platelet aggregation was performed. Blood was collected in 3.8% sodium citrate tubes after 10 d of clopidogrel treatment. Aggregation was assessed with platelet-rich plasma (PRP) and platelet-poor plasma (PPP) by using a turbidimetric method on a LBY-NJ4-channel platelet aggregation analyzer (Beijing Precil Instrument Co., Ltd.). PRP was obtained as a supernatant after the whole blood was centrifuged at 580 $\times$ g for 4 min, and the PPP obtained as the surplus plasma was secondly centrifuged at 2900 $\times$ g for 10 min. The platelet count of PRP was adjusted to $200 \times 10^9/\text{L}$. Light transmission was adjusted to 0% PRP and 100% PPP for each measurement. Results were recorded as light transmission at maximal aggregation ($\text{Agg}_{\text{max}}$) and 5-min aggregation ($\text{Agg}_{\text{late}}$) after the addition of ADP at a final concentration of 5 $\mu\text{mol}/\text{L}$. $\text{Agg}_{\text{max}}$ is considered to reflect the activity of both P2Y1 and P2Y12 receptors. However, clopidogrel only blocks the P2Y12 receptor. Therefore, $\text{Agg}_{\text{max}}$ is more reflective for the P2Y12 receptor activity. Inhibition of platelet aggregation (IPA) was defined as the percent decrease or aggregation values ($\text{Agg}_{\text{max}}$ and $\text{Agg}_{\text{late}}$) between baseline and after treatment, and calculated as follows: IPA = $[(\text{intensity of aggregation at baseline} - \text{intensity of aggregation after treatment})/\text{(intensity of aggregation at baseline})] \times 100\%$. Percentage of platelet disaggregation between $\text{Agg}_{\text{max}}$ and $\text{Agg}_{\text{late}}$ was defined as follows: disaggregation = $[(\text{Agg}_{\text{max}} - \text{Agg}_{\text{late}})/\text{Agg}_{\text{max}}] \times 100\%$. 
1.2.3 Drug therapy All of the patients enrolled in this study were pretreated with a loading dose of 300 mg clopidogrel and aspirin 12 h prior to PCI operation, and followed by 100 mg (300 mg/d for the first month) aspirin and clopidogrel 75 mg/d for one year. Diagnostic and interventional procedures were performed according to standard techniques. For the AMI patients undergoing emergency PCI, hypodermic injection of 0.4 mL of low molecular weight heparin calcium was given per 12 h on the first day, followed by clopidogrel 75 mg/d and 100 mg aspirin twice a day.

1.2.4 Observed indexes Platelet function was assessed at baseline and after 10 d of treatment. According to the TIMI criteria, the primary clinical safety end point of the study was defined as the incidence of combined major and minor bleeding events throughout the follow-up period.

1.2.5 Safety assessment Symptoms and signs such as rash, fever, diarrhea and nausea were observed after patients took the medication. Also, the blood, urine, and stool were tested for safety assessment before and after treatment.

1.2.6 Follow-up The information of patients were collected through outpatient service and the hospital records, and patients were interviewed by telephone after one month about whether they had experienced a bleeding event. Bleeding events were defined according to the TIMI criteria, namely, major bleeding including the intracranial hemorrhage or clinical visible bleeding (including image diagnosis) with hemoglobin concentration decline ≥ 50 g/L, minor bleeding including the clinical visible bleeding (including image diagnosis) with a hemoglobin concentration decline of 3 to 5 g/dL. Bleeding events include gastrointestinal, purpura, hematuria and retinal bleeding and intracranial hemorrhage.

1.3 Statistical analysis Hardy-Weinberg equilibrium was used to test a possible deviation of CYP2C19*17 genotype distribution. Some values of baseline characteristics were presented as mean ± standard deviation which were assessed by one-way analysis of variance (ANOVA), while the other data of baseline characteristics were reported as percentage and were assessed by Chi-square test. The contrast of platelet aggregation in different groups of genotype was assessed by ANOVA followed by SNK-q test. Differences between genotypes with respect to clinical events were assessed by Chi-square test for trend. The odds ratio (OR) and the corresponding 95% confidence interval (CI) were calculated for each variable included in the multivariate model. A multiple logistic regression model was used to test for an independent association of CYP2C19*17 allele carriage with TIMI bleeding events. P < 0.05 was regarded as statistically significant. All statistical analysis was performed with SPSS 13.0 software.

2 Results

2.1 CYP2C19*17 genotype distribution Among the 520 patients, 443 (85.19%) were wild-type homozygous CYP2C19*17 (wt/wt), while only 6 (1.15%) were homozygous CYP2C19*17 ('17/'17). The gene frequency of CYP2C19*17 allele was significantly lower than the wild-type CYP2C19*17. For genotype distribution, no significant deviation from Hardy-Weinberg equilibrium was observed (P > 0.05). See Table 1.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Actual frequency</th>
<th>Expected frequency</th>
<th>Gene frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt/wt (CC)</td>
<td>443 (85.19%)</td>
<td>440.31</td>
<td>37</td>
</tr>
<tr>
<td>Wt/'17 (CT)</td>
<td>71 (13.65%)</td>
<td>76.37</td>
<td>3</td>
</tr>
<tr>
<td>'17/'17 (TT)</td>
<td>6 (1.15%)</td>
<td>3.31</td>
<td>37</td>
</tr>
<tr>
<td>Wt (C)</td>
<td>957 (92.02%)</td>
<td>957.21</td>
<td>37</td>
</tr>
<tr>
<td>'17 (T)</td>
<td>83 (7.98%)</td>
<td>83.79</td>
<td>37</td>
</tr>
</tbody>
</table>

C: cytosine; T: thymine.

2.2 Baseline characteristics of the study population

Data from the patients were recorded for their age, gender, body mass index, etc (Table 2). There was no statistical difference among all the parameters (P > 0.05).

<table>
<thead>
<tr>
<th>Variable</th>
<th>CYP2C19 wt/wt (n=443)</th>
<th>CYP2C19 wt/*17 (n=71)</th>
<th>CYP2C19 *17/*17 (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean±standard deviation, years)</td>
<td>63.5±10.7</td>
<td>63.7±10.8</td>
<td>63.1±10.2</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>261/172</td>
<td>41/30</td>
<td>3/3</td>
</tr>
<tr>
<td>Body mass index (mean±standard deviation, kg/m²)</td>
<td>25.4±4.4</td>
<td>25.1±4.9</td>
<td>27.1±5.4</td>
</tr>
<tr>
<td>Serum creatinine (mean±standard deviation, mg/L)</td>
<td>10.6±5.0</td>
<td>10.5±4.0</td>
<td>10.3±6.0</td>
</tr>
<tr>
<td>Ejection fraction (mean±standard deviation, %)</td>
<td>56.2±10.3</td>
<td>55.7±11.3</td>
<td>54.6±11.5</td>
</tr>
<tr>
<td>Active smoker (n, %)</td>
<td>110 (24.83)</td>
<td>23 (32.39)</td>
<td>2 (33.33)</td>
</tr>
<tr>
<td>Diabetes mellitus (n, %)</td>
<td>119 (26.86)</td>
<td>21 (29.57)</td>
<td>2 (33.33)</td>
</tr>
<tr>
<td>Hypertension (n, %)</td>
<td>376 (84.87)</td>
<td>62 (87.32)</td>
<td>5 (83.33)</td>
</tr>
<tr>
<td>Hypercholesteremia (n, %)</td>
<td>318 (71.78)</td>
<td>52 (73.23)</td>
<td>4 (66.67)</td>
</tr>
<tr>
<td>Previous MI (n, %)</td>
<td>172 (38.82)</td>
<td>28 (39.43)</td>
<td>3 (50)</td>
</tr>
<tr>
<td>Non-STEMI/STEMI (n, %)</td>
<td>57 (12.86)</td>
<td>7 (9.85)</td>
<td>1 (16.67)</td>
</tr>
<tr>
<td>Platelet count (mean±standard deviation, ×10⁴/L)</td>
<td>207±53</td>
<td>205±55</td>
<td>203±58</td>
</tr>
<tr>
<td>Use of PPI (n, %)</td>
<td>94 (21.4)</td>
<td>13 (18.31)</td>
<td>1 (16.67)</td>
</tr>
</tbody>
</table>

MI: myocardial infarction; STEMI: ST-elevation myocardial infarction; PPI: proton pump inhibitor.
2.3 CYP2C19*17 and platelet aggregation  The baseline values of the three groups showed no statistical difference in Aggmax, Aggsize and disaggregation before clopidogrel treatment (P > 0.05). While after 10 d of treatment, the '17/*17 and wt/*17 carriers showed significantly lower ADP-induced platelet aggregation values compared with the wild-type homozygotes (wt/wt) (P < 0.05, P < 0.01). Further analysis revealed that the IPA in extensive metabolizer carriers was higher than those who carried homozygotes (wt/wt) (P < 0.05, P < 0.01), indicating that the inhibition of platelet aggregation in CYP2C19*17 allele carriers (including *17/*17 and wt/*17) was enhanced (P < 0.01), and the same results were found in disaggregation of platelet after 10 d of treatment. CYP2C19*17 carrier status is significantly associated with enhanced response to clopidogrel. See Table 3.

2.4 CYP2C19*17 genotype and bleeding events  The primary safety end point (combined TIMI major and minor bleeding) within the follow-up period occurred in 31 patients (5.96%) of the study population; 15 cases of TIMI major bleeding (2.88%) and 16 cases of TIMI minor bleeding (3.08%) were observed. Only one major bleeding event of intracranial hemorrhage occurred in CYP2C19 allele (*17/*17) carriers confirmed by computed tomography scans. Eleven bleeding events (35.48%) occurred in patients with homozygous or heterozygous CYP2C19*17 allele on the observation of the total of 31 patients. See Table 4.

According to CYP2C19*17 genotype, the risk of bleeding in CYP2C19 wild-type homozygotes was 4.51%, while in heterozygous CYP2C19*17 (wt/*17) was 14.08% (the sample size was small, only 71). For the total study population alone, the bleeding risk was maintained at 5.96%. The bleeding incidence was higher in the CYP2C19*17 allele carriers compared with CYP2C19 wild-type carriers. The risk of bleeding was highest in patients with homozygous CYP2C19*17, though only 1 bleeding event occurred.

Among TIMI major bleeding, three cases of fatal intracranial bleeding, two hematomas and four upper gastrointestinal hemorrhages occurred in wild-type (wt/wt), while two upper gastrointestinal hemorrhages and three subcutaneous hemorrhages occurred in CYP2C19 wt/*17 carriers, and only one intracranial hemorrhage occurred in CYP2C19*17/*17. Among TIMI minor bleeding, seven subcutaneous hemorrhages and four cases of retinal bleeding occurred in wild-type (wt/wt), while five gastrointestinal hemorrhages occurred in CYP2C19*17 allele (wt/*17) carriers.

Results of multiple logistic regression model demonstrated that carriage of the CYP2C19*17 allele was an independent predictor for TIMI bleeding (Table 5); patients with *17 allele of CYP2C19 had a higher ratio of bleeding events than those with the wild-type genotype (P < 0.01). The bleeding incidence was highest in the group of CYP2C19 carriers with both wt/*17 and *17/*17 compared with wild-type and the total risk groups (P < 0.01). See Figure 1.

### Table 3  Difference of platelet aggregation in CYP2C19*17 genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Aggmax Baseline value</th>
<th>Aggmax After 10 d of treatment</th>
<th>Aggsize Baseline value</th>
<th>Aggsize After 10 d of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt/wt (CC)</td>
<td>443</td>
<td>58.02±12.79</td>
<td>41.45±10.27</td>
<td>48.92±14.69</td>
<td>35.87±11.50</td>
</tr>
<tr>
<td>Wt/*17 (CT)</td>
<td>71</td>
<td>55.59±11.43</td>
<td>37.66±8.62**</td>
<td>47.45±11.98</td>
<td>29.12±9.75**</td>
</tr>
<tr>
<td>*17/*17 (TT)</td>
<td>6</td>
<td>54.32±9.45</td>
<td>31.33±7.86*</td>
<td>47.55±9.54</td>
<td>24.87±8.20*</td>
</tr>
</tbody>
</table>

### Table 4  CYP2C19*17 genotype and TIMI bleeding risk

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>TIMI major bleeding events</th>
<th>TIMI minor bleeding events</th>
<th>TIMI total bleeding events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt/wt (CC)</td>
<td>443</td>
<td>9 (2.03)</td>
<td>11 (2.48)</td>
<td>20 (4.51)</td>
</tr>
<tr>
<td>Wt/*17 (CT)</td>
<td>71</td>
<td>5 (7.04)**</td>
<td>5 (7.04)**</td>
<td>10 (14.08)**</td>
</tr>
<tr>
<td>*17/*17 (TT)</td>
<td>6</td>
<td>1 (16.67)**</td>
<td>0</td>
<td>1 (16.67)</td>
</tr>
<tr>
<td>Total</td>
<td>520</td>
<td>15 (2.88)</td>
<td>16 (3.08)</td>
<td>31 (5.96)</td>
</tr>
</tbody>
</table>

* P<0.05, ** P<0.01, wt/wt genotype. Aggmax: maximal aggregation; Aggsize: 5 min aggregation; IPAmax: maximal inhibition of platelet aggregation; IPAsize: inhibition of platelet aggregation after clopidogrel treatment; C: cytosine; T: thymine.
Table 5 Results of a multivariable logistic regression model

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19*17 allele carriage</td>
<td>1.95 (1.31 to 3.16)</td>
</tr>
<tr>
<td>Gender</td>
<td>1.31 (0.68 to 2.54)</td>
</tr>
<tr>
<td>Age (per 10 years increment)</td>
<td>1.57 (1.13 to 2.17)</td>
</tr>
<tr>
<td>BMI (per 5 kg/m² increment)</td>
<td>0.87 (0.61 to 1.25)</td>
</tr>
<tr>
<td>Serum creatinine (per 1 mg/L increment)</td>
<td>0.97 (0.88 to 1.06)</td>
</tr>
</tbody>
</table>

Results of this multiple logistic regression model are shown for combined TIMI major and minor bleeding as the dependent variable. Independent variables including CYP2C19*17 allele carriage are shown. OR: odds ratio; CI: confidence interval; BMI: body mass index; TIMI: thrombolysis in myocardial infarction.

Figure 1 Observation of bleeding risk in different groups

Data are expressed as the rate of TIMI bleeding events in different groups of the total patients, and analyzed by Chi-square test. ** P < 0.01, vs wt/wt genotype; △△ P < 0.01, vs total risk group. TIMI: thrombolysis in myocardial infarction; EM: extensive metabolization.

3 Discussion

In traditional Chinese medicine, CHD belongs to chest impediment, and in the ancient traditional Chinese medical texts, a large number of effective drugs were recorded for promoting blood circulation and removing blood stasis, which were useful for chest impediment. BSS usually refers to the factors such as qi deficiency, qi stagnation, phlegm obstruction and interior heat pattern, which could cause an inability of blood unable to run smoothly. Modern medicine asserts that the main physiological function of platelet is to participate in hemostasis and thrombosis, which plays an important role in atherosclerosis formation and inflammation. Platelet function is closely related to the platelet activation, including adhesion, release and aggregation reactions. This is one of the main mechanisms of MI in CHD. Research on the relationship between blood stasis syndrome and platelet has received more and more attention. Clopidogrel is a commonly used antiplatelet drug, and the metabolism of clopidogrel has a significant correlation with CYPs.

CYPs are responsible for about 80% of all the phase I-dependent metabolism of clinically used drugs, including antiplatelet medication such as clopidogrel and proton pump inhibitors, antidepressants such as citalopram, amitriptyline and clomipramine, and other drugs such as carisoprodol, diazepam, flunitrazepam, progabide, phenytoin and mephénytoin. Clopidogrel, an inactive prodrug, requires metabolism and activation by the hepatic CYP system to generate its active thiol metabolite, which targets and irreversibly inhibits the ADP P2Y1 receptor, but has no effect on P2Y12. Therefore, it restrains the activation of the fibrinogen receptor GpIIb/IIIa, and plays an important role in inhibition of platelet aggregation function. Hepatic metabolism of clopidogrel is achieved by a number of different hepatic CYP isoenzymes including CYP2C19, 3A4/5, 1A2, 2B6, and 2C9. Evidence is accumulating that the polymorphically expressed isoenzyme CYP2C19 constitutes a dominant part in this process. Furthermore, the metabolic activation by CYP2C19 has emerged as a crucial determinant of clopidogrel pharmacodynamic response and clinical efficacy.

CYP2C19 metabolic activity is highly variable among patients because of genetic variation. Sim et al. found that the transcriptional activity of the CYP2C19 gene is significantly up-regulated in the presence of the *17 allele, which is defined by a mutation at position 806 (−806C>T) in the 5'-flanking region of the gene. The *17 allele can specifically bind nuclear proteins to the 5'-flanking region of the gene, which leads to increased gene transcription and expression. Consequently, the presence of the *17 allele is associated with an ultra-rapid metabolism of CYP2C19 substrates, which may lead to an enhanced response to clopidogrel, and increase the risk of bleeding. This has been demonstrated consistently in a number of pharmacological studies.

Our present study suggested that in the Chinese population, the CYP2C19*17 gene frequency is significantly lower than the results obtained by Sibbing et al. in white populations. Besides, the bleeding rate in the total study population was significantly lower compared with the average rate of bleeding events conducted by Serebruany in the analysis of risk of bleeding complications after different doses of aspirin in 192,036 patients enrolled in 31 randomized controlled trials. The incidence of bleeding events was significantly higher in CYP2C19*17 allele carriers than the wild-type carriers due to that the CYP2C19*17 allelic variant causes an increased enzyme function of CYP2C19, and an accelerated metabolism of CYP2C19 substrates. This may lead to an enhanced response to antiplatelet treatment with clopidogrel, meanwhile, it increases the incidence of bleeding.

According to the current research, it is believed that CYP2C19*17 allelic variant is significantly associated with enhanced response to clopidogrel...
and an increased risk of bleeding. By testing the platelet function and analyzing the genetic background of the patients, combining the genotyping of PCR-RFLP and gene sequencing, the genotype and platelet function of the patients could easily determined. The dose of clopidogrel is adjusted according to the results. Besides, commercially available CYP2C19 genetic testing and point-of-care genetic testing have made it possible to use CYP2C19 genotype to guide antiplatelet therapy. However, the safety and efficacy of alternative therapy in response to genotypic or phenotypic testing are entirely unknown. While neither alone adequately describes the global risk profile of an individual patient treated with clopidogrel, point-of-care platelet function testing combined with CYP2C19 genetic testing may be more effective in identifying high-risk patients for alternative antiplatelet therapies than either independently administered. Ultimately, prospective randomized clinical trials will be needed to test specific personalized antiplatelet algorithms for widespread adoption into clinical practice. In conclusion, it is very important to improve the clinical rational for antiplatelet drug uses and avoid the bleeding events for the CYP2C19*17 carriers, and it is strongly recommended that a strengthened antiplatelet scheme should not be applied in CYP2C19*17 allele carriers.

4 Competing interests

The authors declare that they have no competing interests.

REFERENCES


冠心病血瘀证患者细胞色素 P450 2C19*17 基因型分布
与氯吡格雷治疗后血小板聚集率及出血风险的关系

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目的：探讨细胞色素 P450 (cytochrome P450, CYP) 2C19*17 等位基因变异对中国冠心病血瘀证患者经皮冠状动脉介入术 (percutaneous coronary intervention, PCI) 后应用氯吡格雷治疗的血小板聚集率及出血事件的影响。

方法：以限制性片段长度多态性聚合酶链反应基因分析方法检测 CYP2C19*17 基因多态性，研究 2009 年 7 月至 2011 年 4 月福建省立医院心内科择期进行 PCI 成功的冠心病血瘀证患者 520 例。PCI 术前常规服用阿司匹林 300 mg，氯吡格雷 300 mg。术前采血，制备富血小板血浆、贫血小板血浆及二磷酸腺苷 (adenosine diphosphate, ADP) 诱导剂，采用比浊法，以最终浓度为 5 μmol/L ADP 为诱导剂，在氯吡格雷治疗前及治疗后 10 d，检测血小板聚集率。分析最大血小板聚集率 (maximal aggregation, Aggmax) 和残余血小板聚集率 (5-min aggregation, Agg5min)。

结果：试验发现有 5.96% 的患者发生心肌梗死血栓溶解术出血事件，而本试验中病人的 CYP2C19*17 等位基因频率为 7.98%。对于 CYP2C19*17 等位基因携带者，其出血事件发生率远高于野生型 (P < 0.01)；在基线水平，5 μmol/L ADP 诱导的 Aggmax 和 Agg5min 以及血小板聚集率在 CYP2C19*17 各基因型之间并没有显著区别，然而经氯吡格雷治疗 10 d 后，CYP2C19*17 等位基因携带者与野生型相比，上述 3 项指标均明显降低 (P < 0.01 或 P < 0.05)，血小板聚集抑制率显著高于野生型患者 (P < 0.01)；携带者有 CYP2C19*17 等位基因的患者与野生型相比，具有更高的出血风险 (P < 0.01)。

结论：冠心病血瘀证患者 CYP2C19*17 等位基因携带者有着显著的氯吡格雷反应性且其出血风险明显增加。

关键词：细胞色素 P450 酶系统；出血；血小板聚集抑制剂；血瘀