Association of the Platelet Membrane Glycoprotein Ia C807T Gene Polymorphism with Aspirin Resistance

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Summary: To explore the correlation between the C807T polymorphism of platelet membrane glycoprotein Ia (GP Ia) gene and aspirin resistance in Chinese people, 200 patients with high-risk of atherosclerosis took aspirin (100 mg/d) for 7 days. Platelet aggregation function was detected using adenosine diphosphate (ADP) and arachidonic acid (AA) before and after the administration of aspirin. Then the subjects were divided into three groups according to the results of platelet aggregation function: an aspirin resistant (AR) group, an aspirin semi-responder (ASR) group and an aspirin-sensitive (AS) group. Platelet GP Ia gene 807CT polymorphism was examined by means of polymerase chain reaction-sequence specific primers (PCR-SSP). The results showed that T allelic frequency in AR group and ASR group were higher that of AS group (P<0.05), and the prevalence of genotypes (TT+TC) of these two groups was significantly higher than that in AS group (P<0.05). Platelet GP Ia T allele was significantly associated with aspirin resistance as revealed by multiple logistic regression (OR=3.76, 95% CI: 2.87–9.58). The results suggest that inherited platelet GP Ia variations may have an important impact on aspirin resistance and the presence of GP Ia T allele may be a marker of genetic susceptibility to aspirin resistance.

Key words: platelet membrane glycoprotein; aspirin resistance; genetic polymorphism; atherosclerosis

Aspirin is a potent anti-platelet drug which is widely used for primary and secondary prevention of ischemic vascular diseases. It is believed that aspirin can reduce about 20%–40% of ischemic vascular diseases. Despite its well-documented benefits, approximately 30% of the total population has been shown to develop an inadequate response to the platelet inhibitory effect of aspirin, which was named “Aspirin Resistance”.

Recent studies have shown that several prothrombotic genetic variations may contribute to aspirin resistance, and increased risk of cardiovascular events[1] and they include (1) the C807T or A873G polymorphism allied with increased density of platelet GP Ia-IIa collagen-receptor gene, (2) polymorphism PLA1/A2 of the gene encoding glycoprotein IIIa and (3) polymorphism on the cyclooxygenase-1 (COX-1) gene affecting Ser529. Because of the possible increased risk of ischemic vascular events, carriers of these genetic polymorphisms may be resistant to the anti-platelet effects of aspirin.

However, the reports have been scanty on the relationship between the C807T polymorphism of platelet membrane GP Ia gene and the clinical effects on aspirin. The aim of this study was to further investigate the possible association between the platelet GP Ia gene polymorphism and the occurrence of aspirin resistance, as well as the prevalence of patients with aspirin-insensitive platelet aggregation of Chinese population.

1 SUBJECTS AND METHODS

1.1 Samples
The prevalence of the C807T polymorphism of platelet membrane GP Ia gene was assessed in 200 patients with high-risk of atherosclerosis (86 females, 114 males, with age ranging from 54–76 y). They were selected from the Medical Examination Center of the Third Affiliated Hospital of He’nan University of Science and Technology, Luoyang, China, and was compared with 100 healthy blood donors from Luoyang City, from January 2004 to June 2005.

The patients with high-risk of atherosclerosis took aspirin (100 mg/d) for 7 days. Approximately 5 mL of blood was obtained in the morning after an overnight fasting, and then platelet aggregation function was detected with 5 µmol/L adenosine diphosphate (ADP) and 500 µmol/L arachidonic acid (AA) serving as inductor before and after taking aspirin.

Key demographic and clinical features of the subjects were recorded for all recruited individuals, including age, gender, history of diabetes, primary hypertension, hyperlipemia, current smoking habit, platelet count and recently used medication (include ticlopidine, clopidogrel, low molecular weight heparin and other drugs that can effect the function of platelet).

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Exclusion criteria included the history of haemorrhage in the individual or family, platelet count \( \geq 450 \times 10^9/L \) or \( \leq 100 \times 10^9/L \), haematoglobin \( \leq 80 \) g/L, myelosplastic syndrome, malignant plasma cell dyscrasia, having receiving operation within the past month; taking drugs that can affect the function of platelet such as ticlopidine, NSAID, clopidogrel, low molecular heparin, warfarin etc within the preceding 1 week.

1.2 Methods

1.2.1 The Laboratory Criteria for Maximum Agglutination of Platelets

Healthy blood donors (n=100) were taken as control group, and platelet aggregation function was detected by using 5 µmol/L ADP and 500 µmol/L AA as inductor. The normal values of ADP-induced and AA-induced maximum platelet aggregation for healthy adults was set at (52.1±9.0)% and (60.3±5.7)%, respectively.

1.2.2 Evaluation Criteria for Aspirin Resistance

The aspirin resistance was defined as ADP-induced platelet maximum agglutination \( \geq (52.1 \pm 9.0)\% \) and AA-induced platelet maximum agglutination \( \geq (60.3 \pm 5.7)\% \) after the patients with high-risk of atherosclerosis took aspirin (100 mg/d) for 7 days. Aspirin semi-responder (ASR) was defined as those who satisfied either of the above-mentioned criteria.

1.2.3 Genotyping of GP I a C807T Dimorphism

Leukocyte DNA was isolated from whole blood by using standard procedures. The GP I a-specific polymerase chain reaction (PCR) primers used in this study were constructed on the basis of the published GP I a cDNA15 and GP I a gene sequences. About 0.1–1.0 µg genomic DNA was added to a 50 µL of reaction mixture containing 10 mmol/L Tris (pH 8.0), 50 mmol/L KCl, 2.75 mmol/L MgCl₂, 0.125 mmol/L of each dNTP, 0.25 µmol/L each of sense primer (5'-GACAGCCCATATTGAATTGTCA CG-807-3') and 2.5 U Taq DNA polymerase. After initial denaturation at 95°C for 10 min, amplification was performed in a DNA thermocycler for 35 cycles (denaturation at 93°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 30 s). A final extension of 5 min at 72°C completed the PCR. The PCR products were analyzed by electrophoresis on 2.0% agarose gels using Tris-acetate/EDTA buffer for about 30 min (8 V/cm) and visualized by ethidium bromide staining. DNA molecular marker I was used as the standard (Beijing Tianwei Corporation, China). Amplification of genomic DNA yielded 184 bp specific products (detected by United Gene Holdings Ltd, Shanghai, China) (fig. 1).

1.3 Statistical Analysis

Statistical analysis was performed with SPSS software (Version 12.0). Established risk factors of aspirin resistance were identified by multiple logistic regression. The \( \chi^2 \)-square test was used to test deviation of genotype distribution from Hardy-Weinberg equilibrium (a=0.05) to determine the significance of the difference in allele or genotype frequencies in ASR, AS and AR patients. The relationship between the C807T polymorphism and aspirin resistance was determined by multiple logistic regression with adjustment for other aspirin resistance risk factors. A two-sided probability value of less than 0.05 was considered to indicate statistical significance.

2 RESULTS

2.1 Epidemiological Features of the Patients with High-risk of Atherosclerosis

Risk factors such as age, diabetes, primary hypertension, platelet count in ASR and AR patients had no significant differences from AS patients. The number of current smokers and women in AR or AS patients were significantly higher than that in AS patients, which were demonstrated in table 1 (P<0.005).

Table 1 Epidemiological features of aspirin semi-responder (ASR), aspirin-sensitive (AS) and aspirin-Resistant (AR) patients

<table>
<thead>
<tr>
<th>Features</th>
<th>AR (n=9)</th>
<th>ASR (n=41)</th>
<th>AS (n=150)</th>
<th>( \chi^2 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (y)</strong></td>
<td>68.7±10.2</td>
<td>65.1±8.7</td>
<td>64.6±10.6</td>
<td>( \chi^2 )</td>
<td>( P )</td>
</tr>
<tr>
<td><strong>Women (%)</strong></td>
<td>44.4</td>
<td>34.1</td>
<td>14.7</td>
<td>11.156</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>Current smokers (%)</strong></td>
<td>33.3</td>
<td>31.7</td>
<td>11.3</td>
<td>11.639</td>
<td>0.003</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>22.2</td>
<td>19.5</td>
<td>15.3</td>
<td>0.632</td>
<td>0.729</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>55.6</td>
<td>53.7</td>
<td>51.3</td>
<td>0.117</td>
<td>0.943</td>
</tr>
<tr>
<td>Platelet count (10^9/L)</td>
<td>276±103</td>
<td>237±97</td>
<td>265±112</td>
<td>( \chi^2 )</td>
<td>12.848</td>
</tr>
</tbody>
</table>

\( P<0.05 \) (analysis of variance, \( \bar{x} \pm s \)) \( **P<0.005 \) (\( \chi^2 \)-square test, \( \chi^2 \)=12.848)

Fig. 1 Representative results of GP I a genotypes determined by PCR-SSP of three individuals

lanes 1, 2: person 1 (heterozygous CT); lanes 3, 4: person 2 (homozygous TT); lanes 5, 6: person 3 (homozygous CC); lanes 7, 8 negative controls

Genomic DNA was amplified by specific primer for C807 (right panel) or for T807 allele (left panel) and was analyzed on 2.0% agarose gel electrophoresis. DNA molecular marker I was used as standard (lane M).