A Meta-analysis of the Association of COX-1 Gene rs3842788 and rs1330344 Polymorphism with Aspirin Resistance in Chinese

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Abstract

The cyclooxygenase-1 (COX-1) gene rs3842788 variant (128G>A) and rs1330344 variant (1676G>A) have been associated with aspirin resistance in patients with cardiovascular disease. However, there is not enough evidence to demonstrate whether the A or G allele of COX-1 gene is indeed a genetic factor that can lead to aspirin resistance, with many of the studies coming to opposite conclusion. Here, we identified 10 articles on COX-1 in a literature search, and conducted a meta-analysis on the rs3842788 and rs1330344 genotype difference between aspirin resistance and aspirin sensitive patients. We found that there is no significant difference between cases and controls on GA+AA and GG genotypes of rs3842788 (OR, 1.22; 95% CI, 0.85-1.75; P, 0.29). The results provide additional evidence that rs3842788 of COX-1 gene maybe not a factor to cause aspirin resistance. However, we found that there is a significant difference between cases and controls on GA+GG and AA genotypes of rs1330344 (OR, 1.48; 95% CI, 1.15-1.90; P, 0.002). The results provide evidence that rs1330344 of COX-1 gene may be a factor to cause aspirin resistance. Therefore, more researches are needed to show the relationship between COX-1 gene and aspirin resistance.

Keywords: Aspirin resistance; COX-1; Allele; Genotype; Meta-analysis

Introduction

Acetylsalicylic acid (aspirin) is commonly used as an antiplatelet treatment for cardiovascular disease [1]. By inhibiting cyclooxygenase (COX) activity, a prostaglandin formation of ltb4 and hlen spirin impairs the formation of prostaglandin, and thromboxane A2 [2], which is critical to platelet aggregation. Long-term aspirin use therefore reduces the risks of heart attack and stroke [1]. A concept of “aspirin resistance (AR)” describes the inability of aspirin to thromboxane (TX) biosynthesis and produces an anticipated effect on one or more in vitro tests of platelet function [2]. Clinically, aspirin resistant patients still develop adverse vascular events despite aspirin intake [3].

This phenomenon indicates that aspirin may have unequal antiplatelet effects on individuals. While the causative factors of aspirin resistance have not been determined, speculations of possible influences are plentiful. Generally, there is agreement that the mechanisms for aspirin resistance are multifactorial, but genetic polymorphism is potentially one of the direct causes [4].

Abundant literature suggested that there exist genetic basis for aspirin resistance and evaluated whether polymorphisms on the candidate genes can be identified for antiplatelet resistance [5]. Specifically, single nucleotide polymorphisms (SNPs) on COX may be the most reported genetic determinants. Because of the heterogeneity of the population studied, the diverse methods used, and the inadequate sample size, current studies often provide inconsistent and irreproducible results [6-16].

Therefore, there remains a need to systematically analyze whether the COX-1 genetic polymorphisms can be identified as biomarkers for aspirin resistance. In order to investigate the impact of COX-1 polymorphisms on aspirin resistance, we present here a meta-analysis of individual participant data, evaluating the association of two COX-1 polymorphisms (rs3842788: G128A, 128G>A rs1330344: A1676G, 1676AG) with the risk of having aspirin resistance. The result encompasses only patients with cardiovascular disease. The selection of COX-1 enzyme is based on its critical role in platelet regulation, and the SNPs are selected for their relevance to studies concerning aspirin resistance. The polymorphism has more than 1000 subjects for sufficient data synthesis.

Materials and method

Data sources

PubMed, Web of Science, Wanfang (http://www.wanfangdata.com.cn), and CNKI (http://www.cnki.net) electronic databases were searched up until 1 August 2017 for all articles evaluating the association between the genetic polymorphism of rs3842788 on COX-1 with aspirin resistance. Search terms used for the primary search were “aspirin resistance” and “AR” in combination
with "rs3842788", "G128A", and "128G>A" or "rs1330344", "A1676G" and "1676AG". Searches were limited to published English and Chinese language articles.

A secondary search was performed on all potentially relevant articles for any additional articles. Eligibility of the retrieved articles was evaluated by reading the titles and the abstracts if necessary. The search results were limited to human. Studies were required to have measured aspirin resistance using laboratory methods described previously.

### Inclusion/exclusion criteria

Articles were included if (i) They evaluated the association of the genetic polymorphisms rs3842788 or rs1330344 with the risk of having aspirin resistance (ii) They were conducted on a case-control or nested case-control study design (iii) They provided the genotype and/or allele counts of examined polymorphisms between patients with aspirin resistance and controls in order to estimate odds ratio (OR) and 95% confidence interval (95% CI) (iv) The study contained a clear description of the method used to establish the effects of aspirin on platelet reactivity to compare patients with laboratory aspirin resistance with those without.

Articles were excluded if (i) They did not provide the genotype or allele counts of examined polymorphisms; (ii) They lacked either patient group or control group (iii) They were performed on nonhuman subjects (iv) They were meeting abstracts, case reports/series, editorials, review articles, or non-English and non-Chinese publications.

### Data extraction

Data were extracted independently by different authors on a standardized Excel template and were verified with disagreements settled by consensus. For each article, information was extracted on the first author, publication year, age, aspirin dosages used, sample size of type of aspirin reactivity (aspirin resistance and aspirin sensitive), the genotypes/alleles of examined polymorphisms, study design, ethnicity as well as population characteristics.

### Statistical analysis

Data were analyzed using Cochrane Review Manager, version 5.3 (Cochrane Collaboration, Syracuse, NY, USA). Risk estimate was expressed as a pooled odds ratio (OR) calculated using fixed and random effects model, along with the 95% CI to measure the strength of association. Fixed-effects summary ORs were calculated using the Mantel–Haenszel method. Test for heterogeneity were performed. For assessment of publication bias graphically, we used funnel plot on ORs.

### Results

#### Qualified articles

The initial search identified 204 papers. When we refined the search by viewing the title, the number of papers was reduced to 97. We excluded 16 papers that are replicated. We reviewed 81 abstracts for evidence of data related to aspirin resistance and its relation to rs3842788 or rs1330344. We further excluded papers that contain only nonhuman subjects, have no data, or associate only with other gene locus. Overall, 10 papers met the inclusion criteria.

#### Study characteristics

We included 10 full-text articles. Aspirin dosages used in included studies are between 75 and 100 mg daily. Different studies apply various criteria to distinguish whether the patients were aspirin resistant or sensitive. Various sequencing methods were used to examine the sample characteristics. Sequenom Mass ARRAY iPLEX was used in three studies. (Peng Zhang LiXL). Three studies utilized Sequencing, PCR-RELP was used in three studies. And genechip were adopted in the other one study. All of the subjects in the meta-analysis are Chinese patients with cardiovascular disease. Five studies included Cerebral Ischemic Stroke patients. (Peng Zhang Cao). One study paid attention to Chronic Stable Angina patients. The other four studies involved patients with respectively Coronary Atherosclerotic Heart Disease and Ischemic Cerebrovascular. The aspirin resistance frequency of patients with genetic polymorphism rs3842788 (AA+AG) was 14.88% in patients, and 17.76% in controls, and rs1330344 (GG +AG) was 69.78% in patients, and 60.80% in controls. The 6 studies on the association between aspirin resistance and rs3842788 analyzed statistically included 1610 subjects sensitive, and 330 subjects resistant, to aspirin. An OR of 1.22 was observed for aspirin resistance in subjects carrying the rs3842788 SNP (AG+AA). Interstudy OR heterogeneity was measured (χ²=14.90, df=5 (P=0.01) I²=66%). Moreover, The 5 studies on the association between aspirin resistance and rs1330344 analyzed statistically included 1541 subjects sensitive, and 471 subjects resistant, to aspirin. An OR of 1.48 (95% CI 0.83, 1.90, P=0.002) was observed for aspirin resistance in subjects carrying the rs1330344 SNP (AG+GG). Interstudy OR heterogeneity was measured (χ²=3.86, df=4 (P=0.43) I²=0%).

### Meta-analysis

Figure 2 presents the OR and P values for the pooled analyses. The overall OR of the GA+AA genotypes compare to the GG genotype was
1.22 (95% CI 0.85-1.75, P=0.29). It indicates that there is no significant difference between AR and AS groups on COX-1 gene rs3842788. There was no publication bias (not shown). Figure 3 presents the OR and P values for the pooled analyses. The overall OR of the AG+GG genotypes compare to the AA genotype was 1.48 (95% CI 0.83, 1.90; P=0.002). It indicates that there is significant difference between AR and AS groups on COX-1 gene rs1330344. There was no publication bias (not shown).

### Table 1: The general situation of the study.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Ethnicity</th>
<th>Average age ± (sd)</th>
<th>Drug dose</th>
<th>Sample size</th>
<th>Gene detection method</th>
<th>Sample characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ping LL (2014)</td>
<td>Chinese</td>
<td>64.35 ± 11.88</td>
<td>100 mg/d</td>
<td>192 18</td>
<td>174</td>
<td>Sequenom Mass ARRAY IPLEX</td>
</tr>
<tr>
<td>XU JJ (2017)</td>
<td>Chinese</td>
<td>58.59 ± 11.35</td>
<td>100 mg/d</td>
<td>366 166</td>
<td>200</td>
<td>PCR-RFLP</td>
</tr>
<tr>
<td>XUE M (2016)</td>
<td>Chinese</td>
<td>61.80 ± 11.35</td>
<td>100 mg/d</td>
<td>207 51</td>
<td>156</td>
<td>Sequencing</td>
</tr>
<tr>
<td>Wang B (2009)</td>
<td>Chinese</td>
<td>57.16 ± 9.59</td>
<td>100 mg/d</td>
<td>220 19</td>
<td>201</td>
<td>genechip</td>
</tr>
<tr>
<td>ZHANG SS (2015)</td>
<td>Chinese</td>
<td>64.35 ± 11.88</td>
<td>100 mg/d</td>
<td>96 9</td>
<td>87</td>
<td>Sequenom Mass ARRAY IPLEX</td>
</tr>
<tr>
<td>CAO LP (2014)</td>
<td>Chinese</td>
<td>64.21 ± 12.71</td>
<td>100 mg/d</td>
<td>859 67</td>
<td>792</td>
<td>Sequencing</td>
</tr>
</tbody>
</table>

### Figure 2: The forest plots in meta-analysis of the relationship between COX-1 gene rs3842788 polymorphism and aspirin resistance.

### Figure 3: The forest plots in meta-analysis of the relationship between COX-1 gene rs1330344 polymorphism and aspirin resistance.

### Discussion

Aspirin blocks the aggregation of platelet by inhibiting the acetylation of the 529 serine of cox-115. Therefore, we evaluate the association of two common polymorphisms (rs3742788 and rs1330344) from COX-1 with aspirin resistance via the meta-analysis.

### Table 2: Distribution of rs3842788 genotype in COX-1 gene in patients and controls.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>AR</th>
<th>AS</th>
<th>P-value</th>
<th>Allelic frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ping LL (2014)</td>
<td>2</td>
<td>16</td>
<td>0.045</td>
<td>0.950/0.050</td>
</tr>
<tr>
<td>XU JJ (2017)</td>
<td>140</td>
<td>26</td>
<td>0.887</td>
<td>0.915/0.085</td>
</tr>
<tr>
<td>XUE M (2016)</td>
<td>42</td>
<td>9</td>
<td>0.016</td>
<td>0.954/0.046</td>
</tr>
<tr>
<td>Wang B (2009)</td>
<td>172</td>
<td>29</td>
<td>0.22</td>
<td>0.914/0.086</td>
</tr>
<tr>
<td>ZHANG SS (2015)</td>
<td>1</td>
<td>8</td>
<td>0.045</td>
<td>0.950/0.050</td>
</tr>
<tr>
<td>CAO LP (2014)</td>
<td>56</td>
<td>11</td>
<td>0.457</td>
<td>0.934/0.066</td>
</tr>
</tbody>
</table>

RS3842788

In this systematic review and meta-analysis of six studies and 1940 subjects, there is no consistent association between a SNP (rs3842788) on COX-1 and the resistance of aspirin.

One possible explanation for the negative association is that encoded Glutamine does not change because of the polymorphism. Since the mutant site is the third position of the codon, the mutation from G to A is synonymous.

Moreover, from the data shown in Table 2, we found that Homozygous mutation is rare, which might potentially reduce the effect of rs3842788. One of the reasons that some studies suggested positive correlation between this SNP and AR is the change of Motif structure [8].
RS1330344

In this systematical review and meta-analysis of five studies and 1958 subjects, there is a significant association between rs1330344 and aspirin resistance. The possible reason is that rs1330344 locate in the promoter region of the COX-1 gene, and the GG type promotes the aggregation of platelets by increasing the expression of COX-1 proteins and produces phenomenon of aspirin resistance [6]. The study shows that the average frequency of G alleles is 40%, and that the distribution frequencies of rs1330344 in Japanese and Chinese are the same, so the rs1330344 may be a marker SNP for aspirin resistance in Asians 14.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>AR</th>
<th>AS</th>
<th>Allelic frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huang JJ (2013)</td>
<td>13</td>
<td>53</td>
<td>CG: 0.411/0.589</td>
</tr>
<tr>
<td>Fan L (2012)</td>
<td>65</td>
<td>135</td>
<td>GA+AA: 0.405/0.595</td>
</tr>
<tr>
<td>Li XL (2012)</td>
<td>17</td>
<td>42</td>
<td>0.399/0.601</td>
</tr>
<tr>
<td>Cao LP (2014)</td>
<td>25</td>
<td>42</td>
<td>0.385/0.615</td>
</tr>
<tr>
<td>Li X (2016)</td>
<td>6</td>
<td>19</td>
<td>0.407/0.593</td>
</tr>
</tbody>
</table>

Table 3: Distribution of rs1330344 genotype in COX-1 gene in patients and controls.

Strengths and limitations of the study

Aspirin is a common drug in the treatment of cardiovascular and cerebrovascular diseases, but a phenomenon called "aspirin resistance" inhibits the therapeutic effect of aspirin. Previously, the association of aspirin resistance and genetic polymorphism was analyzed by a collection of clinical trials, but the results of these studies were sometimes inconsistent, and the sample size too small [12]. Therefore, we conducted a meta-analysis to combine results from different studies and did a comprehensive summary analysis. Despite the significance of our study, the meta-analysis has several potential limitations. First of all, although we have searched for all the published literature, the sample size is still relatively limited so that subgroup analyses cannot be taken into consideration. Second, since we only retrieved Chinese and English literatures, sample selection bias could not be excluded. At the same time, all the studied subjects are Chinese, while the occurrence of aspirin resistance may have diversity between Chinese and other ethnicities. Third, sometimes COX-2 is also associated with AR. Therefore, the implication of the interaction between different genes that may lead to aspirin resistance cannot be assessed. These possible explanations indicated the need for further research.

Conclusions

In spite of these limitations, the study established that rs1330344 in COX-1 is associated with aspirin resistance directly while rs3842788 isn’t in Chinese populations. This meta-analysis offers contribution to the further study of genetic mutations to aspirin resistance. Along with the proposal of the Human Genome Project and the development of gene-sequencing technology, practitioners could find more effective therapy for individuals based on their genetic characteristics. This kind of method that connects individual's genes with disease treatment and prevention is defined as precision medicine. This meta-analysis provides a basis for the doctor’s diagnose aspirin resistance.

Acknowledgement

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References