P2Y12 Polymorphisms and the Risk of Adverse Clinical Events in Patients Treated with Clopidogrel: A Meta-Analysis

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ABSTRACT

Background and study aim Some studies have reported an association between P2Y12 gene polymorphisms and clopidogrel adverse outcomes with inconsistent results. We aimed to explore the relationship between P2Y12 polymorphisms and the risk of adverse clinical events in patients treated with clopidogrel through a meta-analysis.

Methods A systematic search of PubMed, Web of Science and the Cochrane Library was conducted. Retrieved articles were comprehensively reviewed and eligible studies were included, and the relevant data was extracted for this meta-analysis. All statistical tests were performed by the Review Manager 5.3 software.

Results A total of 14 studies involving 8,698 patients were included. In the Han Chinese population, ischemic events were associated with P2Y12 T744C polymorphism in the CC vs TT + CT genetic model (OR = 3.32, 95%CI = 1.62-6.82, P = 0.001), and the events were associated with P2Y12 C34T polymorphism in the TT + TC vs CC genetic model (OR = 1.70, 95%CI = 1.22-2.36, P = 0.002). However, ischemic events were not related to P2Y12 G52T polymorphism (TT + TG vs GG: OR = 1.13, 95%CI = 0.76-1.68, P = 0.56; TT vs GG + TG: OR = 2.02, 95%CI = 0.65-6.28, P = 0.22). The associations between the P2Y12 polymorphism and ischemic events were not significant in T744C, G52T and C34T genotype for another subgroup of the Caucasian population (P > 0.05). Only two studies referring to bleeding events were included in this analysis of C34T polymorphism, and no significant association was found (TT + TC vs CC: OR = 1.07, 95%CI = 0.65-1.74, P = 0.90).

Conclusions In the Caucasian population, P2Y12 gene polymorphisms are not associated with clinical events. However, in the Chinese Han population, P2Y12 T744C and C34T polymorphisms are significantly associated with adverse clinical events.


Supporting Information for this article is available online at http://www.thieme-connect.de/products
Introduction

Clopidogrel is an important antiplatelet drug, inhibiting the adenosine diphosphate (ADP)-induced platelet aggregation. Aspirin plus clopidogrel are recommended for the treatment and prevention of cerebrovascular and cardiovascular diseases[1]. Clopidogrel has been widely used for ischemic stroke (IS) and acute coronary syndrome (ACS), particularly in patients undergoing percutaneous coronary intervention (PCI)[2]. However, some patients still have adverse clinical outcomes with clopidogrel treatment, including composite ischemic events [transient ischemic attack (TIA), myocardial infarction (MI), target vessel revascularization (TVR), stent thrombosis (ST), vascular-related mortality, etc.] and bleeding events[3–5].

Genetic polymorphisms play a vital role in the clopidogrel response variability[6]. Clopidogrel inhibits the platelet P2Y12 (purinergic receptor P2Y, G-protein coupled, 12) receptor, and the receptor gene polymorphisms may be an important factor in the drug response. T744C (rs2046934), G52T (rs6809699) and C34T (rs6785930) polymorphisms have been reported frequently for the association of the P2Y12 receptor gene and adverse clinical events. Haplotype (H1/H2) could be generated by the C139T, T744C, in-s801A and G52T polymorphisms, as the four single nucleotide polymorphisms (SNPs) are in complete linkage disequilibrium. The C34T polymorphism is not linked to the four SNPs[7].

A number of studies have reported the association of P2Y12 gene polymorphisms with clopidogrel adverse clinical events. For T744C, Li XQ considered that the T allele was associated with an increased risk of major adverse cardiac events[8]. While Sun B showed that the impact of T744C polymorphism on the recurrence of ischemic events was not significant[9]. Studies of other mutation alleles had similarly inconsistent results. This led us to conduct a meta-analysis of the published studies, and we aimed to systematically identify the association of P2Y12 gene polymorphisms with the risk of adverse clinical events in clopidogrel treated patients.

Materials and Methods

Literature search

The electronic databases of PubMed, Web of Science and the Cochrane Library were searched. Search terms were “P2RY12 OR P2Y12” AND “clopidogrel OR antiplatelet OR platelet” AND “single nucleotide polymorphisms OR SNP OR polymorphism OR variant OR variation”. All eligible studies were retrieved by full texts on March 23, 2018. The bibliographies of the included articles were searched to identify other pertinent articles.

Inclusion and exclusion criteria

The inclusion criteria were: (1) published in English, (2) case-control studies, (3) patients with cardiovascular or cerebrovascular diseases, (4) patients receiving a maintenance dose 75 mg/day of clopidogrel and (5) with complete data about P2Y12 genotyping and adverse clinical events. Animal trials, case reports, reviews, studies with incomplete data about P2Y12 gene and adverse clinical events, conference papers, and studies with a follow-up < 3 months were excluded.

Data extraction

Two reviewers independently screened all retrieved studies by titles and abstracts, and then by the full texts. Eligible studies were obtained by using the inclusion and exclusion criteria. Any disagreements were resolved by discussion. Standardized forms were used for data extraction, including the first author of a study, publication year, country, race, studied population, treatment protocol, sample size, P2Y12 genotype, follow-up time, and all adverse clinical outcomes. The Newcastle-Ottawa Scale (NOS) was used for assessing the quality of the included studies[10].

Statistical analysis

All statistical tests for this meta-analysis were performed by the Review Manager 5.3 (The Cochrane Collaboration, Oxford, UK). The odds ratio (OR) with 95% confidence interval (CI) was used to assess the strength of the association between P2Y12 polymorphisms and adverse clinical events in clopidogrel-treated patients. The test for heterogeneity was performed for each meta-analysis. The fixed-effects model (FE) was adopted if there was no statistical difference (P > 0.1, I² < 50 %), otherwise the random-effects model (RE) was chosen[11]. Sensitivity analyses were conducted by an individual exclusion of each study to assess its effect on the pooled outcome OR[12]. The publication bias was assessed by the funnel plot. P < 0.05 indicates a significant difference among different genotypes[13].

Results

Study characteristics

A total of 668 articles were identified through database searching, of which 210 duplicate articles were excluded. Additionally, 434 articles (238 irrelevant studies, 119 reviews, 8 conference papers, 59 studies without adverse events, 4 animal trials, 4 case reports, and 2 non-English-language study) were excluded by title and abstract screening. Then, six studies[14–18] with incomplete data, one study[19] with incorrect data, one study[20] with a follow-up < 3 months and one study[21] without full-text were excluded respectively. Fifteen studies[8, 9, 22–34] met the inclusion criteria. However, one study[26] was not included, because the genotypes of all recruited population were of homozygous wild-type (GG).

A total of 14 studies were included in this meta-analysis (Supporting Material 1), including 8,698 patients. All patients received aspirin in these studies with pre-specified ischemic or bleeding events. The NOS value of each article is greater than 5. The baseline characteristics (race, patient population, treatment protocol, sample size, genetic locus, follow-up period, outcomes, NOS score etc.) of the studies are presented in the Supporting Material 2.

Meta-analysis results

Association of the P2Y12 T744C polymorphism with ischemic events.

Eight of the fourteen included studies reported an association between the P2Y12 T744C polymorphism and adverse clinical events in clopidogrel-treated patients. In the CC + CT vs TT genetic model, a total of 4,348 patients were enrolled: 435 had ischemic events and 1,329 were carriers of the C allele. In another genetic model (CC vs
TT + CT), seven studies including 4,012 patients were analyzed. The heterogeneity test was performed in two different genotype genetic models (CC + CT vs TT: \( P = 0.03, I^2 = 54\% \) and CC vs TT + CT: \( P = 0.42, I^2 = 1\% \)), and the random-effects model and the fixed-effects model was respectively used for this analysis (▶ Fig. 1). In the analysis of CC + CT vs TT genetic model, no significant association was found between the P2Y12 T744C polymorphism and ischemic events either in the Han Chinese population (OR = 1.59, 95% CI = 0.88-2.84, \( P = 0.12 \)) or in the Caucasian population (OR = 0.99, 95% CI = 0.75-1.31, \( P = 0.95 \)). However, in another analysis of CC vs TT + CT genetic model, the correlation between the P2Y12 T744C polymorphism and ischemic events was significant in the Han Chinese population (OR = 3.32, 95% CI = 1.62-6.82, \( P = 0.001 \)), but it was not significant in the Caucasian population (OR = 1.42, 95% CI = 0.71-2.83, \( P = 0.32 \)).
Association of the P2Y12 G52T polymorphism with ischemic events.

Five studies reported an association of the P2Y12 G52T polymorphism with adverse clinical outcomes. Only one study was conducted in the Caucasian population, and the other four studies were in the Han Chinese population. Of the 3327 subjects, 443 had ischemic events, and 865 were carriers of the T allele in the TT + TG vs GG genetic model. However, four studies with complete data were enrolled in another genetic model, including a total of 2,829 subjects. The fixed-effects model was performed because a lower heterogeneity was identified ($I^2 = 42\%$ and $I^2 = 0\%$) in different genotype genetic models (Fig. 2). The analysis indicated that ischemic events were not associated with the P2Y12 G52T polymorphism (TT + TG vs GG: OR = 1.13, 95% CI = 0.76-1.68, $P = 0.56$; TT vs GG + TG: OR = 2.02, 95% CI = 0.65-6.28, $P = 0.22$) in the Han Chinese population.

Association of the P2Y12 C34T polymorphism with ischemic events

Seven studies reporting an association of the P2Y12 C34T polymorphism with ischemic events were included. A total of 4,176 patients were enrolled in the TT + TC vs CC genetic model, while only four studies provided complete information in the TT vs CC + TC genetic model.
The fixed-effects model and the random-effects model were adopted respectively after heterogeneity testing (Fig. 3).

In the Han Chinese population, a significant association between the P2Y12 C34T polymorphism and ischemic events was found under the TT + TC vs CC genetic model (OR = 1.70, 95%CI = 1.22-2.36, P = 0.002). However, the association was not found in the Caucasian population (OR = 0.92, 95%CI = 0.73-1.16, P = 0.46). Meanwhile, the P2Y12 C34T polymorphism was unrelated to ischemic events under the TT vs CC + TC genetic model in both populations.

Association of the P2Y12 C34T polymorphism with bleeding events

Two studies referring to bleeding events were included in the analysis of the C34T polymorphism. The association of the P2Y12 C34T polymorphism with bleeding events was analyzed under the TT + TC vs CC genetic model. The random-effects model was chosen on the basis of a higher heterogeneity (I^2 = 82 %, Fig. 4). No significant association was found in this analysis (OR = 1.07, 95%CI = 0.37-3.15, P = 0.90).

### Table 1: Meta-analysis of the P2Y12 C34T polymorphism and ischemic events under two genotype genetic models.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>TT + TC</th>
<th>CC</th>
<th>Total</th>
<th>Events</th>
<th>M-H, Random</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li MN 2017</td>
<td>34</td>
<td>51</td>
<td>105</td>
<td>34</td>
<td>1.80</td>
<td>0.62</td>
<td>0.37-3.13</td>
<td>0.46</td>
</tr>
<tr>
<td>Lin YJ 2014</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>4</td>
<td>0.80</td>
<td>0.36</td>
<td>0.11-3.21</td>
<td>0.46</td>
</tr>
<tr>
<td>Ou W 2016</td>
<td>14</td>
<td>31</td>
<td>45</td>
<td>14</td>
<td>1.17</td>
<td>0.49</td>
<td>0.27-4.39</td>
<td>0.27</td>
</tr>
<tr>
<td>Tang XF 2013</td>
<td>13</td>
<td>220</td>
<td>233</td>
<td>13</td>
<td>2.43</td>
<td>1.02</td>
<td>0.58-2.90</td>
<td>0.78</td>
</tr>
<tr>
<td>Zhang JH 2015</td>
<td>19</td>
<td>215</td>
<td>234</td>
<td>19</td>
<td>1.76</td>
<td>0.87</td>
<td>0.35-5.56</td>
<td>0.56</td>
</tr>
<tr>
<td>Subtotal (95%CI)</td>
<td>672</td>
<td>1090</td>
<td>1762</td>
<td>672</td>
<td>1.70</td>
<td>1.22</td>
<td>2.36</td>
<td>0.002</td>
</tr>
<tr>
<td>Total events</td>
<td>85</td>
<td>97</td>
<td>182</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterogeneity: Tau^2 = 0.00; Chi^2 = 2.70, df = 4 (P = 0.61); I^2 = 0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Test for overall effect: Z = 3.16 (P = 0.002)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3.1.2 Caucasian</th>
<th>TT + TC</th>
<th>CC</th>
<th>Total</th>
<th>Events</th>
<th>M-H, Random</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Siasos G 2016</td>
<td>25</td>
<td>124</td>
<td>149</td>
<td>25</td>
<td>1.22</td>
<td>0.62</td>
<td>0.37-2.39</td>
<td>0.46</td>
</tr>
<tr>
<td>Simon T 2009</td>
<td>138</td>
<td>1107</td>
<td>1245</td>
<td>138</td>
<td>0.88</td>
<td>0.69</td>
<td>0.46-1.43</td>
<td>0.13</td>
</tr>
<tr>
<td>Subtotal (95%CI)</td>
<td>1231</td>
<td>1183</td>
<td>2414</td>
<td>1231</td>
<td>0.92</td>
<td>0.73</td>
<td>1.16</td>
<td>0.61</td>
</tr>
<tr>
<td>Total events</td>
<td>163</td>
<td>168</td>
<td>331</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterogeneity: Tau^2 = 0.00; Chi^2 = 0.80, df = 1 (P = 0.37); I^2 = 0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Test for overall effect: Z = 0.74 (P = 0.46)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Total (95%CI)      | 1903    | 2273| 4176  |        | 1.33        | 0.95       | 1.88    | 0.002 |
| Total events       | 248     | 265 | 513   |        |             |            |        |    |
| Heterogeneity: Tau^2 = 0.10; Chi^2 = 12.52, df = 6 (P = 0.05); I^2 = 52% |
| Test for overall effect: Z = 1.55 (P = 0.10) |
| Test for suborobous differences: Chi^2 = 9.02, df = 1 (P = 0.003); I^2 = 88.9% |

### Fig. 3: Meta-analysis of the P2Y12 C34T polymorphism and ischemic events under two genotype genetic models. a: TT + TC vs CC model; b: TT vs CC + TC model.
The P2Y12 gene is located in the q25.1 region of chromosome 3, and it belongs to the G-protein coupled receptor family. The coupling of the P2Y12 protein with the inhibitory G alpha protein subunit results in the inhibition of cyclic adenosine monophosphate (cAMP) production, and therefore a subsequent reduction in the intracellular cAMP content. Furthermore, P2Y12 receptor induces a platelet aggregation through a weak activation of glycoprotein IIb/IIIa integrin via the phosphoinositide kinase-3 pathway [7]. Platelet activation by ADP plays a crucial role in hemostasis and thrombosis. Numerous studies have established the key role of this receptor in the ADP-dependent amplification of platelet aggregation induced by other agonists, such as thromboxane A2 and thrombin [38, 39]. The antithrombotic effect of clopidogrel and the ADP-induced platelet response are considerably variable. Therefore, the P2Y12 gene is screened for possible variants.

Many studies investigated the relationship of the P2Y12 gene polymorphism and adverse clinical events, but these studies [8, 9, 22–34] had divergent results. It is therefore necessary to perform a meta-analysis exploring this association. A total of 14 articles were included, and the NOS value of each article is greater than 5. Three genotypes (T744C, G52T and C34T) of the P2Y12 gene were analyzed in our study.

T744C is located at the 744 nucleotide after the 5′ intron start site and consists of a T-to-C transition [7]. Four frequent polymorphisms (i-C139T (rs6809699), i-T744C (rs2046934), i-ins801A (rs5853517), G52T (rs6809699)) were in total linkage disequilibrium, determining haplotypes H1 and H2, with respective allelic frequencies of 0.86 and 0.14. The presence of the minor haplotype H2 was associated with an enhanced ADP-induced platelet aggregation in healthy volunteers. The molecular mechanism by which the H2 haplotype increases ADP-induced platelet aggregation remains unclear. Based on the investigated sequence, an amino acid substitution affecting the protein structure or splice variants could be ruled out. Thus, an increase in the number of platelet surface receptors is most likely the explanation [7]. C34T is not part of the haplotype and no functional research was found until now.

Based on the analysis of subgroups, significant associations between the P2Y12 T744C/C34T polymorphism and ischemic events were found (P < 0.05), but the association of the P2Y12 G52T polymorphism and ischemic events is not observed (P > 0.05) in the Han Chinese population. Ischemic events are related to the P2Y12 T744C polymorphism under the CC vs TT + CT genetic model (P = 0.001), therefore the T744C genotype may predict the risk of ischemic events in the Han Chinese population.
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The P2Y12 T744C and C34T polymorphisms are found to be associated with ischemic events in Han Chinese patients treated with clopidogrel, and the P2Y12 G52T polymorphism has no significant impact on ischemic events. Patients with the CC homozygote of the T744C genotype have a higher risk of recurrent ischemic events, and the carriers of the mutant T allele of the C34T genotype are more likely than non-carriers to have ischemic events. However, the risk of clinical ischemic events is not related to P2Y12 T744C, G52T and C34T polymorphisms in the Caucasian population. Bleeding events are not significantly associated with the P2Y12 C34T polymorphism in the Han Chinese population. Further research on the relationship of other genetic factors of P2Y12 and the risks of bleeding events in patients treated with clopidogrel is needed.

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Conflict of Interest

No conflict of interest has been declared by the author(s).

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