Association of CRP gene rs1130864 polymorphism with ischemic stroke and coronary artery disease: a meta-analysis

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Abstract: Objective: CRP is a most commonly used inflammatory marker with atherosclerotic lesions and during the ischemic event. The reported results of the association of SNPs of CRP gene with ischemic stroke and coronary artery disease are not always consistent. We aimed to examine the association of rs1130864 polymorphism with risk of ischemic stroke and coronary artery disease. Methods: We searched human case-control studies in PubMed, Embase, Google Scholar, China National Knowledge Infrastructure and Wanfang database date to April 2017, then summarized the data and used Odds ratios (ORs) and 95% confidence intervals (CIs) to evaluate risk of ischemic stroke and coronary artery disease. We also performed subgroup analyses, sensitivity analyses and publication bias. Results: A total of 11 eligible articles were analyzed. We found rs1130864 polymorphism was not associated with risk of ischemic stroke in dominant model (OR=0.974, 95% CI=0.873-1.086), recessive model (OR=0.886, 95% CI=0.726-1.082), homozygous model (OR=0.889, 95% CI=0.723-1.094), heterozygous model (OR=0.992, 95% CI=0.885-1.111), and allelic model (OR=0.968, 95% CI=0.891-1.052). No significant association between rs1130864 polymorphism and coronary artery disease risk was observed in dominant model (OR=1.018, 95% CI=0.941-1.102), recessive model (OR=1.035, 95% CI=0.908-1.180), homozygous model (OR=1.040, 95% CI=0.907-1.193), heterozygous model (OR=1.016, 95% CI=0.935-1.104), and allelic model (OR=1.007, 95% CI=0.947-1.070). Subgroup analysis suggested no relationship between rs1130864 polymorphism and ischemic stroke and coronary artery disease risk was found either in Asian or Caucasian population. Conclusions: This meta-analysis indicated that the CRP gene rs1130864 variant examined may not modulate ischemic stroke and coronary artery disease risk. However, further studies with larger sample sizes and gene-gene interactions as well as gene-environment interactions are warranted.

Keywords: Coronary artery disease, CRP gene, ischemic stroke, meta-analysis, polymorphism

Introduction

Both ischemic stroke (IS) and coronary artery disease (CAD) are considered multiple-factorial diseases, and share risk factors including genetic and environmental factors. In clinical practice, conventional risk factors for IS and CAD can be prevented by changing their lifestyle in most cases [1]. In addition, genetic factors play a vital role in IS and CAD susceptibility and many candidate genes have been reported for their potential roles in IS and CAD, such as ZNF208 gene, GLO1 gene, and CX3CL1 gene [2-4]. However, the recognition on the heritability of IS and CAD remains quite limited.

C-reactive protein (CRP), plays a significant role in acute and chronic inflammation [5]. CRP is a biomarker for atherothrombotic disease and may reflect the level of inflammatory activity within atherosclerotic plaques [6]. Plasma CRP levels are under genetic influence [7]. CRP gene variations may act alone or synergistically to increase risk for IS and CAD, due to its involvement in the regulation of CRP levels. However, Meta-analyses demonstrated little evidence to support a role of CRP gene rs2794521, rs1205, and rs1800947 polymorphisms in IS [8, 9] or CAD risk [10].

It is well-known that rs1130864 locates in the 3’ untranslated region of the CRP gene and the
major allele of rs1130864 has been associated with lower circulating CRP levels [11]. CRP gene rs1130864 variation is proposed as a possible genetic biomarker, the T allele of rs1130864 is suggested to be used to predict IS and CAD risk [12, 13, 18], as well as post-stroke functional outcome [14]. However, other studies did not find this polymorphism playing a role in IS and CAD risk [15, 17, 19, 36]. For the above-mentioned inconsistent reports, we performed a meta-analysis to determine whether the risks of IS and CAD were associated with rs1130864 polymorphism.

Methods

Literature retrieval strategy

This meta-analysis was according to the recommendations of the PRISMA statement, and ethical approval was not required. To identify eligible studies on the association of rs1130864 polymorphism and risk of IS and CAD, we performed a literature search of published studies in PubMed, Embase, Google Scholar, China National Knowledge Infrastructure and Wanfang database from 1980 to April 2017. The terms we used are as follows: C-reactive protein or CRP, combined with polymorphism or genetic variants or mutation, as well as ischemic stroke or cerebral infarction or stroke or coronary artery disease or coronary heart disease or ischemic heart disease. Besides, references of all identified studies were inspected for further research.

Selected criteria

Studies were included in the meta-analysis if they met all of the following criteria: (1) used a case-control design with healthy individuals as controls; (2) investigated the association of rs1130864 polymorphism with IS and/or CAD risk; (3) could calculate an odds ratio (OR) and 95% confidence intervals (CI) by crude data; (4) restricted the publication language in English and/or Chinese. The exclusion criteria were as follows: (1) case reports, duplicate studies, comments or animal model studies; (2) studies without detailed genotype frequency distribution; (3) studies focus on IS and CAD recurrence or pharmacogenetics.

Data extraction

Two investigators (Z.F. and G.T.) independently extracted information from each study. All authors discussed to reach a consensus when disagreements arise. Main characteristics including the following data: first author, publication year, subjects’ locations, number of cases and controls, genotyping methods, Hardy-Weinberg equilibrium (HWE) in controls, and allele and genotype frequencies. If genotype frequencies were not available in the text of the respective publications, we would contact the authors of primary articles by email. Newcastle-Ottawa Scale (NOS) scores were used to assess the quality of included studies, in which NOS scores > 5 shows a good quality.

Statistical analysis

The HWE of genotype distribution of the controls for all studies was tested using the Chi-square test. Crude ORs with 95% CI were performed to evaluate the relationship between rs1130864 and IS and CAD risk. Pooled ORs were calculated according to the following genetic models: allele (T/C), homozygote (TT/CC), heterozygous (CT/CC), dominant (TT+CT/CC), and recessive model (TT/CT+CC).

The Chi-square-based Q test and the I² metric were served to test heterogeneity. If p > 0.10 or...
rs1130864 polymorphism and risk of IS and CAD

Table 1. Characteristics of studies included in the meta-analysis for the association of rs1130864 polymorphism with the risk of IS or CAD

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Ethnic group</th>
<th>Nation</th>
<th>Disease</th>
<th>Genotyping Methods</th>
<th>Cases, CC/CT/TT</th>
<th>Controls, CC/CT/TT</th>
<th>NOS score</th>
<th>P_{HWE}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Du et al [15]</td>
<td>2015</td>
<td>Asian</td>
<td>China</td>
<td>IS</td>
<td>PCR-RFLP</td>
<td>142/16/0</td>
<td>248/42/0</td>
<td>6</td>
<td>0.184</td>
</tr>
<tr>
<td>Andersson et al [34]</td>
<td>2009</td>
<td>Caucasian</td>
<td>Sweden</td>
<td>IS</td>
<td>TaqMan</td>
<td>139/111/17</td>
<td>284/214/46</td>
<td>7</td>
<td>0.529</td>
</tr>
<tr>
<td>Zacho et al [35]</td>
<td>2008</td>
<td>Caucasian</td>
<td>Denmark</td>
<td>IS</td>
<td>TaqMan</td>
<td>356/319/66</td>
<td>4550/4036/949</td>
<td>7</td>
<td>0.218</td>
</tr>
<tr>
<td>Huang et al [19]</td>
<td>2016</td>
<td>Asian</td>
<td>China</td>
<td>IS</td>
<td>qPCR</td>
<td>69/27/4</td>
<td>74/30/2</td>
<td>6</td>
<td>0.600</td>
</tr>
<tr>
<td>Morita et al [13]</td>
<td>2006</td>
<td>Asian</td>
<td>Japan</td>
<td>IS</td>
<td>TaqMan</td>
<td>132/19/1</td>
<td>265/38/1</td>
<td>7</td>
<td>0.768</td>
</tr>
<tr>
<td>Ladenvall et al [29]</td>
<td>2006</td>
<td>Caucasian</td>
<td>Sweden</td>
<td>IS</td>
<td>TaqMan</td>
<td>287/257/56</td>
<td>281/258/61</td>
<td>6</td>
<td>0.875</td>
</tr>
<tr>
<td>Brull et al [18]</td>
<td>2003</td>
<td>Caucasian</td>
<td>UK</td>
<td>CAD</td>
<td>PCR-RFLP</td>
<td>86/78/22</td>
<td>122/92/13</td>
<td>7</td>
<td>0.420</td>
</tr>
<tr>
<td>Grammar et al [16]</td>
<td>2008</td>
<td>Caucasian</td>
<td>Germany</td>
<td>CAD</td>
<td>PCR-RFLP</td>
<td>1150/1124/281</td>
<td>305/316/76</td>
<td>8</td>
<td>0.664</td>
</tr>
<tr>
<td>Pai (1) et al [17]</td>
<td>2008</td>
<td>Caucasian</td>
<td>USA</td>
<td>CAD</td>
<td>TaqMan</td>
<td>125/94/18</td>
<td>238/178/51</td>
<td>7</td>
<td>0.047</td>
</tr>
<tr>
<td>Pai (2) et al [17]</td>
<td>2008</td>
<td>Caucasian</td>
<td>USA</td>
<td>CAD</td>
<td>TaqMan</td>
<td>126/98/24</td>
<td>235/205/52</td>
<td>7</td>
<td>0.466</td>
</tr>
<tr>
<td>Yan et al [36]</td>
<td>2006</td>
<td>Asian</td>
<td>China</td>
<td>CAD</td>
<td>PCR</td>
<td>114/14/0</td>
<td>107/12/0</td>
<td>7</td>
<td>0.562</td>
</tr>
<tr>
<td>Zacho et al [35]</td>
<td>2008</td>
<td>Caucasian</td>
<td>Denmark</td>
<td>CAD</td>
<td>TaqMan</td>
<td>835/767/184</td>
<td>407/3588/831</td>
<td>7</td>
<td>0.324</td>
</tr>
<tr>
<td>Sun et al [37]</td>
<td>2011</td>
<td>Asian</td>
<td>China</td>
<td>CAD</td>
<td>PCR-RFLP</td>
<td>84/13/1</td>
<td>111/13/1</td>
<td>6</td>
<td>0.383</td>
</tr>
</tbody>
</table>

Abbreviations: CAD, coronary artery disease; HWE, Hardy-Weinberg equilibrium; IS, ischemic stroke; NOS, Newcastle-Ottawa scale; PCR-RFLP, polymerase chain reactions restriction fragment length polymorphism.

Results

Selection and characteristics of studies

Study selection process is graphically represented in Figure 1. An initial screening of 224 records matching the search terms were obtained, of which 207 were discarded owing to various reasons and 17 potential articles were identified through carefully reading the titles and abstracts. Of the 17 studies, 1 study did not show baseline age and gender, as well as genotype method; 4 studies were repeated researches; and 1 study did not show genotype frequencies. Therefore, these 6 studies were excluded according to the criteria. Finally, leaving 11 eligible articles met the inclusion criteria and thus were included in the meta-analysis.

The main information of the eligible studies is summarized in Table 1. There were 2024 IS cases and 11373 controls from 6 case-control studies to estimate the relationship between rs1130864 polymorphism and IS risk, and 5238 CAD cases and 10617 controls from 7 case-control studies for CAD risk. Deviation from HWE was identified in the genotype distribution of 1 study [17]. Moreover, there was well homogeneity in genotyping measurement, matching age and gender across studies.

Meta-analysis

Table 2 shows the principal results of fixed-effect or random-effect meta-analysis. In general, we did not find any association of rs1130864 polymorphism with IS risk in dominant model (OR=0.974, 95% CI=0.873-1.086), recessive model (OR=0.886, 95% CI=0.726-1.082), homozygous model (OR=0.889, 95% CI=0.723-1.104), heterozygous model (OR=0.992, 95% CI=0.885-1.111), and allelic model (OR=0.968, 95% CI=0.891-1.104). Subgroup analysis by ethnicity and HWE deviation suggested a similar non-significant trend toward IS.
rs1130864 polymorphism and risk of IS and CAD

Table 2. Association of rs1130864 polymorphism with risk of ischemic stroke and coronary artery disease

<table>
<thead>
<tr>
<th>Genetic model</th>
<th>Overall and subgroups</th>
<th>Ischemic stroke</th>
<th>Coronary artery disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>T/C</td>
<td>Overall</td>
<td>6</td>
<td>0.968 (0.891-1.052)</td>
</tr>
<tr>
<td></td>
<td>All in HWE</td>
<td>6</td>
<td>0.968 (0.891-1.052)</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>3</td>
<td>0.982 (0.901-1.071)</td>
</tr>
<tr>
<td></td>
<td>Asian</td>
<td>3</td>
<td>0.805 (0.588-1.103)</td>
</tr>
<tr>
<td>TT/CC</td>
<td>Overall</td>
<td>6</td>
<td>0.889 (0.723-1.094)</td>
</tr>
<tr>
<td></td>
<td>All in HWE</td>
<td>6</td>
<td>0.889 (0.723-1.094)</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>3</td>
<td>0.873 (0.708-1.077)</td>
</tr>
<tr>
<td></td>
<td>Asian</td>
<td>3</td>
<td>2.109 (0.486-9.156)</td>
</tr>
<tr>
<td>CT/CC</td>
<td>Overall</td>
<td>6</td>
<td>0.992 (0.885-1.111)</td>
</tr>
<tr>
<td></td>
<td>All in HWE</td>
<td>6</td>
<td>0.992 (0.885-1.111)</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>3</td>
<td>1.009 (0.894-1.138)</td>
</tr>
<tr>
<td></td>
<td>Asian</td>
<td>3</td>
<td>0.862 (0.609-1.221)</td>
</tr>
<tr>
<td>TT+CT/CC</td>
<td>Overall</td>
<td>6</td>
<td>0.974 (0.873-1.086)</td>
</tr>
<tr>
<td></td>
<td>All in HWE</td>
<td>6</td>
<td>0.974 (0.873-1.086)</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>3</td>
<td>0.983 (0.876-1.103)</td>
</tr>
<tr>
<td></td>
<td>Asian</td>
<td>3</td>
<td>0.895 (0.637-1.259)</td>
</tr>
<tr>
<td>TT/CT+CC</td>
<td>Overall</td>
<td>6</td>
<td>0.886 (0.726-1.082)</td>
</tr>
<tr>
<td></td>
<td>All in HWE</td>
<td>6</td>
<td>0.886 (0.726-1.082)</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>3</td>
<td>0.871 (0.712-1.066)</td>
</tr>
<tr>
<td></td>
<td>Asian</td>
<td>3</td>
<td>2.125 (0.492-9.171)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HWE, Hardy-Weinberg equilibrium; N, number of studies; N/A, not applicable; OR, odds ratio; P_H, P value for heterogeneity.

and CAD risk. For IS risk meta-analysis, we observed high homogeneity (P_H > 0.05, I²=0) in all of the five genetic models. A notable homogeneity (P_H > 0.05, I² < 50%) existed in each of genetic models and subgroups analysis for CAD risk. Figure 2 shows the results of IS risk (A) and CAD risk (B) under the dominant model (TT+CT/CC).

Sensitivity analysis

We excluded one single study from the overall pooled analysis and recalculated the pooled ORs to check whether the pooled ORs were materially changed. No individual study significantly affected the recalculated ORs, indicating our results were valuable. Figure 3 shows the results of sensitivity analysis on the association between rs1130864 polymorphism and IS risk (A) and CAD risk (B) under the dominant model (TT+CT/CC).

Publication bias

Publication bias for IS and CAD risk were evaluated by Begg's test and Egger's test. Funnel plots appeared to be symmetrical and Egger's test did not show statistically significant asymmetry both for IS (dominant model, P_Egger =0.532) and CAD (dominant model, P_Egger =0.691). Figure 4 shows symmetrically distributed funnel plot of the association between rs1130864 polymorphism and IS (A) and CAD (B) under the dominant model (TT+CT/CC).

Discussion

Though alterations in plasma CRP levels have biological significance including reflection of inflammation stages and elevated CRP levels are associated with an increased risk of IS and CAD [6, 20-22]. In present meta-analysis, however, we did not find any association in five genetic models between rs1130864 polymorphism and IS risk neither in Asian nor Caucasian population. We also evaluated the association of rs1130864 polymorphism with CAD risk, and the consequences were likely to be the same.

As a well-characterized biomarker of inflammation, CRP is of importance in the initiation, pro-
rs1130864 polymorphism and risk of IS and CAD

Figure 2. Forest plot of rs1130864 polymorphism and IS risk (A) and CAD risk (B) under the dominant model (TT+CT/CC). Abbreviations: CAD, coronary artery disease; CI, confidence interval; IS, ischemic stroke; OR, odds ratio.

Figure 3. Sensitivity analysis on the association between rs1130864 polymorphism and IS risk (A) and CAD risk (B) under the dominant model (TT+CT/CC). Abbreviations: CAD, coronary artery disease; CI, confidence interval; IS, ischemic stroke.

Figure 4. Funnel plot of the association of rs1130864 polymorphism with IS risk (A) and CAD risk (B) under the dominant model (TT+CT/CC). Abbreviations: CAD, coronary artery disease; IS, ischemic stroke; OR, odds ratio.

Preservation, and clinical outcome of atherosclerosis [5], which through its complications is the leading cause of IS and CAD [21, 34]. In adults, higher CRP levels are positively associated with
larger infarction volumes in acute IS [23], but cannot help in distinguishing TOAST subtypes [24]. Moreover, CRP levels in the very early phase of acute IS is a predictor for the outcome [25]. Among children, the multicenter VIPS (Vascular effects of Infection in Pediatric Stroke) study found that higher levels of CRP were associated with increased recurrent IS [26]. These observations illustrate a clear positive relationship between CRP and ischemic events. In addition, the absolute level of CRP also influenced by age, BMI, smoking, and other clinical or environmental risk factors [7, 27, 28].

Given that SNPs of CRP gene have been showed to be significantly associate with plasma CRP levels in prior association studies [18, 25], which might lead to a significantly increased susceptibility of IS and CAD, a number of researches have been performed to assess the possible relations between CRP gene polymorphisms and IS and CAD risk. Kotlega et al [31] suggested that AA genotype of rs2794521 promotes improvement of neurological state in IS patients. Similarly, Wang et al [32] reported that the A allele frequency for rs2794521 performed a protective effect for against IS. The minor alleles of 2667C associated with lower plasma CRP concentrations in Caucasian were associated with decreased risk of cerebrovascular disease mortality [33]. These findings indicate that some of the CRP gene variations may protect against the malignant progression of IS through decreasing the plasma CRP levels. Nonetheless, several studies suggest completely opposite results. The T allele of 1919A/T is associated with plasma CRP levels and cerebrovascular disease risk in older adults [36]. Kuhlenbaeumer et al [38] suggested that rs1130864 variant was not associated with IS as a whole but strongly associated with microangiopathic stroke. The T allele of rs1130864 showed a significant association with poor functional outcome in IS patients [14], and TT homozygotes variant increases susceptibility to CAD [18].

Although several previous studies indicated that rs1130864 was associated with IS and CAD risk [13, 14, 18, 38], the majority of studies did not show associations among them in all genetic models. Ladenvall et al [29] demonstrated that rs1130864 polymorphism was associated with CRP levels but no association was detected for overall or TOAST subtypes of IS. Shi et al [10] reported that CAD risk was associated with rs2794521 polymorphism but not with rs1130864 polymorphism. In brief, the relationship between rs1130864 polymorphism and IS or CAD risk is not causal [17, 31, 34-37]. In view of these mixed findings, we performed meta-analysis, the pooled results showed that rs1130864 variant examined may not directly modulate IS and CAD risk.

Ethnicity is a well-known factor in determining the genetic variation, as different genetic backgrounds may result in various consequences. Through racial subgroup analysis, the genetic backgrounds among subgroup studies were genetically homogenous. Our results showed a notable homogeneity (P_H > 0.05, I^2 < 50%) in all of the five genetic models for IS and CAD risk. Therefore, subgroup results added credibility to the correlation between rs1130864 polymorphism and IS and CAD risk. Subsequently, we performed sensitivity analysis to evaluate the stability of the pooled effect sizes due to baseline of percentage of males and mean age are not exactly the same between-studies. No individual study significantly affected the recalculated ORs, indicating our results were valuable.

This study presents several limitations. Firstly, the pathogenesis of IS and CAD are complex and involves multiple genes, though these studies included some of the same SNPs, estimated haplotypes could not directly pool and summarize. Secondly, most of those studies containing small sample size, which reduced the statistical power. Therefore, comprehension of CRP genetic variation in IS and CAD risk remains unclear. Thirdly, clinical subtype and severity of the diseases were not studied. Lastly, the language of qualified research is limited to English and Chinese, despite the fact that some of our statistical tests do not show evidence of publication bias, some may still exist.

In conclusion, this meta-analysis indicated that rs1130864 polymorphism may not act as modifiers of IS and CAD risk. Genetic variation in such single SNP may not directly relate to the occurrence of IS and CAD since they are considered multiple-factorial diseases. To validate the results, larger scale studies on gene-gene interactions as well as gene-environment interactions are warranted.
Disclosure of conflict of interest

None.

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