Genetic polymorphisms of interleukin-6 gene and susceptibility to coronary artery disease in Chinese population: Evidence based on 4582 subjects

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A B S T R A C T

The aim of this study was to explore whether interleukin-6 (IL-6) gene (−174 G/C and −572 C/G) polymorphisms are associated with susceptibility to coronary artery disease (CAD) risk in Chinese population. All the statistical tests were performed using Stata version 11.0. Twelve articles involving 16 studies were included in this meta-analysis, covering a total of 2309 CAD cases and 2273 controls. For IL-6 gene −572 C/G polymorphism, the results showed evidence for significant association between IL-6 gene −572 C/G polymorphism and CAD risk (for G allele vs. C allele: OR = 1.48, 95% CI = 1.26–1.74, \( p < 0.001 \); for G/G vs. C/C: OR = 2.60, 95% CI = 1.54–4.39, \( p < 0.001 \); for G/G vs. G/C + C/C: OR = 2.15, 95% CI = 1.35–3.42, \( p = 0.001 \); for G/G + G/C vs. C/C: OR = 1.55, 95% CI = 1.29–1.85, \( p < 0.001 \)). However, for IL-6 gene −174 G/C polymorphism, no significant association was found between this variation and CAD risk. In summary, our meta-analysis showed evidence that IL-6 gene −572 C/G polymorphism may be a risk factor for CAD susceptibility. For IL-6 gene −174 G/C polymorphism, no significant association was found between this variation and CAD risk.

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1. Introduction

Coronary artery disease (CAD) is the most common cardiovascular disease that causes significant morbidity and mortality worldwide [1]. The World Health Organization (WHO) estimates that 17.3 million people died from this disease worldwide per year and most of these deaths occur in the developing countries, and this number will increase to 23.6 million by 2030 [1]. Despite it is well established that a poor diet, advanced age, smoking, diabetes, hypertension, and dyslipidemia are associated with increased risk of CAD, a detailed etiology underlying CAD is still obscure. It is well known that inflammatory response is an essential part of the pathogenesis of atherosclerosis [2–4], and inflammatory response has been implicated in increasing the risk of CAD [5,6]. In addition, several epidemiological studies have demonstrated that increased serum levels of inflammatory markers, such as interleukin-6 (IL-6) and interleukin-1 (IL-1), being associated with increased risk of CAD [7,8], suggesting genetic factors involved in cytokines may play an important role in the development of CAD.

IL-6 is an important proinflammatory cytokine produced by many different cells, such as adipocytes, fibroblasts, myocytes, lymphocytes, monocytes and endothelial cells. The human IL-6 gene, located on chromosome 7p21, the common single nucleotide polymorphisms (SNPs) at position −174 and −572 of the IL-6 promoter regions have been identified [9,10], and consequent evidences demonstrated that these SNPs in the promoter regions could affect IL-6 gene transcription and its secretion [9,10]. To date, a variety of molecular epidemiological studies have focused on the associations between IL-6 gene polymorphisms and CAD risk. However, results of different studies have been inconsistent. In 2012, Zheng et al. performed a meta-analysis of worldwide studies including 27 studies showed that no significant association between IL-6 gene −174 G/C polymorphism and CAD risk [13]. In addition, they also found significant association between IL-6 gene
572 C/G polymorphism and CAD risk. Considering that potential ethnic difference might be associated with the distribution of genotypes, we conducted a meta-analysis by collecting and sorting the previously published studies in Chinese population.

2. Materials and methods

2.1. Publication search

We performed this meta-analysis in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) criteria [14]. A computerized literature search was conducted for the relevant available studies from PubMed, Embase, Web of Science, Cochrane database, Clinicaltrials.gov, Current Controlled Trials, Chinese Clinical Trial Registry, CBMdisc, CNKI and Google Scholar (updated to February 12, 2014). These computer searches were limited to English and Chinese language articles. The following keywords and subject terms were used for searching: “interleukin-6” OR “IL-6” AND “polymorphism” OR “mutation” OR “variant” OR “genotype” AND “coronary artery disease” OR “CAD” OR “coronary heart disease” OR “CHD”. The equivalent Chinese terms were used in the Chinese databases. Additionally, we also screened references of the retrieved articles and review articles by a hand search.

2.2. Inclusion criteria

The studies included in the meta-analysis must meet all the following inclusion criteria: (1) human studies; (2) studies on the relationships between IL-6 gene (−174 G/C and −572 C/G) polymorphisms and CAD; (3) published case-control studies; (4) studies with full text articles; (5) sufficient data for estimating an odds ratio (OR) with 95% confidence interval (CI); (6) at least two comparison groups (CAD group vs. control group), and (7) not republished data.

2.3. Data extraction

Data was carefully extracted from all eligible publications independently by two authors (Yin YW and Sun QQ) of this article. In case of disagreement, a third author (Hu AM) examined such articles, and the disagreement was resolved until consensus was reached. For each study, data was extracted including (1) name of the first author; (2) date of publication; (3) country of origin; (4) ethnicity of the studied population; (5) source of controls; (6) sample size; (7) genotype number in cases and controls and (8) evidence of Hardy–Weinberg equilibrium (HWE, \( p < 0.05 \) was considered significant deviation from HWE).

2.4. Quality score assessment

The quality of included studies were assessed independently by two authors (Hu AM and Sun QQ) using the Newcastle–Ottawa Scale (NOS) [15]. The NOS ranges between zero (worst) up to nine stars (best). Studies with a score of seven stars or greater were considered to be of high quality. Disagreement was settled as described above.

2.5. Statistical analysis

All of the statistical tests used in the present study were performed by Stata version 11.0, which has been widely used in the meta-analysis [16,17]. The strength of associations between IL-6 gene polymorphisms and CAD risk was measured by ORs with 95% CIs. For IL-6 gene −572 C/G polymorphism, the combined ORs were respectively calculated for four genetic models (allelic model: G allele vs. C allele, additive model: G/G vs. C/C, recessive model: G/G vs. G/C + C/C, and dominant model: G/G + G/C vs. C/C). For IL-6 gene −174 G/C polymorphism, only two genetic models (allelic model: C allele vs. G allele, and codominant model: G/C vs. G/G) were used to calculate the combined ORs due to the fact that no C/C genotype was found in Chinese population. Cochran’s Q statistic and the \( I^2 \) statistic were used to assess...
Table 1
Characteristics of studies included in this meta-analysis.

<table>
<thead>
<tr>
<th>Position</th>
<th>First author</th>
<th>Year</th>
<th>Geographical location</th>
<th>Source of controls</th>
<th>Criteria for CAD</th>
<th>Sample size (case/control)</th>
<th>Genotypes distribution (case/control)</th>
<th>HWE Y/N(P)</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>−174 G/C</td>
<td>Wang [21]</td>
<td>2005</td>
<td>Hubei PB</td>
<td>CA</td>
<td>50/60</td>
<td>G/G 50/58 , G/C 0/2 , C/C 0/0</td>
<td>100/118 , G 0/2 , C 0/1</td>
<td>Y(0.896) 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Li [22]</td>
<td>2005</td>
<td>Hubei PB</td>
<td>1979 WHO</td>
<td>199/189</td>
<td>197/185 , 2/4 , 0/0</td>
<td>396/374 , G/C 2/4 , C/C 0/1</td>
<td>Y(0.883) 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fan [24]</td>
<td>2010</td>
<td>Guangdong HB</td>
<td>CA</td>
<td>174/130</td>
<td>173/129 , 1/1 , 0/0</td>
<td>347/259 , G/C 1/1 , C/C 0/1</td>
<td>Y(0.959) 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liu [25]</td>
<td>2011</td>
<td>Shaxi PB</td>
<td>1979 WHO, CA</td>
<td>126/150</td>
<td>123/148 , 3/2 , 0/0</td>
<td>249/298 , G/C 3/2 , C/C 0/1</td>
<td>Y(0.904) 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tong [32]</td>
<td>2013</td>
<td>Beijing PB</td>
<td>CA</td>
<td>326/341</td>
<td>201/220 , 87/98 , 38/23</td>
<td>489/538 , G/C 163/144 , C/C 0/1</td>
<td>N(0.011) 8</td>
<td></td>
</tr>
<tr>
<td>−572 C/G</td>
<td>Wei [26]</td>
<td>2005</td>
<td>Guangxi PB</td>
<td>1979 WHO</td>
<td>128/145</td>
<td>6/1 , 50/43 , 2/101</td>
<td>62/45 , G/C 194/245 , C/C 0/1</td>
<td>Y(0.115) 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fu [27]</td>
<td>2006</td>
<td>Henan PB</td>
<td>CA</td>
<td>245/260</td>
<td>16/4 , 101/90 , 128/166</td>
<td>133/98 , G/C 357/422 , C/C 0/1</td>
<td>N(0.034) 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zhao [28]</td>
<td>2006</td>
<td>Henan PB</td>
<td>CA</td>
<td>157/168</td>
<td>9/2 , 67/60 , 81/106</td>
<td>85/64 , G/C 229/272 , C/C 0/1</td>
<td>N(0.040) 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liu [23]</td>
<td>2007</td>
<td>Jilin PB</td>
<td>1979 WHO, CA</td>
<td>90/95</td>
<td>2/0 , 39/26 , 49/68</td>
<td>43/26 , G/C 137/162 , C/C 0/1</td>
<td>Y(0.120) 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gao [29]</td>
<td>2008</td>
<td>Jiangsu PB</td>
<td>1979 WHO, CA</td>
<td>126/108</td>
<td>10/4 , 51/32 , 65/72</td>
<td>71/40 , G/C 181/176 , C/C 0/1</td>
<td>Y(0.850) 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liang [30]</td>
<td>2010</td>
<td>Shaxi PB</td>
<td>CA</td>
<td>434/417</td>
<td>14/8 , 161/126 , 259/283</td>
<td>189/142 , G/C 679/692 , C/C 0/1</td>
<td>Y(0.156) 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jia [31]</td>
<td>2010</td>
<td>Beijing PB</td>
<td>CA</td>
<td>231/210</td>
<td>22/15 , 130/107 , 79/88</td>
<td>174/137 , G/C 288/283 , C/C 0/1</td>
<td>N(0.021) 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fan [24]</td>
<td>2010</td>
<td>Guangdong HB</td>
<td>CA</td>
<td>197/130</td>
<td>10/3 , 101/95 , 101/136</td>
<td>174/137 , G/C 293/222 , C/C 0/1</td>
<td>Y(0.873) 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liu [25]</td>
<td>2011</td>
<td>Shaxi PB</td>
<td>1979 WHO, CA</td>
<td>126/150</td>
<td>11/3 , 52/55 , 63/92</td>
<td>74/61 , G/C 178/239 , C/C 0/1</td>
<td>Y(0.107) 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tong [32]</td>
<td>2013</td>
<td>Beijing PB</td>
<td>CA</td>
<td>326/341</td>
<td>37/41 , 110/120 , 179/180</td>
<td>184/202 , G/C 468/480 , C/C 0/1</td>
<td>N(0.004) 8</td>
<td></td>
</tr>
</tbody>
</table>


Table 2
Meta-analyses of IL-6 gene −174 G/C and −572 C/G polymorphisms and risk of CAD in each subgroup.

<table>
<thead>
<tr>
<th>Position</th>
<th>Sample size (case/control)</th>
<th>Allelic model</th>
<th>Additive/codominant model</th>
<th>Recessive model</th>
<th>Dominant model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P&lt;sub&gt;Q&lt;/sub&gt;</td>
<td>OR (95% CI)</td>
<td>P&lt;sub&gt;Q&lt;/sub&gt;</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Overall analysis</td>
<td>−174 G/C</td>
<td>965/965</td>
<td>1.91 [0.93, 1.52]</td>
<td>0.669 (55.1%)</td>
<td>0.93 [0.67, 1.28]</td>
</tr>
<tr>
<td>Sensitive analysis</td>
<td>−174 G/C</td>
<td>639/624</td>
<td>0.66 [0.25, 1.70]</td>
<td>0.747 (0%)</td>
<td>0.65 [0.25, 1.70]</td>
</tr>
<tr>
<td></td>
<td>−572 C/G</td>
<td>2254/2147</td>
<td>1.48 [1.26, 1.74]</td>
<td>0.018 (55.1%)</td>
<td>2.60 [1.54, 4.39]</td>
</tr>
</tbody>
</table>

P<sub>Q</sub>: P values for heterogeneity from Q-test.

* Significant heterogeneity: the random-effects model was chosen to summarize the result.
statistical heterogeneity among studies \( (p < 0.10 \text{ and } I^2 > 50\% \) indicated evidence of heterogeneity) \[18,19\]. A random-effects model or fixed-effects model was used to calculate pooled effect estimates in the presence or absence of heterogeneity, respectively \[20,21\]. Sensitivity analysis was conducted by limiting the meta-analysis to studies conforming to HWE \( (p < 0.05 \text{ of HWE} \) was considered significant). The estimates of potential publication bias were evaluated by Begg’s funnel plot and Egger’s regression test \( (p < 0.05 \) was considered representative of statistically significant publication bias) \[22\].

3. Results

3.1. Study characteristics

The study selection process is detailed in Fig. 1. Our initial search identified a total of 761 potentially eligible articles. A total of 12 articles involving 16 studies fulfilling the inclusion criteria were identified \[23–34\], containing 2309 CAD cases and 2273 controls. Six of these studies involved the association between IL-6 gene \( \text{−}174 \text{ G/C polymorphism and CAD risk} \), and 10 studies involved IL-6 gene \( \text{−}572 \text{ C/G polymorphism} \). These eligible studies were from nine provinces of China including Beijing, Guangdong, Guangxi, Henan, Hubei, Jiangsu, Jilin, and Shanxi. Controls were population-based in 14 studies \[23–25,27–34\], hospital-based in two studies \[26\]. Five studies did not follow the HWE \[29,30,33,34\]. The NOS results showed that the average score was 8.2, which indicated that the methodological quality was generally good. Tables 1 and 2 show the detailed characteristics of these studies.

3.2. Quantitative synthesis

For IL-6 gene \( \text{−}174 \text{ G/C polymorphism} \), six studies were combined and no significant association between IL-6 gene \( \text{−}174 \text{ G/C polymorphism and CAD risk} \) was found in the two genetic models (for C allele vs. G allele: \( \text{OR} = 1.91, 95\% \text{ CI} = 0.93–1.52, p = 0.164; \) for G/C vs. G/G: \( \text{OR} = 0.93, 95\% \text{ CI} = 0.67–1.28, p = 0.647 \)). The main results of meta-analysis were shown in Table 2 and Fig. 2, respectively.

For IL-6 gene \( \text{−}572 \text{ C/G polymorphism} \), 10 studies were combined showing evidence for significant association between this variation and CAD risk (for G allele vs. C allele: \( \text{OR} = 1.48, 95\% \text{ CI} = 1.26–1.74, p < 0.001; \) for G/G vs. C/C: \( \text{OR} = 2.60, 95\% \text{ CI} = 1.54–4.39, p < 0.001; \) for G/G vs. G/C + C/C: \( \text{OR} = 2.15, 95\% \text{ CI} = 1.35–3.42, p = 0.001; \) for G/G + G/C vs. C/C: \( \text{OR} = 1.55, 95\% \text{ CI} = 1.29–1.85, p < 0.001 \)). The main results of meta-analysis were shown in Table 2 and Fig. 3, respectively.

3.3. Sensitivity analysis

Sensitivity analysis was performed to assess the influence of the studies without HWE on the pooled OR by removal of these studies. Five studies \[29,30,33,34\] without HWE were excluded \( (p < 0.05) \). The corresponding pooled ORs were not materially altered in overall comparisons, either for IL-6 gene \( \text{−}174 \text{ G/C polymorphism} \) or for IL-6 gene \( \text{−}572 \text{ C/G polymorphism} \). The results of the sensitivity analysis were shown in Table 2.

3.4. Publication bias

Begg’s funnel plot and Egger’s regression test were performed to assess the publication bias. For IL-6 gene \( \text{−}174 \text{ G/C polymorphism} \), no obvious asymmetry was observed in any genetic model according to the visual assessment of funnel plot (i.e. Fig. 4A: Funnel plot for allelic model). In addition, the results of Egger’s regression test still did not provide any evidence of publication bias for this variation \( (p = 0.135 \text{ for allelic model, and } p = 0.342 \text{ for codominant model}) \). However, the effect size was asymmetrically distributed with publication bias visually present in all genetic models of IL-6 gene \( \text{−}572 \text{ C/G polymorphism} \) (i.e. Fig. 4B: Funnel plot for allelic model). Also, the result of Egger’s regression test provided evidence for publication bias \( (p = 0.005 \text{ for allelic model, } p = 0.001 \text{ for additive model, } p = 0.001 \text{ for recessive model, and } p = 0.018 \text{ for dominant model, respectively}) \).

4. Discussion

Recently, meta-analysis has been widely used in genetic association studies because it has the potential to detect small effects between gene polymorphism and human disease \[35–37\]. A previous meta-analysis of worldwide studies showed that IL-6 gene \( \text{−}174 \text{ G/C polymorphism} \) was associated with increased CAD risk among Asians \[13\]. Here, we focused on the associations between IL-6 gene \( \text{−}174 \text{ G/C and } \text{−}572 \text{ C/G polymorphisms and CAD risk} \) in Chinese population. Although the associations between these gene variations and CAD risk have been intensively studied in Chinese population, the results remain inconclusive \[23–34\]. Therefore, we designed this meta-analysis to derive a more precise associations between IL-6 gene \( \text{−}174 \text{ G/C and } \text{−}572 \text{ C/G polymorphisms and CAD risk} \) in Chinese population.

To the best of our knowledge, this is the first comprehensive meta-analysis to date investigating the associations between IL-6

Fig. 2. Forest plots for IL-6 gene \( \text{−}174 \text{ G/C polymorphism and CAD risk} \) in the overall study. (A) Allelic model: C allele vs. G allele. (B) Codominant model: G/C vs. G/G.
The IL-6 gene –174 G/C and –572 C/G polymorphisms and CAD risk in Chinese population. In the present meta-analysis, the combined evidence showed that IL-6 gene –174 G/C polymorphism was not associated with increased risk of CAD in Chinese population. Furthermore, significant association was found between IL-6 gene –572 C-to-G mutation and CAD risk, suggesting the risk of developing CAD in G allele carriers was 1.48-fold higher than those without. Moreover, the individuals with IL-6 gene –572 G/G genotype had a significantly higher risk for developing CAD (for OR = 2.60) compared to those with C/C genotype. Therefore, it is reasonable to assume that the IL-6 gene –572 G allele is an independent risk factor for the development of CAD in Chinese population. In comparison with the previous meta-analysis of worldwide studies, this meta-analysis obtained the same conclusions on the relationships between IL-6 gene –174 G/C and –572 C/G polymorphisms and CAD risk. In addition, considering the results produced from genetic association case–control studies may be spurious when the genotype distribution of controls deviates from HWE [38], we also performed sensitivity analysis restricted to the studies conforming to HWE, and similar results to that of overall study were obtained between IL-6 gene –174G/C and –572 C/G polymorphisms and CAD risk. The corresponding pooled ORs were not materially altered, suggesting that our overall results were statistically robust. Also, HWE should not be considered as a factor influencing the overall results.

Publication bias is one of the most important sources of bias in meta-analysis, which might influence the interpretation of our final results supporting the role of IL-6 gene polymorphisms in
CAD. As shown in Fig. 4B, asymmetrical "missing" data, which was in the lower part of the funnel plot (Funnel plot for allelic model of IL-6 gene –572 C/G polymorphism), suggesting obvious publication bias in the comparison of G allele vs. C allele. The present meta-analysis only focused on studies with full text articles and published in English and Chinese, missing some eligible studies. It may be an important reason for publication bias. Furthermore, we did not find publication bias in overall comparisons of IL-6 gene –174 G/C polymorphism. Therefore, projections from the literature of who is at risk for IL-6 gene –572 C/G polymorphism attributable CAD and who would benefit from IL-6 gene-targeted therapies should therefore be approached with caution.

For better interpreting the results, some limitations of this meta-analysis should be acknowledged. First, publication bias in our analysis should be noted, which may affect the overall results of this meta-analysis. Second, subgroup analysis was not performed by the factors such as gender, age, smoking status and lifestyle because insufficient data could be extracted from the primary article. Third, CAD is a complex disease with various involved factors including environmental and genetic factors. However, many eligible studies included in this meta-analysis did not consider most of the important environmental factors. Moreover, China is a multi-ethnic country. We were unable to perform subgroup analysis by ethnic group because the studies in Chinese minority are relatively few and constitute small sample sizes.

In conclusions, our meta-analysis suggests that IL-6 gene –572 C/G polymorphism may be a risk factor for CAD susceptibility in Chinese population. In addition, no significant association was found between IL-6 gene –174 G/C polymorphism and CAD risk. However, the result should be interpreted with caution because of its limitations. Further studies with large sample size are needed in the future.

References
