Calculate the meta-analysis of the association between IL-18 rs1946518, rs187238 polymorphisms and coronary artery diseases

Li-Ping Dong1,2, Jian-Ming Li1,3, Wen-Qi Luo1, Liang Tang4, Heng Yuan4, Guan-Lan Liu4, Xiao-Dong Zhang4, Guang-Yi Li4, Mei-Hua Bao4

1Department of Anatomy, Histology and Embryology, Institute of Neuroscience, Changsha Medical University, Changsha 410219, China; 2Department of Histology and Embryology, School of Basic Medicine, Central South University, Changsha 410013, China; 3Department of Neurology, Xiangya Hospital, Central South University, Changsha 410008, China

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Abstract: Coronary artery disease (CAD) is one of the major health problems worldwide. The polymorphisms of Interleukin-18 (IL-18), including rs1946518 (-607 C/A) and rs187238 (-137 G/C), have been found to be probably associated with the risk of CAD. However, the results were inconsistent and controversial. To determine the association between IL-18 promoter rs1946518 and rs187238 polymorphisms with CAD, we conducted the meta-analysis of all relevant studies cited in Pubmed, Embase, CENTRAL, CBM and CNKI before 6 July 2016. A total of 7 studies, including 2636 cases and 1664 controls, were identified for the meta-analysis. The results showed that the CC genotype of rs1946518 was associated with a significantly higher risk of CAD in allelic model (OR = 1.38), homozygous model (OR = 2.06), heterozygous model (OR = 1.44) and dominant model (OR = 1.56) in the overall analysis. But in subgroup analysis by ethnicity, the CC of rs1946518 had no significant associations with risk of CAD in Caucasians. The results also showed a significant higher risk of CAD in GG genotype of rs187238 in heterozygous model (OR = 1.45) and dominant model (OR = 1.19) in the overall analysis. Subgroup analysis by ethnicity discovered GG genotype of rs187238 was associated with a significantly higher risk of CAD in Asian, while only in heterozygous model in Indian. In conclusion, our results suggested higher risks of CAD for CC of rs1946518 and GG of rs187238 in Asian. However, the results of this meta-analysis are hypothesis-generating results which should be interpreted with caution because of the heterogeneity and publication bias among study designs.

Keywords: IL-18, rs1946518, rs187238, polymorphism, coronary artery disease, meta-analysis

Introduction

Cardiovascular Diseases (CVDs) is the major cause of death worldwide. According to the World Health Organization (WHO) report in 2014, more than 17.5 million individuals died from cardiovascular disease in 2012, accounting for 46.2% of noncommunicable diseases deaths [1]. Coronary artery disease (CAD), one of the CVDs, has a complex pathophysiology generated by interactions between genes and environment. Besides the environmental risk factors, the single nucleotide polymorphisms (SNPs) were associated with the risk of CAD.

Inflammation is involved in almost all stages of atherosclerosis, from initiation of lesion to successive progression and final plaque destabilization. The best proved inflammatory biomarker is CRP, other markers include soluble CD40 ligand, interleukin 18 (IL-18), and matrix metalloproteinase 9 (MMP-9), etc. They also give additional information for risk prediction of CAD [2].

IL-18, a member of the IL-1 superfamily, is a pro-inflammatory cytokine. The IL-18 gene is located on chromosome 11q22.2-q22.3, and comprises six exons and five introns [3]. The polymorphisms in IL-18 have been found to be probably associated with the risk of CAD. However, the results were inconsistent and controversial results. Some studies have demonstrated that the IL-18 polymorphisms, including
rs1946518 and rs187238, were related to the risk of CAD [4-8], while others considered they were not [9, 10]. Thus, in the present study, we included 7 studies (2636 cases and 1664 controls) to get a more precise and comprehensive estimation of the association between these polymorphisms and CAD.

**Experimental section**

**Publication search strategy and inclusion criteria**

We used the following electronic databases to search studies: Pubmed, Embase, Cochrane Central Register of Controlled Trials (CENTRAL), Chinese Biomedical Literature Database (CBM), and Chinese National Knowledge Infrastructure (CNKI). The search terms and key words were (“Coronary artery disease”, or “CAD”), (“interleukin-18” or “IL-18”), and (“polymorphism” or “mutation” or “SNP” or “single nucleotide polymorphism”), without restrictions on language. The deadline for publication was 6 July 2016.

Inclusion criteria: (1) case-control design; (2) the association of interleukin-18 rs1946518 or rs187238 polymorphisms with CAD risks should be investigated and reported; (3) sufficient data should be provided for the present estimation. Exclusion criteria: (1) repeat publications; (2) abstracts, letters, reviews and master degree thesis; (3) studies not meeting all of the inclusion criteria.

**Data collection**

We collected information from each included study by two independent investigators. Any discrepancy between the reviewers was resolved by consensus or a third investigator. For each study, the collected information included: first authors' name, publishing year, country, ethnicity, genotyping method, sex ratio, sex and age match, genotype numbers of cases and controls, and the source of controls.

**Quality assessment**

The quality of the included publications was evaluated according to the predefined scale for quality assessment [11]. The score scale includes source of cases, source of controls, specimens used for determining genotypes, total sample size, and evidence of Hardy-Weinberg equilibrium (HWE). The quality scores range from 0-15. Reports scoring ≥10 were classified as “high quality”, and those <10 as “low quality”.

**Statistical methods**

We used $\chi^2$-test to evaluate the HWE of the control group polymorphism. If $P<0.05$, it was considered to be deviated from HWE.

To evaluate the association between IL-18 polymorphisms and CAD risk, the odds ratio (OR) with 95% confidence interval (CI) was used. The pooled ORs were calculated in homozygous, heterozygous, dominant, and allelic genetic model. The statistical significance was determined by the Z-test, $P<0.05$ was considered to be statistically significant. Subgroup analysis was conducted by ethnicity.

The statistical heterogeneity between studies was evaluated by an $I^2$-square statistical test, which was not dependent on the number of studies in the meta-analysis [12]. If there was an obvious heterogeneity among the studies ($I^2>50$%), the random-effects model was used for the meta-analysis [13]. Otherwise, the fixed-effect model was used [14].

Sensitivity analysis was performed to assess the effect of individual study on pooled results and the stability of results.

The publication bias was detected by using Begg's funnel plot and Egger's linear regression method, and $P<0.05$ was considered to be statistically significant [15].

All statistical analysis was performed using the STATA 12.0 software and Revman 5.3.

**Results**

**Characteristics of eligible studies**

A total of 40 studies were obtained from the literature search after duplicates were removed. Among them, 11 studies were excluded for irrelevance, 6 were master’s degree theses, 4 were reviews, 1 was abstract, 1 was editorial comments and 10 full text articles excluded according to inclusion and exclusion criteria. Finally, 7 studies met the criteria, including 2636 cases and 1664 controls [4-10]. Among them, 5 studies described the associations between IL-18
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rs1946518 and CAD, and 6 studies described the associations between IL-8 rs187238 and CAD. The PRISMA flowchart is shown in Figure 1 and the information for included studies is presented in Table 1.

Results of meta-analysis

The results of the meta-analysis for the associations between IL-18 rs1946518, rs187238 and CAD risks were shown in Table 2; Figures 2, 3.

IL-18 rs1946518 and CAD

5 studies, with 2090 cases and 1219 controls, were included in this study. Significant associations were found in overall analysis for all genetic models. A significant increase in CAD risk susceptibility was found for the CC genotype in homozygous model (CC vs. AA, OR = 2.06, 95% CI = 1.44-2.94, P = 0.00001), heterozygous model (CC vs. CA, OR = 1.44, 95% CI = 1.03-2.00, P = 0.03), dominant model (CC vs. CA/AA, OR = 1.56, 95% CI = 1.08-2.25, P = 0.02), and allelic model (C vs. A, OR = 1.38, 95% CI = 1.11-1.73, P = 0.004). But in subgroup analysis by ethnicity, the CC of rs1946518 had no significant associations with risk of CAD in Caucasians.

IL-18 rs187238 and CAD

6 studies, with 2506 cases and 1437 controls, were included in this study. Significant increase in CAD risk susceptibility was found for the GG genotype in heterozygous model (GG vs. GC, OR = 1.45, 95% CI = 1.11-1.91, P = 0.007) and dominant model (GG vs. GC/CC, OR = 1.19, 95% CI = 1.03-1.38, P = 0.02). No significant associations were found between rs187238 and CAD risk in other genetic models. Subgroup analysis by ethnicity discovered GG genotype of rs187238 was associated with a significantly higher risk of CAD in all genotypes in Asian and in heterozygous model in Indian, while had no significant associations with risk of CAD in Caucasians.

Sources of heterogeneity

For IL-18 rs1946518 polymorphism, there was significant heterogeneity between studies in all comparison models. To clarify the source of heterogeneity, we performed subgroup analysis by ethnicity. The results indicate that ethnicity was the source of heterogeneity for rs1946518 in all genetic models. For rs187238, ethnicity also was the source of the heterogeneity in all genetic models.

Sensitivity analysis

The influence of each study on the pooled OR and 95% CI was evaluated by excluding one single study at a time using STATA 12.0 software. The analysis showed that no single individual study significantly affected the pool OR in all genetic models for rs1946518 and rs187238 (only the allelic model of rs1946518 results are shown in Figure 4).

Publication bias

To evaluate the publication bias, the Begg's funnel plot and Egger's test were performed. The p values for Begg's and Egger's tests are shown in Tables 3, 4. Obvious publication bias was observed for IL-18 rs187238 in dominant and allelic models in Egger's test. For IL-18
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Table 1. Characteristics of eligible studies included in the meta-analysis

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Ethnicity</th>
<th>Genotyping method</th>
<th>Sex ratio (male: female) (case/control)</th>
<th>Sex/age match</th>
<th>Quality score</th>
<th>Sample size (case/control)</th>
<th>Case</th>
<th>Control</th>
<th>HWE of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opstad TB [9]</td>
<td>2011</td>
<td>Norway</td>
<td>Caucasians</td>
<td>TaqMan</td>
<td>783:218/147:57 scientific</td>
<td>match</td>
<td>10</td>
<td>996/204</td>
<td></td>
<td></td>
<td>0.762</td>
</tr>
</tbody>
</table>

Table 2. Pooled ORs and 95% CIs of the association between IL-18 rs1946518, rs187238, and CAD risks

<table>
<thead>
<tr>
<th>Genetic Model</th>
<th>N</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>P (%)</th>
<th>Model</th>
<th>Genetic Model</th>
<th>N</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>P (%)</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC vs. AA</td>
<td>6</td>
<td>2.06 [1.44, 2.94]</td>
<td>&lt;0.00001</td>
<td>57</td>
<td>R</td>
<td>CC vs. CA</td>
<td>5</td>
<td>1.44 [1.03, 2.00]</td>
<td>0.03</td>
<td>71</td>
<td>R</td>
</tr>
<tr>
<td>Asian</td>
<td>3</td>
<td>2.69 [2.04, 3.54]</td>
<td>&lt;0.00001</td>
<td>0</td>
<td>F</td>
<td>Asian</td>
<td>3</td>
<td>1.89 [1.50, 2.38]</td>
<td>&lt;0.00001</td>
<td>0</td>
<td>F</td>
</tr>
<tr>
<td>Caucasians</td>
<td>2</td>
<td>1.36 [0.92, 2.00]</td>
<td>0.12</td>
<td>31</td>
<td>F</td>
<td>Caucasians</td>
<td>2</td>
<td>0.98 [0.75, 1.29]</td>
<td>0.90</td>
<td>0</td>
<td>F</td>
</tr>
<tr>
<td>CC vs. AA/CA</td>
<td>5</td>
<td>1.56 [1.08, 2.25]</td>
<td>0.02</td>
<td>78</td>
<td>R</td>
<td>CC vs. A</td>
<td>5</td>
<td>1.38 [1.11, 1.73]</td>
<td>0.004</td>
<td>75</td>
<td>R</td>
</tr>
<tr>
<td>Asian</td>
<td>3</td>
<td>2.11 [1.70, 2.63]</td>
<td>&lt;0.00001</td>
<td>0</td>
<td>F</td>
<td>Asian</td>
<td>3</td>
<td>1.66 [1.45, 1.90]</td>
<td>&lt;0.00001</td>
<td>0</td>
<td>F</td>
</tr>
<tr>
<td>Caucasians</td>
<td>2</td>
<td>1.02 [0.79, 1.32]</td>
<td>0.88</td>
<td>0</td>
<td>F</td>
<td>Caucasians</td>
<td>2</td>
<td>1.05 [0.88, 1.26]</td>
<td>0.57</td>
<td>0</td>
<td>F</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genetic Model</th>
<th>N</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>P (%)</th>
<th>Model</th>
<th>Genetic Model</th>
<th>N</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>P (%)</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG vs. CC</td>
<td>6</td>
<td>1.10 [0.79, 1.55]</td>
<td>0.56</td>
<td>44</td>
<td>F</td>
<td>GG vs. GC</td>
<td>6</td>
<td>1.45 [1.11, 1.91]</td>
<td>0.007</td>
<td>66</td>
<td>R</td>
</tr>
<tr>
<td>Asian</td>
<td>3</td>
<td>2.72 [1.03, 7.21]</td>
<td>0.04</td>
<td>0</td>
<td>F</td>
<td>Asian</td>
<td>3</td>
<td>1.37 [1.10, 1.71]</td>
<td>0.004</td>
<td>30</td>
<td>F</td>
</tr>
<tr>
<td>Caucasians</td>
<td>2</td>
<td>1.26 [0.80, 1.98]</td>
<td>0.32</td>
<td>26</td>
<td>F</td>
<td>Caucasians</td>
<td>2</td>
<td>2.57 [1.68, 3.93]</td>
<td>&lt;0.00001</td>
<td>-</td>
<td>F</td>
</tr>
<tr>
<td>Indian</td>
<td>1</td>
<td>0.60 [0.33, 1.12]</td>
<td>0.11</td>
<td>-</td>
<td>F</td>
<td>Indian</td>
<td>1</td>
<td>1.00 [0.65, 1.10]</td>
<td>0.21</td>
<td>-</td>
<td>F</td>
</tr>
<tr>
<td>GG vs. CC/GC</td>
<td>6</td>
<td>1.19 [1.03, 1.38]</td>
<td>0.02</td>
<td>48</td>
<td>F</td>
<td>G vs. C</td>
<td>6</td>
<td>1.21 [1.03, 1.41]</td>
<td>0.007</td>
<td>66</td>
<td>R</td>
</tr>
<tr>
<td>Asian</td>
<td>3</td>
<td>3.14 [1.14, 1.75]</td>
<td>0.002</td>
<td>30</td>
<td>F</td>
<td>Asian</td>
<td>3</td>
<td>1.38 [1.14, 1.68]</td>
<td>0.0011</td>
<td>19</td>
<td>F</td>
</tr>
<tr>
<td>Caucasians</td>
<td>2</td>
<td>2.13 [0.88, 1.44]</td>
<td>0.33</td>
<td>14</td>
<td>F</td>
<td>Caucasians</td>
<td>2</td>
<td>1.11 [0.92, 1.34]</td>
<td>0.27</td>
<td>38</td>
<td>F</td>
</tr>
<tr>
<td>Indian</td>
<td>1</td>
<td>0.90 [0.65, 1.24]</td>
<td>0.51</td>
<td>-</td>
<td>F</td>
<td>Indian</td>
<td>1</td>
<td>0.85 [0.65, 1.10]</td>
<td>0.21</td>
<td>-</td>
<td>F</td>
</tr>
</tbody>
</table>

R: Random model; F: Fixed model.
rs1946518 polymorphism, there was no publication bias in all models. These results were also demonstrated by the shape of the funnel plot (only the allelic model results are shown in Figure 5).

Discussion

Previous studies have reported the importance of inflammation in the risk of atherosclerosis [16]. IL-18 is a proinflammatory cytokine pro-
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produced by activated monocytes/macrophages and Kupffer cells and can promote interferon-γ (IFN-γ) production [17]. The variation in the IL-18 gene was associated with IL-18 serum concentrations [18], and serum levels of IL-18 may influence the risk of CAD [19], multiple

Figure 3. Forest plots of odds ratios for the association of IL-18 rs187238 with risk of CAD.

GG vs. CC

GG vs. GC

GG vs. CC/GC

G vs. C

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Recently, several SNPs in IL-18 have been studied to investigate their relations with CAD risk. Rs1946518 has gained the greatest attention in these involved studies, some of which found a significant association between rs1946518 and CAD, while others denied it.

The main findings of this study showed that the CC genotype of IL-18 rs1946518 (C/A) was associated with a significantly higher risk of CAD in allelic model (OR = 1.38), homozygous model (OR = 2.06), heterozygous model (OR = 1.44) and dominant model (OR = 1.56) in the overall analysis. But in subgroup analysis by ethnicity, the CC of rs1946518 had no significant associations with risk of CAD in Caucasians. GG genotype of IL-18 rs187238 (G/C) was associated with a significantly higher risk of CAD in heterozygous model (OR = 1.45) and dominant model (OR = 1.19), while no significant associations in allelic model (OR = 1.19), homozygous model (OR = 1.10) in the overall analysis. However, in subgroup analysis by ethnicity, GG genotype of rs187238 was associated with a significantly higher risk of CAD in all genotype in Asian and in heterozygous model in Indian, while GG genotype of rs187238 had no significant associations with risk of CAD in Caucasians.

Significant heterogeneity was found in this study for rs1946518 and rs187238. Thus, we conducted subgroup analysis by ethnicity and found that it was the sources of the heterogeneity for rs1946518 and rs187238. Thus, further studies are needed to confirm these results. In the sensitivity analysis, no significant changes were found when omitting each study one at a time, indicating the relative stability and credibility of the results of this study.

An obvious asymmetry in funnel plots and significant p-values for rs187238 through Egger's test were found in this study. According to the Cochrane Handbook (version 5.1.0, Section 10), the cause includes the following aspects: selection bias (publication bias of selective outcome reporting), small-sample effects, true heterogeneity, and chance. In this study, 6 studies were small sample sizes. There are not large-scale studies available. Therefore, the small-sample effect might be one reason for this asymmetry. Furthermore, studies only in English or Chinese have been searched. There might be studies in other languages which are not included, which might be another reason for the asymmetry.

This study has several limitations that should be considered. Firstly, the number of studies...
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was too small to reach to definite conclusion. Secondly, publication bias and heterogeneity may have distorted the meta-analysis. Thirdly, the ethnicity of the subjects was predominantly Asian. We included 7 studies in our present meta-analysis, while only 2 studies were conducted in Caucasians, which estimated the associations of rs1946518, rs187238 with risk of CAD. However, no obvious relationships were found in all of these SNPs in Caucasians. The positive results in our meta-analysis are all related with Asians. Thus, the conclusion in the present meta-analysis might be only of generalizability for Asians. The last limitation is that CAD is a multifactorial disease influenced by both genetic and environmental factors, but most studies lack the information on multiple SNPs in haplotypes and environmental exposure.

Conclusions

In conclusion, this study suggested a higher risk of CAD for the CC genotype of IL-18 rs1946518 in Asians, and a higher risk of CAD for the GG genotype of IL-18 rs187238 in Asians, but not in Caucasians. Thus, rs1946518 and rs187238 might be recommended as predictors for susceptibility of CAD in Asians. However, the results of this meta-analysis are hypothesis-generating results which should be interpreted with caution because of the heterogeneity and publication bias among study designs. Further studies are required to evaluate the association between IL-18 polymorphisms and CAD in various ethnic groups.

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Disclosure of conflict of interest

None.

Authors’ contribution

Li-Ping Dong and Mei-Hua Bao planned and designed the experiments; Jian-Ming Li, Liang Tang, Wen-Qi Luo, Guang-Yi Li and Li-Ping Dong performed experiments; Heng Yuan, Guan-Lan Liu and Xiao-Dong Zhang analyzed the data; Li-Ping Dong and Mei-Hua Bao wrote the paper.

Address correspondence to: Li-Ping Dong and Mei-Hua Bao, Department of Anatomy, Histology and Embryology, Institute of Neuroscience, Changsha Medical University, Changsha 410219, China. Tel: +86-731-8488-4488; Fax: +86-731-8849-8866; E-mail: ddongliping@163.com (LPD); mhbao78@163.com (MHB)

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