Meta-analysis of myeloperoxidase gene polymorphism and coronary artery disease susceptibility

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ABSTRACT

Objective: To assess the association between myeloperoxidase (MPO) gene polymorphism and coronary artery disease (CAD).

Methods: Several databases were used to retrieve relevant literature up to March 2013 by keywords. A Meta-analysis was performed by Stata12.0 software to estimate the pooled odds ratio (OR) and the 95% confidence interval (CI). Heterogeneity among studies was tested and sensitivity analysis was applied. Publication bias was examined using Begg's funnel plot and Egger's linear regression test.

Results: A total of 17 studies were included in this Meta-analysis. For MPO -463 G/A polymorphism, the pooled OR of A allele vs G allele was 0.58 [95% CI (0.47-0.72)] and the pooled OR of genotypes AA+AG vs GG was 0.58 [95% CI (0.46-0.72)]. In subgroup analysis of study population, AA and AG genotypes were significantly associated with CAD in Asians but not in Europeans. The MPO -463 G/A polymorphism in the stable angina pectoris subgroup was evaluated in 3 studies and the pooled OR of A allele vs G allele and genotypes AA+AG vs GG for proven CAD was 0.45 [95% CI (0.15-1.37)] and 0.57 [95% CI (0.19-1.65)]. For MPO -129 A/G gene polymorphism, the pooled OR of genotype GG vs AA+AG was 0.91 [95% CI (0.74-1.10)].

Conclusion: A allele of MPO -463 G/A gene is associated with decreased risk of CAD except in the Europeans. There is no association between MPO -129 A/G gene polymorphisms and CAD risk.

KEY WORDS: coronary artery disease; myeloperoxidase; gene polymorphism; Meta-analysis

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Biography: CHEN Luyao, M.D., Physician, mainly engaged in the research of oxidative stress and cardiovascular disease.

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髓过氧化物酶基因多态性与冠心病易感性的 Meta 分析

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【摘要】目的：分析髓过氧化物酶 (myeloperoxidase, MPO) 基因多态性与冠心病易感性的关系。方法：利用多个网络数据库及相应关键词检索自建立以来至 2013 年 3 月的相关文献。用 Stata12.0 对纳入文献的相关数据进行 Meta 分析，评估相对危险度 (odds ratio, OR 值) 及 95% 置信区间 (confidence interval, CI)。同时，对文献中各研究的异质性及敏感性进行分析。发表偏倚通过 Begg’s 漏斗图和 Egger’s 线性回归图进行检测。结果：共纳入 17 篇文献，Meta 分析显示各基因型合并后的 OR 值及 95%CI，其中在 MPO -463 G/A 基因中，A 等位基因与 G 等位基因比较，OR=0.58，95%CI (0.47~0.72)；基因型 AA+AG 与 GG 比较，OR=0.58，95%CI (0.46~0.72)。在欧洲人群亚组中，AA+AG 对 GG 的 OR=0.79，95% CI (0.58~1.08)；在稳定型心绞痛亚组中，AA 对 GG 的 OR=0.45，95% CI (0.15~1.37)；AA+AG 对 GG 的 OR=0.57，95% CI (0.19~1.65)。在 MPO -129 A/G 基因中，G 对 AA+AG 的 OR=0.91，95% CI (0.74~1.10)。结论：除欧洲人群外，携带 MPO -463 G/A 基因中 A 等位基因者冠心病的发病风险降低，MPO -129 A/G 基因与冠心病易感性无相关性。

【关键词】冠心病；髓过氧化物酶；基因多态性；Meta 分析

Coronary artery disease (CAD) is still one of the leading causes of death and disability [1-2]. It is well-known that the disease results from an interaction between genetic background and environmental factors [3-4]. Despite the established facts that smoking, dyslipidemia, hypertension, diabetes, physical inactivity, obesity, and diet are associated with increased risk of CAD, an exact etiology underlying CAD is still obscure. A growing body of evidence has demonstrated the roles of oxidative stress and inflammation in the pathogenesis of CAD [5-6].

Myeloperoxidase (MPO) has emerged as a potential participant in the promotion and/or propagation of atherosclerosis [5]. As a member of the heme peroxidase super family, MPO generates numerous reactive oxidants and diffusible radical species that are capable of both initiating lipid peroxidation and promoting an array of post-translational modifications to target proteins, including halogenation, nitration, and oxidative cross-linking [8]. It has been identified in human atherosclerotic plaques where it exerts a proatherogenic effect through the reduction of nitric oxide bioavailability, the oxidation of low-density lipoprotein and the generation of dysfunctional high-density lipoprotein [5,8]. Previous study [8] has demonstrated that elevated levels of MPO are associated with the prevalence of CAD. Furthermore, studies [3,10] also found that elevated concentrations of MPO are not only independently associated with increased risk of CAD, but also predicted future risk of CAD in apparently healthy individuals.

A guanosine (G) to adenosine (A) nucleotide substitution, −G463A (rs2333227) was found to elevate MPO transcriptional activity, via promoting SPI transcription factor binding [11]. The high-activity −463G allele is associated with increased MPO activity in several diseases [12-13] including lung cancer [14-15]. The low-activity A allele which is associated with lower levels of polycyclic aromatic hydrocarbons [16] and ROS production elicits decreased risk in diseases such as Alzheimer’s disease [17], multiple sclerosis [12], myeloid leukemia [18], esophageal [19], and lung cancers [14,20-21]. Accumulating evidence also suggests association of MPO −463 G/A gene with CAD development although discrepancies exist. And another most frequently studied polymorphisms, MPO −129 A/G also has been reported to be associated with CAD but the results are inconsistent [17,23].

In this study, we performed a Meta-analysis to evaluate the association between the MPO −463 G/A and −129 A/G variant and risk of CAD, also taking into consideration the potential modifying influences of the different study population.

I Methods

1.1 Literature search
To identify eligible studies for this Meta-analysis, 2 investigators (ZHAO Shushan and CHEN Luyao) searched the Pubmed, Science Citation Index, CNKI and WANGFANG databases in all languages which were published up to March 2013. The search strategy was based on Boolean combinations of the keywords [(MPO...
or myeloperoxidase) and (genetic polymorphism or gene or polymorphism) and (coronary artery disease or CAD)]. As the review progressed, we improved the research strategy when necessary. All references cited in these studies were also reviewed to identify additional studies.

1.2 Inclusion and exclusion criteria

All relevant case-control studies were included, regardless of publication status, language, or sample size. In this Meta-analysis, the following inclusive criteria were set and reviewed by 2 independent investigators: 1) Full-text publications were available; 2) the study aimed to investigate the relationship between MPO genetic polymorphism and CAD risk in human subjects; 3) CAD patients according to the diagnosis standard of coronary angiography (>50% stenosis in at least one major coronary vessel) as well as clinical symptoms were included; 4) the association between MPO gene polymorphism and CAD was examined based on case-control design; and 5) original data were presented (If there were multiple publications from the same study group, the most complete and recent results were extracted).

Exclusion criteria included: 1) Contrary to the inclusion criteria; 2) duplicate publications; 3) insufficient data, such as meeting abstracts and conference preceding; and 4) non-report of the genotype frequency.

1.3 Datum extraction

Data was extracted independently by 2 of the authors (CHEN Luyao and ZHAO Shushan), using a pre-designed datum extraction form, and the information was subsequently entered into Review Management or Stata 12.0 software. For each study, the following information was record: first author, year of publication, country or region of origin, study design, participants (such as age range, simple size, gender, and some other characters) and MPO genotype counts in CAD patients and controls. Discrepancies between the extracted data were resolved by discussion, or counsel to the third investigators. When the data of a study was not clear or not presented by the author in the full-test publications, we contacted the authors for further details.

1.4 Datum analysis

Management and analysis of data were performed with the use of the Stata 12.0 and Review Manager 5.1 software. Heterogeneity was explored by a Chi-square test using a 10% significance level, and the quantity of heterogeneity was measured by value $I^2$, which represents the proportion of inter-study variability that can contribute to heterogeneity rather than chance. If $I^2>50\%$ indicating significant heterogeneity between the studies, a random-effect model was conducted for Meta-analysis; otherwise a fixed effect model was used. If statistically significant heterogeneity was presented or there was a considerable variation in results, the trials were not combined in a Meta-analysis, but presented in a forest plot. All the studies were dichotomous data expressed as odds ratios (OR) with 95% confidence intervals (CI) to measure the strength of the genetic association. Subgroup analysis by ethnicity and genotype methods were carried out, strictly for exploratory and hypothesis-generating purposes. Sensitivity analysis was performed through omitting each study to assess the quality and consistency of the results. Publication bias was examined by the visual inspection of funnel plot, Begg’s test and Egger’s regression test ($P<0.05$ was considered representative of statistically significant publication bias). The overall effect was tested using $z$ scores calculated by Fisher’s $z’$ transformation, with the significance set at $P<0.05$.

2 Results

2.1 Search results

Based on strategy mentioned above, we searched relevant literature, and finally a total of 209 studies were identified (PubMed: 68; CNKI: 103; WEB OF SCIENCE: 28; WANFANG database: 10). After review of the title by 2 independent reviewer authors, 125 studies were excluded as they were not irrelevant to CAD ($n=46$), MPO ($n=59$), gene polymorphism ($n=12$), or association between MPO gene polymorphism and CAD. Forty-nine articles were excluded after the abstract review including 9 reviews and 40 indexes, and 35 articles were retrieved for further evaluation. According to the inclusion and exclusion criteria, 17 studies [24, 28, 32, 35, 37-38, 47] were ultimately included for the Meta-analysis. The remaining studies were excluded because of the following reasons. Two studies [40-41] lacked sufficient data, and emails were sent to the authors for further information, but no replies. Five studies [42-46] used other study method. Literature [47] is the translation of literature [17] in Chinese, literature [27] and literature [48], literature [33] and literature [49], literature [35] and literature [50] are duplicate publications. Literature [24, 28, 32, 35, 37-38, 47] were retrieved in more than 2 databases. The publication year of involved studies ranged from 2001 to 2011. A flow diagram schematized the process of selecting and excluding articles with specific reasons, as shown in Figure 1.
2.2 Study characteristics
Among these 17 involved studies including 3821 CAD patients and 3,407 controls, 5 studies [17,26,29,31,36] recruited Caucasians including Canada [17], Sweden [26,31], Norway [29], and Turkish [36], while the other studies [24-25,15,28,30-39] included Asians. Two single nucleotide polymorphisms (SNPs) in the MPO gene were considered, including MPO -129 A/G in 3 studies and MPO -463 G/A in 15 researches. For one study [32] involving 2 stage groups, each group was analyzed separately. Genotyping for -129 A/G or -463 G/A polymorphism across all studies was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The genotype frequencies of -129 A/G and -463 G/A in CAD and control groups followed Hardy-Weinberg Equilibrium in all studies. The data including numbers of cases and controls, numbers of genotype and allele in each group were extracted. The baseline characteristics of all included studies in this Meta-analysis are summarized in Table 1.

2.3 Meta analysis
2.3.1 Association between MPO -463 G/A polymorphism and CAD risk
Fifteen studies [17,24-28,30-36,38-39] including 3,449 cases and 3,082 controls investigated the association between MPO -463 G/A genotype and CAD risk. According to Chi-squared statistic and I square ($I^2=80.5\%$, $P<0.001$), the heterogeneity obviously existed which might be a result of the difference in ethnicity, country, sources of controls and genotype methods, so a random-effect approach was used to estimate the OR of CAD patients versus controls. The pooled frequency of the A allele in CAD patients and controls was 0.213 and 0.265, respectively. The Meta-analysis results showed that A allele of MPO -463 G/A polymorphism was significantly correlated with the reduced risk of CAD (A allele vs G allele: OR=0.58, 95%CI: 0.47–0.72, $P<0.001$; AA vs AG+GG: OR=0.38, 95%CI: 0.26–0.57, $P<0.001$; AA+AG vs GG: OR=0.58, 95%CI: 0.46–0.72, $P<0.001$; Figure 2). In the stratified
analysis by ethnicity, MPO −463 G/A was significantly related to reduced risk of CAD among Asians (A allele vs G allele: OR=0.52, 95%CI: 0.42–0.64, P<0.001; AA vs AG+GG: OR=0.34, 95%CI:0.23–0.51, P<0.001; AA+AG vs GG: OR=0.50, 95%CI: 0.40–0.64, P=0.031; respectively). However, there were no correlation among Caucasians (A allele vs G allele: OR=0.79, 95%CI:0.58–1.08, P=0.015; AA vs AG+GG: OR=0.54, 95%CI: 0.24–1.23, P<0.025; AA+AG vs GG: OR=0.82, 95%CI:0.61–1.11, P<0.061; Figure 2).

2.3.2 Association between MPO −463 G/A polymorphism and the risk of SAP risk

Three studies[26,33,40] recruiting 207 CAD patients and 278 controls investigated the correlation between MPO −463 G/A polymorphism and SAP subgroup of CAD risk. The random-effect model was used because the heterogeneity was also obvious under genetic models (I²=88.8%, P<0.001). But no association was found between MPO −463 G/A polymorphism and SAP risk in this Meta-analysis (A allele vs G allele: OR=0.45, 95%CI: 0.15–1.37, P<0.001; AA vs AG+GG: OR=0.71, 95%CI: 0.11–4.77, P=0.003; AA+AG vs GG: OR=0.57, 95%CI: 0.19–1.65, P<0.011; Figure 3). All of the 3 studies were performed in Asia, so no subgroup analysis was performed.

Table 1 Main characteristics of studies included in the Meta-analysis of the relationship between MPO −463 G/A, MPO −129 A/G gene polymorphism and CAD

<table>
<thead>
<tr>
<th>Authors</th>
<th>Years</th>
<th>Regions</th>
<th>Study design</th>
<th>Genes</th>
<th>SNPs</th>
<th>Ages/years</th>
<th>Numbers (male/ female)</th>
<th>Study design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nikpoor B, et al[37]</td>
<td>2001</td>
<td>Canada</td>
<td>229</td>
<td>217</td>
<td>MPO −463</td>
<td>MPO −463G/A</td>
<td>59.3 ± 9.6</td>
<td>177/52</td>
</tr>
<tr>
<td>Hao L, et al[34]</td>
<td>2006</td>
<td>China</td>
<td>105</td>
<td>105</td>
<td>MPO −463</td>
<td>MPO −463G/A</td>
<td>58.2 ± 10.4</td>
<td>80/25</td>
</tr>
<tr>
<td>Grahl DA, et al[28]</td>
<td>2007</td>
<td>Sweden</td>
<td>81</td>
<td>176</td>
<td>MPO −463</td>
<td>MPO −463G/A</td>
<td>6.2 ± 50.0</td>
<td>60/51</td>
</tr>
<tr>
<td>Li Hui, et al[37]</td>
<td>2007</td>
<td>China</td>
<td>79</td>
<td>69</td>
<td>MPO −463</td>
<td>MPO −463G/A</td>
<td>62.2 ± 9.9</td>
<td>60/19</td>
</tr>
<tr>
<td>Yin Qianzhong, et al[24]</td>
<td>2008</td>
<td>China</td>
<td>134</td>
<td>145</td>
<td>MPO −463</td>
<td>MPO −463G/A</td>
<td>51.5 ± 5.8</td>
<td>65/69</td>
</tr>
<tr>
<td>Berg KK, et al[29]</td>
<td>2009</td>
<td>Norway</td>
<td>130</td>
<td>100</td>
<td>MPO −129</td>
<td>MPO −129A/G</td>
<td>60.0 ± 57.0</td>
<td>106/24</td>
</tr>
<tr>
<td>Chen Z, et al[36]</td>
<td>2009</td>
<td>China</td>
<td>229</td>
<td>230</td>
<td>MPO −463</td>
<td>MPO −463G/A</td>
<td>52.3 ± 7.0</td>
<td>131/98</td>
</tr>
<tr>
<td>Zotova E, et al[31]</td>
<td>2009</td>
<td>Sweden</td>
<td>1213</td>
<td>1561</td>
<td>MPO −129</td>
<td>MPO −129A/G</td>
<td>60.2 ± 62.1</td>
<td>852/361</td>
</tr>
<tr>
<td>Zhang Hua, et al[36]</td>
<td>2009</td>
<td>China</td>
<td>69(ACS)</td>
<td>76</td>
<td>MPO −463</td>
<td>MPO −463G/A</td>
<td>52.2 ± 5.1</td>
<td>40/29</td>
</tr>
<tr>
<td>Zhao Jili, et al[35]</td>
<td>2009</td>
<td>China</td>
<td>220</td>
<td>105</td>
<td>MPO −463</td>
<td>MPO −463G/A</td>
<td>70.2 ± 8.7</td>
<td>114/106</td>
</tr>
<tr>
<td>Du Yongsheng, et al[34]</td>
<td>2010</td>
<td>China</td>
<td>191</td>
<td>95</td>
<td>MPO −463</td>
<td>MPO −463G/A</td>
<td>65.0 ± 9.5</td>
<td>106/86</td>
</tr>
<tr>
<td>Li Aihua, et al[35]</td>
<td>2010</td>
<td>China</td>
<td>219</td>
<td>70</td>
<td>MPO −463</td>
<td>MPO −463G/A</td>
<td>64.5 ± 9.8</td>
<td>126/93</td>
</tr>
<tr>
<td>Ergen A, et al[36]</td>
<td>2011</td>
<td>Turkish</td>
<td>100</td>
<td>100</td>
<td>MPO −463</td>
<td>MPO −463G/A</td>
<td>58.2 ± 11.1</td>
<td>76/24</td>
</tr>
<tr>
<td>Hu Junping, et al[37]</td>
<td>2011</td>
<td>China</td>
<td>267</td>
<td>78</td>
<td>MPO −129</td>
<td>MPO −129A/G</td>
<td>64.0 ± 11.0</td>
<td>158/109</td>
</tr>
<tr>
<td>Han Lili, et al[34]</td>
<td>2011</td>
<td>China</td>
<td>157</td>
<td>78</td>
<td>MPO −463</td>
<td>MPO −463G/A</td>
<td>62.9 ± 11.1</td>
<td>125/32</td>
</tr>
<tr>
<td>Lin Zhangzhao, et al[39]</td>
<td>2011</td>
<td>China</td>
<td>296</td>
<td>91</td>
<td>MPO −463</td>
<td>MPO −463G/A</td>
<td>63.0 ± 10.4</td>
<td>172/124</td>
</tr>
</tbody>
</table>

All the studies included in this Meta-analysis are case-control design. Mean value was used in all the included patients. ACS: Acronym for acute coronary syndromes; SAP: Stable angina pectoris.
**Figure 2** Subgroup analysis by ethnicity of ORs with a random-effect model for association between MPO –463 G/A gene polymorphism and CAD risk.

Figure 3 Subgroup analysis by ethnicity of ORs with a random-effect model for association between MPO −463 G/A gene polymorphism and SAP risk
A: Association between MPO −463 G/A gene polymorphism and SAP risk (A allele vs G allele); B: Association between MPO −463 G/A gene polymorphism and SAP risk (AA+AG vs GG); C: Association between MPO −463 G/A gene polymorphism and CAD risk (AA vs AG+GG)
2.3.3 Association between MPO −129 GG/AG + AA genotype polymorphism and the risk of CAD

Despite of MPO −463 G/A polymorphism, 3 articles [30, 32, 38] of all eligible case-control studies explored the role of MPO −129 GG/AG + AA polymorphism in CAD susceptibility. No comparison between allele and other genotypes were mentioned. So we only illustrated the relationship between MPO −129 AA + AG/GG and the risk of CAD. The heterogeneity was not obvious ($I^2=32\%$, $P=0.23$), thus fixed-effect model was applied. Nevertheless, MPO −129 GG/AG + AA genotype polymorphism did not show any statistical association with the risk of CAD (AA+GA vs GG: OR=0.91, 95%CI: 0.74–1.10, $P=0.23$).

In comparison of the subgroup analysis between Caucasian (AA+AG vs GG: OR=0.74, 95%CI: 0.48–1.14, $P=0.17$) and Asian (AA+AG vs GG: OR=0.95, 95%CI: 0.77–1.19, $P=0.67$) based on ethnicity, the result was similar (Figure 4).

2.4 Sensitivity analysis

Sensitivity analysis was performed to assess the influence of each individual study on the pooled ORs by omission of individual study. The analysis results showed that no individual study significantly affected the pooled ORs in both MPO −463 G/A and MPO −129 GG/AG + AA genotype polymorphism under the dominant model (Figure 5).

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Figure 4  Subgroup analysis by ethnicity of ORs with a fixed-effect model for association between MPO −129 A/G genotype polymorphism and CAD risk

A: Overall Meta-analysis of ORs with a fixed-effect model for association between MPO −129 A/G genotype polymorphism and CAD risk (GG vs AG+AA); B: Subgroup analysis of Caucasians by ethnicity of ORs with a fixed-effect model for association between MPO −129 A/G genotype polymorphism and CAD risk (GG vs AG+AA); C: Subgroup analysis of Asians by ethnicity of ORs with a fixed-effect model for association between MPO −129 A/G genotype polymorphism and CAD risk (GG vs AG+AA)
2.5 Publication bias

Begger’s funnel plot and Egger’s linear regression test were performed to estimate the publication bias of included studies. The shapes of the funnel plots did not reveal any evidence of significant asymmetry under the dominant model (Figure 6). Egger’s test also did not show any obvious statistical evidence of publication bias under the dominant model (MPO-463 G/A: t=0.69, P=0.501; MPO-129 GG/AG+AA: t=0.16, P=0.899)

2.6 Cumulative Meta-analysis

In the cumulative Meta-analysis of all trials, an evidence suggested the consistent result in both MPO-463 G/A and MPO-129 GG/AG+AA genotype polymorphism. The more studies were included, the more stable results were obtained (Figure 7).

A summary of the Meta-analysis findings on the association between MPO gene polymorphism and CAD risk is presented in Table 2.

Figure 6  Begger’s and Egger’s funnel plot of the Meta-analysis of MPO gene polymorphism with CAD risk

A: MPO-463 G/A gene polymorphism with CAD risk; B: MPO-129 A/G gene polymorphism with CAD risk
Figure 7  Cumulative analysis of the Meta-analysis of MPO gene polymorphism with CAD risk
A: MPO –463 G/A gene polymorphism with CAD risk; B: MPO –129 A/G gene polymorphism with CAD risk
Discussion

MPO, a member of the heme peroxidase superfamily, has been reported to be involved in the pathogenesis of CAD via inducing oxidative stress and inflammation. As shown in Figure 8, MPO exerts a proatherogenic effect through the reduction of nitric oxide bioavailability, the oxidation of low-density lipoprotein, and the generation of dysfunctional high-density lipoprotein and other REDOX signaling pathways \[^{[3,7-8]}\]. In recent years, several studies \[^{[9-10]}\] have confirmed that elevated concentrations of MPO are independently associated with increased risk of CAD. Both MPO \(-463\) G/A and MPO \(-129\) A/G gene are located upstream of the translation initiation codon of MPO gene.

The 2 genes have been reported that variant A allele could disrupt the SP1-binding site in an Alu hormone-responsive element and subsequently induce the down-regulation of MPO expression, which, in turn, likely decrease the enzyme levels \[^{[11]}\]. Therefore, it is reasonable to assume that the observation of an association between the MPO \(-463\) G/A and MPO \(-129\) A/G variant genotypes and the decreased risk of CAD might be related to the modulation effects of the variant genotypes on MPO concentrations. All of those mentioned above may lead to the individual differences in a variety of CAD susceptibility. However, to date, a number of studies \[^{[17,24-49]}\] on the relationship between MPO gene polymorphism and CAD have demonstrated the inconsistent results.

| Table 2 Meta-analysis of the association between MPO polymorphism and CAD risk |
|---------------------------------|--------|---------|-----------|--------|---------|-----------|--------|---------|-----------|
| Subgroups                       | A allele vs G allele | AA vs AG + GG | AA + AG vs GG |
| (sample size)                   | OR     | 95%CI   | P         | OR     | 95%CI   | P         | OR     | 95%CI   | P         |
| MPO \(-463\) and CAD risk      |        |         |           |        |         |           |        |         |           |
| Overall(15)                     | 0.58   | (0.47,0.72) | <0.001   | <0.01  | 0.38   | (0.26,0.57) | <0.001   | <0.001  | 0.58   | (0.46,0.72) | <0.001   | <0.001  |
| Ethnicity                       |        |         |           |        |         |           |        |         |           |
| Caucasians(4)                   | 0.79   | (0.58,1.08) | 0.015   | 0.11  | 0.54   | (0.24,1.23) | 0.025   | 0.018  | 0.82   | (0.61,1.11) | 0.061   | 0.075  |
| Asians(11)                      | 0.52   | (0.42,0.64) | <0.001   | 0.003 | 0.34   | (0.23,0.51) | <0.001   | 0.031  | 0.50   | (0.40,0.64) | <0.001   | 0.031  |
| MPO \(-129\) and CAD risk      |        |         |           |        |         |           |        |         |           |
| Overall(13)                     | 0.45   | (0.15,1.37) | <0.001   | <0.001 | 0.71   | (0.11,4.77) | 0.003   | 0.006  | 0.57   | (0.19,1.65) | 0.011   | 0.001  |
| Ethnicity                       |        |         |           |        |         |           |        |         |           |
| Caucasians(2)                   | 0.74   | (0.48,1.14) | 0.17   | 0.18  | 0.91   | (0.74,1.10) | 0.32   | 0.230  | 0.95   | (0.77,1.19) | 0.67   | 0.075  |
| Asians(11)                      |        |         |           |        |         |           |        |         |           |

\(Ph\): \(P\) value of heterogeneity test

Figure 8 Relationship between MPO and CAD
In the present study, we performed a Meta-analysis to explore the association of the SNPs, genotypes, and alleles in MPO gene with CAD. Fifteen studies about MPO –463 G/A including 3 449 cases and 3 082 controls and 3 articles about MPO –129 GG/AG + AA genotype polymorphism including 1 535 cases and 1 655 controls met the inclusion criteria. When all the eligible studies were pooled into the Meta-analysis, the results (Table 2) showed that there was a strong genetic effect of the MPO –463 G/A polymorphism on CAD whereas the MPO –463 G/A polymorphism had no effect on SAP and the MPO –129 GG/AG + AA polymorphisms had no effect on CAD as well.

Considering the existence of heterogeneity, we performed a stratified analysis based on ethnicity. For the MPO –463 G/A gene, the results showed that the MPO –463 G/A polymorphism might be closely correlated to CAD risk among Asians, while not among Caucasians. Special care must be taken when interpreting these results because only 4 studies about European were involved in the present study. So MPO –463 G/A polymorphism and CAD susceptibility in Europeans need further investigation. This analysis also did not find any association between MPO –463 G/A gene and SAP. It is consistent with the previous study[11] which found no significant changes of serum MPO level in SAP patients compared with the controls. No expression variation may not illustrate the existence of relationship between SNP polymorphism and SAP. It is possible that the pathogenesis of SAP may have less inflammation and oxidative stress compared with other types of CAD, and the serum MPO level did not significantly increased in SAP patients compared with normal controls[11]. Thus it is reasonable to speculate that the inexistence of MPO –463 G/A SNP polymorphism may illustrate the unchanged plasma MPO level in SAP patients.

For the MPO –129 gene, we found that there was no association between the MPO –129 GG/AG + AA polymorphisms and CAD in subgroup studies. Even though, the sensitivity analysis found that one study[31] influences the overall results. If this study was eliminated, the MPO –129 A/G gene polymorphism was associated with CAD. The reason may be that the number of CAD patients and controls in the study was the largest, which including 1 138 CAD patients and 1 477 controls, while all of the 3 articles recruited 1 535 patients and 1 655 controls. It has a power of 82% to detect associations between MPO –129 A/G gene polymorphism and CAD and it is likely to cause a false negative result. Therefore, the negative result in this study would obviously affect the overall evaluation. In addition, the discrepancy may be related to genetic differences between the Sweden and other ethnic populations, or the result which consisted with the Meta-analysis result represents a real relationship in all populations. In the cumulative Meta-analysis, the association was initially significant difference, but it tends toward null association after adding study reported by Zotova et al[31]. With the data increasing, the 95% CI for OR became narrower, indicating the strength of our result. Therefore, these results should be verified by large, well-designed epidemiologic population-based studies. Based on the results all above, ethnic differences in CAD susceptibility probably result from both genetic and epidemiological factors. The genetic factors refer to mutations in rare genes that confer high risk and mutation in specific genes and it may contribute to increased risks.

Some limitations of this Meta-analysis should be addressed. Firstly, there are potential pitfalls which could distort the results through the weakness and biases of genetic association studies such as study design, genotyping errors, population stratification, and publishing biases[31-33]. Secondly, a publication bias is another potentially important limitation. Although neither Egger’s test nor funnel plots found evidence for a publication bias in the current study (data not shown), negative studies are less likely to be published or take longer to be published, which may affect the validity of analysis[34]. Thirdly, the sample size is still relatively small and may not provide sufficient power to estimate the association between MPO gene polymorphism and CAD risk, especially the MPO –129 gene studied by 3 articles with only one genotype. Besides, the including studies in this Meta-analysis were from Asia (China) and Europe (Norway, Turkish, and Sweden). The data about other populations were limited, such as those from Oceania and Africa. So, considering the potential limitations of studies included in the current Meta-analysis, our results should be interpreted with caution. Nonetheless, it is well acknowledged that many other factors, such as gene-gene or gene-environment interactions may affect the risk of CAD.

In spite of these limitations, our Meta-analysis still have some merits and values. To the best of our knowledge, this is the first Meta-analysis which systematically investigates the association between 2 SNPs of MPO gene polymorphism (MPO –463 and MPO –129 GG/AG + AA genotype) and CAD risk, especially the analysis in SAP patients and subgroups based on ethnicity. Moreover, we have established an efficient searching strategy based on computer-assisted programs and manual searches, and checked more than 3 times, which allowed us to include as many studies as possible. On the basis of our
selection criteria, the quality of studies included in this Meta-analysis is sufficient. Explicit methods for study selection and the extraction and analysis of data were well designed before initiating. Finally, there were no evidence of publication bias in this Mate-analysis and the sensitivity analysis indicates that the results are statistically robust.

In conclusion, our Meta-analysis suggests that MPO –436 A variant genotype reduces the risk of CAD, especially in Asians, but has no effect on SAP subgroup. No sufficient evidence demonstrates that MPO –129 A/G gene polymorphism is related to the CAD risk. Numerous investigators have studied changes of serum MPO level in CAD patients. The concentration of MPO could be served as a diagnostic and prognostic indicator of CAD in clinic[6-14]. Combining cellular and genetic studies together may provide a more comprehensive mechanism of how MPO functions in the development of CAD, as well as promise a more effective method to prevent, timely diagnose, and treat this chronic disease. However, further studies are still needed to warrant and validate the association between MPO gene polymorphisms or other genetic polymorphism and CAD risk.

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Conflict of interest

The authors do not have any conflict of interest.

References


29. LIN Zhangchao. Relationship between myeloperoxidase 463G/A polymorphism and coronary heart disease[D]. Shijiazhuang: Hebei Medical University, 2011.


