PRISMA-combined Myeloperoxidase -463G/A gene polymorphism and coronary artery disease
A meta-analysis of 4744 subjects

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Abstract
Background: Myeloperoxidase (MPO) -463G/A gene polymorphism may be associated with an increased risk of developing coronary artery disease (CAD). Studies on the subject, however, do not provide a clear consensus. This meta-analysis was performed to explore the relationship between MPO gene -463G/A polymorphism and CAD risk.

Methods: This meta-analysis combines data from 4744 subjects from 9 independent studies. By using fixed or random effect models, the pooled odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were assessed.

Results: Our analysis found a significant association between MPO gene -463G/A polymorphism and CAD in the whole population under all genetic models: allelic (OR: 0.68, 95% CI: 0.54–0.85, P = 0.0009), recessive (OR: 0.41, 95% CI: 0.22–0.76, P = 0.005), dominant (OR: 0.682, 95% CI: 0.534–0.871, P = 0.002), homozygous (OR: 0.36, 95% CI: 0.16–0.79, P = 0.01), heterozygous genetic model (OR: 0.832, 95% CI: 0.733–0.945, P = 0.004), and additive (OR: 0.64, 95% CI: 0.46–0.90, P = 0.01), especially in the Chinese subgroup (P < 0.05). On the contrary, we found no such relationship in the non-Chinese subgroup (P > 0.05).

Conclusion: The MPO gene -463G/A polymorphism is associated with CAD risk, especially within the Chinese population. The A allele of MPO gene -463G/A polymorphism might protect the people from suffering the CAD risk.

Abbreviations: A = adenine, ACS = acute coronary syndrome, Apo A-I = ApolipoproteinA-I, CAD = coronary artery disease, CIs = confidence intervals, Cl- = chloride anion, G = guanine, H2O2 = hydrogen peroxide, HOCl = hypochlorous acid, HWE = Hardy-Weinberg equilibrium, LDL/HDL = low and high-density lipoprotein, MPO = myeloperoxidase, ORs = odds ratios, ox-LDL = oxidized low density lipoprotein, SP1 = specificity protein 1.

Keywords: -463G/A, coronary artery disease, gene, myeloperoxidase, polymorphism

1. Introduction
Coronary artery disease (CAD) describes a family of ischemic diseases characterized by the occlusion of the coronary artery lumen by atheromatous plaque. Plaque from acute coronary syndrome (ACS) patients has increased levels of myeloperoxidase (MPO) and its oxidative products, suggesting that MPO may be involved in the atherosclerotic process.

MPO, a member of the heme-peroxidase superfamily, is secreted by activated phagocytes and plays a crucial role in the antimicrobial innate immune response. It has a 150-kDa homodimeric structure consisting of two 15-kDa light chains and 2 variable-weight heavy chains with a prosthetic heme group. The primary reaction catalyzed by MPO is the degradation of hydrogen peroxide (H2O2) to hypochlorous acid (HOCl) and...
chloride anion (Cl\textsuperscript{–}). Although HOCl has strong anti-microbial and detoxification properties, it can also cause oxidative damage to host tissue. MPO is secreted primarily by myelocytes, with up to 95% of the circulating MPO found in the blood vessels derived from neutrophils.

The MPO gene, located in the 17q23.1, spans 14.638 kb and contains 12 exons and 11 introns.\textsuperscript{[4]} The -463G/A gene polymorphism involves a substitution of a guanine (G) for an adenine (A) in the upstream promoter region and acts in the cis-acting element for specificity protein 1 (SP1), a transcription factor. Four G\!u repetitive sequences are included in the cis-acting element. The G nucleotide in this MPO -463G/A gene polymorphism establishes the central binding site for the SP1 transcription factor that can induce up to a 25-fold increase in MPO gene transcription. Substitution of this central guanine with adenine significantly decreases the MPO gene transcription by reducing the promoter’s binding affinity, providing a plausible mechanism of how MPO gene transcription could influence CAD susceptibility.\textsuperscript{[9]}

Many studies on the association between the -463G/A gene polymorphism and CAD have been conducted, but have not provided a clear consensus. In 2006, Hao and Lu\textsuperscript{[6]} also identified the G allele as an independent risk factor for CAD and the A allele as protective against CAD in the Chinese population, an effect attributed to the polymorphism’s impact on transcription. Similarly, Nikpoor et al\textsuperscript{[7]} found the A allele of the MPO -463G/A gene polymorphism to be associated with fewer CAD cases and concluded that the MPO gene -463 G/A polymorphism influences the CAD risk. On the contrary, Lin and Zhang\textsuperscript{[8]} found no significant association of MPO -463G/A gene polymorphism with CAD in a separate Chinese population.

Given the lack of consensus on the relationship between MPO -463G/A gene polymorphism and CAD, we produced the current meta-analysis of 2454 CAD patients and 2290 controls.\textsuperscript{[9]}

2. Methods

2.1. Publication search and inclusion criteria

The current study was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University. The terms “coronary heart disease,” “myeloperoxidase,” “coronary artery disease,” “coronary heart disease,” and “polymorphism” were used to collect studies. The China Biological Medicine Database, China National Knowledge Infrastructure, Embase, PubMed, and the Web of Science were searched. Retrieved studies were published between 2001 and 2011 with the latest paper updated on February 21, 2017.

The selected studies had to conform to the following criteria: Assessment of the MPO -463G/A gene polymorphism and CAD; Diagnosis of CAD conducted in a manner consistent with criteria proposed by World Health Organization in 1979; and Genotypes in the control group follow the Hardy–Weinberg equilibrium (HWE).

2.2. Data extraction

Data were extracted as per a standardized protocol. Studies published in duplicates, those that did not meet inclusion criteria, and those with insufficient data were removed from the study. Overlapping data sets applied in separate publications were applied singly. The extracted data comprised the following items: the first author’s name, publication year, region, number of genotypes, genotyping, matching criteria, total number of cases and controls.

2.3. Statistical analysis

In the present meta-analysis, 6 genetic models as the allelic (A allele distribution frequency of MPO -463G/A gene polymorphism), recessive (AA vs GA+GG), dominant (AA+GA vs GG), homozygous (AA vs GG), heterozygous (GA vs GG), and additive genetic models (A vs G) were used. The odds ratio (OR) and their corresponding 95% confidence interval (CI) were used to compare the relationship between MPO -463G/A gene polymorphism and CAD. The Chi-square based Q-test was used to calculate the heterogeneity between studies and the significance was set at P < 0.05 level.\textsuperscript{[10]} The heterogeneity variation was evaluated by calculating the inconsistency index I\textsuperscript{2}. If heterogeneity was detected, the random-effects model (Der Simonian and Laird method) would be used to estimate the pooled OR.\textsuperscript{[11]} If no heterogeneity is detected, the fixed-effects model (Mantel–Haenszel method) would be adopted.\textsuperscript{[12]} The pooled OR would be determined by using the Z test with significance set at P < 0.05 level.

The HWE was evaluated by using the Fisher exact test and the significance was set at P < 0.05 level. Potential publication bias would be estimated by using funnel plot. The Egger linear regression test on the natural logarithm scale of the OR was used to assess the funnel plot asymmetry and the significance was set at P < 0.05 level.\textsuperscript{[13]} The STATA 12.0 software (StataCorp, College Station, TX) and review manager 5.0 were used to perform the statistical analysis.

3. Results

3.1. Studies and populations

Among the 19 papers produced from our initial search, 9 papers met the inclusion criteria. In total, data were extracted from 2454 CAD patients and 2290 controls (Table 1).\textsuperscript{[6–8,14–19]} Patients

| Author          | Year | Region   | Ethnicity | GG  | GA | AA  | GG  | GA | AA  | Matching criteria | Sample size (CAD/control) |
|-----------------|------|----------|-----------|-----|----|-----|-----|----|-----|-------------------|---------------------------|
| Zotova et al\textsuperscript{[14]} | 2009 | Sweden   | Non-Chinese | 682 | 368 | 47  | 854 | 488 | 72  | Age, sex, ethnicity | 1097/1414                  |
| Nikpoor et al\textsuperscript{[7]}  | 2001 | Canada   | Non-Chinese | 151 | 75  | 3   | 120 | 76  | 19  | Age, sex, ethnicity | 220/217                   |
| Li and Bao\textsuperscript{[8]}     | 2007 | China    | Chinese   | 68  | 10  | 1   | 48  | 20  | 1   | Age, sex, ethnicity | 79/69                     |
| Ergen et al\textsuperscript{[16]}   | 2011 | Turkey   | Non-Chinese | 27  | 52  | 21  | 33  | 47  | 20  | Age, sex, BMI, ethnicity | 100/100                   |
| Zhang et al\textsuperscript{[17]}   | 2009 | China    | Chinese   | 148 | 69  | 3   | 55  | 44  | 6   | Age, sex, ethnicity | 220/215                   |
| Lin and Zhang\textsuperscript{[8]}  | 2011 | China    | Chinese   | 217 | 70  | 9   | 66  | 22  | 3   | BMI, ethnicity       | 296/91                    |
| Hao and Lu\textsuperscript{[6]}     | 2006 | China    | Chinese   | 68  | 35  | 2   | 53  | 39  | 13  | Age, sex, ethnicity | 105/105                   |
| Han and Shen\textsuperscript{[18]}  | 2007 | China    | Chinese   | 101 | 49  | 7   | 39  | 28  | 11  | Age, ethnicity       | 157/78                    |
| Zhang and Ma\textsuperscript{[10]}  | 2006 | China    | Chinese   | 135 | 36  | 0   | 71  | 38  | 2   | Age, ethnicity       | 171/111                   |

Case-control study design and PCR-RFLP genotyping method were used in all of the above studies.

BMI = body mass index, CAD = coronary artery disease, MPO = myeloperoxidase, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, Year = publication year.
were from China, Sweden, Canada, and Turkey and were of either Chinese or non-Chinese descent. Of the 10 studies removed from our initial search, 2 papers were duplicates and 3 were reviews on the topic. Two other papers did not address either MPO-463G/A gene polymorphism or CAD. Three studies were excluded for deviating from HWE.\(^\text{[20–22]}\)

### 3.2. Pooled analyses

There was a significant association between MPO gene -463G/A polymorphism and CAD in the whole population under allelic (OR = 0.68, 95% CI: 0.54–0.85, \(P = 0.0009\)), recessive (OR = 0.41, 95% CI: 0.22–0.76, \(P = 0.005\)), dominant (OR = 0.682, 95% CI: 0.534–0.871, \(P = 0.002\)), homozygous (OR = 0.36, 95% CI: 0.16–0.79, \(P = 0.01\)), heterozygous genetic model (OR = 0.832, 95% CI: 0.733–0.945, \(P = 0.004\)), and additive genetic models (OR = 0.64, 95% CI: 0.46–0.90, \(P = 0.01\)) (Table 2, Figs. 1–6).

Under the heterozygous genetic model, no heterogeneity was detected in the whole population. Although the whole population displayed significant heterogeneity under all genetic models (\(P_{\text{heterogeneity}} < 0.05\)), heterogeneity was only detected under the

### Table 2

Summary of meta-analysis of association of MPO-463G/A gene polymorphism and CAD.

| Genetic model | Pooled OR (95% CI) | Z value | \(P\) | Literature number | CAD size | Control size | \(P_{\text{heterogeneity}} (I^2\%)\) |
|---------------|-------------------|---------|------|-----------------|---------|-------------|------------------|
| Allelic genetic model | 0.68 (0.54–0.85) | 3.32 | 0.0009 \(^\text{a}\) | 9 | 2454 | 2200 | 0.0004 (72.0%) |
| Chinese subgroup | 0.58 (0.47–0.71) | 5.09 | 3.58×10^{-4} | 9 | 2454 | 2200 | 0.31 (16.0%) |
| Non-Chinese subgroup | 0.85 (0.62–1.18) | 0.95 | 0.34 | 3 | 1426 | 1731 | 0.02 (76.0%) |
| Recessive genetic model | 0.41 (0.22–0.70) | 2.84 | 0.005 | 6 | 1028 | 559 | 0.004 (64.0%) |
| Chinese subgroup | 0.29 (0.16–0.50) | 3.81 | 0.0004 \(^\text{a}\) | 6 | 1028 | 559 | 0.38 (6.0%) |
| Non-Chinese subgroup | 0.58 (0.24–1.41) | 1.20 | 0.23 | 3 | 1426 | 1731 | 0.01 (78.0%) |
| Dominant genetic model | 0.682 (0.534–0.871) | 3.06 | 0.002 | 9 | 2454 | 2200 | 0.007 (61.8%) |
| Chinese subgroup | 0.573 (0.452–0.725) | 4.64 | 3.48×10^{-4} | 6 | 1028 | 559 | 0.37 (7.3%) |
| Non-Chinese subgroup | 0.883 (0.642–1.219) | 0.77 | 0.44 | 6 | 1028 | 559 | 0.00 (58.5%) |
| Hetero genetic model | 0.36 (0.16–0.70) | 2.56 | 0.01 | 9 | 2454 | 2200 | 0.0002 (73.0%) |
| Chinese subgroup | 0.24 (0.11–0.50) | 3.77 | 0.0002 \(^\text{a}\) | 6 | 1028 | 559 | 0.26 (24.0%) |
| Non-Chinese subgroup | 0.61 (0.16–2.34) | 0.72 | 0.47 | 3 | 1426 | 1731 | 0.001 (85.0%) |
| Hetero genetic model | 0.832 (0.773–0.945) | 2.94 | 0.004 | 9 | 2454 | 2200 | 0.057 (47.1%) |
| Chinese subgroup | 0.629 (0.496–0.793) | 3.91 | 9.23×10^{-4} | 6 | 1028 | 559 | 0.4 (2.6%) |
| Non-Chinese subgroup | 0.934 (0.803–1.068) | 0.88 | 0.37 | 3 | 1426 | 1731 | 0.32 (17.7%) |
| Additive genetic model | 0.64 (0.46–0.90) | 2.59 | 0.01 \(^\text{a}\) | 9 | 2454 | 2200 | <0.00001 (84.0%) |
| Chinese subgroup | 0.53 (0.36–0.70) | 3.16 | 0.002 | 6 | 1028 | 559 | 0.004a (71.0%) |
| Non-Chinese subgroup | 0.91 (0.49–1.69) | 0.29 | 0.77 | 3 | 1426 | 1731 | <0.0001a (90.0%) |
| Additive genetic model: total A vs total G; Allelic genetic model: distribution of A allelic frequency of MPO-463G/A polymorphism; CAD = coronary artery disease; CAD size = the total number of CAD cases; CI = confidence interval; control size: the total number of control group; Dominant genetic model: AA+GA vs GG; hetero genetic model: heterozygous genetic model, GA vs GG; homo genetic model: homozygous genetic model: AA vs GG; OR = odds ratio; recessive genetic model: AA vs GA+GG; MPO = myeloperoxidase.

\(^{a}\)P < 0.05.

**Figure 1.** Forest plot of CAD associated with MPO-463G/A gene polymorphism stratified by ethnicity under an allelic genetic model (distribution of A allelic frequency of MPO-463G/A gene polymorphism). A = adenine, CAD = coronary artery disease, G = guanine, MPO = myeloperoxidase.
Figure 2. Forest plot of CAD associated with MPO -463G/A gene polymorphism stratified by ethnicity under a recessive genetic model (AA vs GA+GG). A = adenine, CAD = coronary artery disease, G = guanine, MPO = myeloperoxidase.

Figure 3. Forest plot of CAD associated with MPO -463G/A gene polymorphism stratified by ethnicity under a dominant genetic model (AA+GA vs GG). A = adenine, CAD = coronary artery disease, G = guanine, MPO = myeloperoxidase.
Figure 4. Forest plot of CAD associated with MPO -463G/A gene polymorphism stratified by ethnicity under a homozygous genetic model (AA vs GG). A = adenine, CAD = coronary artery disease, G = guanine, MPO = myeloperoxidase.

Figure 5. Forest plot of CAD associated with MPO -463G/A gene polymorphism stratified by ethnicity under a heterozygous genetic model (GA vs GG). A = adenine, CAD = coronary artery disease, G = guanine, MPO = myeloperoxidase.
additive model when looking at the Chinese subgroup ($P_{\text{heterogeneity}} > 0.05$). In contrast, the non-Chinese subgroup still showed significant heterogeneity under the allelic, recessive, homozygous, and additive genetic models ($P_{\text{heterogeneity}} < 0.05$). This suggests ethnicity as a primary source of the heterogeneity in the current meta-analysis (Table 2, Figs. 1–6).

In the Chinese subgroup, a significant association between them was found under the allelic (OR: $0.58$, 95% CI: $0.47$–$0.71$, $P = 3.38 \times 10^{-7}$, $P_{\text{heterogeneity}} = 0.31$), recessive (OR: $0.29$, 95% CI: $0.16$–$0.55$, $P = 0.0001$, $P_{\text{heterogeneity}} = 0.38$), dominant (OR: $0.573$, 95% CI: $0.452$–$0.725$, $P = 3.48 \times 10^{-6}$, $P_{\text{heterogeneity}} = 0.37$), homozygous (OR: $0.24$, 95% CI: $0.11$–$0.50$, $P = 0.0002$, $P_{\text{heterogeneity}} = 0.26$), heterozygous (OR: $0.629$, 95% CI: $0.498$–$0.793$, $P = 9.23 \times 10^{-5}$, $P_{\text{heterogeneity}} = 0.40$), and additive genetic models (OR: $0.53$, 95% CI: $0.36$–$0.79$, $P = 0.002$, $P_{\text{heterogeneity}} = 0.004$).

In the non-Chinese subgroup, no significant association between them was found under the allelic (OR: $0.85$, 95% CI: $0.62$–$1.18$, $P = 0.34$, $P_{\text{heterogeneity}} = 0.02$), recessive (OR: $0.58$, 95% CI: $0.24$–$1.41$, $P = 0.23$, $P_{\text{heterogeneity}} = 0.01$), dominant (OR: $0.883$, 95% CI: $0.642$–$1.213$, $P = 0.441$, $P_{\text{heterogeneity}} = 0.09$), homozygous (OR: $0.61$, 95% CI: $0.16$–$2.34$, $P = 0.47$, $P_{\text{heterogeneity}} = 0.001$), heterozygous (OR: $0.934$, 95% CI: $0.803$–$1.086$, $P = 0.577$, $P_{\text{heterogeneity}} = 0.322$), and additive genetic models (OR: $0.91$, 95% CI: $0.49$–$1.69$, $P = 0.77$, $P_{\text{heterogeneity}} < 0.0001$).

3.3. Bias diagnostics

The publication bias of the individual studies was evaluated by the funnel plot and Egger test. No visual publication bias was detected in the funnel plot under the allelic genetic model (Fig. 7).

No statistically significant difference was detected in the Egger test that implies no publication bias in the present meta-analysis existed by using additive genetic model ($T = -2.08$, $P = 0.076$) (Fig. 8).

4. Discussion

Our meta-analysis on the relationship between MPO gene -463G/A polymorphism and CAD found significant correlation under allelic (OR: $0.68$), recessive (OR: $0.41$), dominant (OR:...


1. Introduction

Methylenecyclohexane peroxidase (MPO) is a key enzyme in oxidizing low-density lipoprotein (LDL) and high-density lipoprotein (HDL) to oxidized LDL (ox-LDL) and HDL, respectively.

2. Results

Summarily, our data suggest that the MPO -463G/A gene polymorphism under an additive genetic model (total A vs total G). The horizontal and vertical axis correspond to the OR and confidence limits. A = adenine, CAD = coronary artery disease, G = guanine, MPO = myeloperoxidase, OR = odds ratio, SE = standard error.

3. Discussion

Although MPO is an important component of the innate immune response, but its expression is closely linked to atherosclerotic formation. It is used clinically as a marker for local inflammation in the coronary artery and has even demonstrated to be an independent marker for myocardial infarction.

4. Conclusion

The present meta-analysis indicates that the MPO gene -463G allele might increase CAD risk, especially in the Chinese population. It is our hope that the conclusion assists researchers in the search for an individualized treatment for CAD and that

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6. Limitations

Despite the limitations of the current meta-analysis, we hope that this study will offer other researchers a comprehensive and improved perspective on the relationship between the MPO -463G/A polymorphism and CAD over past meta-analyses.

7. Future directions

In addition to the inevitable measurement errors, the large-scale research on the relationship between CAD and MPO gene -463G/A polymorphism are still needed. Inevitably, plasma MPO levels are influenced by other genetic polymorphisms, such as the MPO gene -129A/G polymorphism and environmental factors, such as ethnicity, smoke, hypertension, hyperlipidemia, and diabetes mellitus.

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future studies will build upon this study to further elucidate this important area of research.

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