MMP-9-C1562T polymorphism and susceptibility to chronic obstructive pulmonary disease
A meta-analysis
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Abstract
Background: To comprehensively evaluate the association between the polymorphism of matrix metalloproteinase-9 (MMP-9)-C1562T (rs3918242) and susceptibility to chronic obstructive pulmonary disease (COPD) in middle-aged and elderly patients through Meta-analysis.

Methods: PubMed, EMBASE, CNKI, Wanfang, VIP, and other databases were searched by computer in the inception to August 2019 to collect all the case-control studies that met the inclusion criteria in this literature. Meta-analysis was performed using Stata 15.0, including the OR value calculations of the association between the merged MMP-9-C1562T polymorphism and the COPD susceptibility. Subgroup analysis, sensitivity analysis, and publication bias test were also performed. A total of 13 literature were included in this Meta-analysis with a total of 2512 cases and 2716 controls.

Results: The results have shown that the OR of MMP-9-C1562T T allele to C allele was 0.35 (95% confidence interval [CI]: 0.23–0.52, P < .01). The subgroup analysis of ethnicity result showed that the merged OR of MMP-9-C1562T T allele to C allele was 0.24 (95% CI: 0.17–0.34, P < .01) in Caucasian while the merged OR was 0.62 (95% CI: 0.22–1.70, P > .05) in Asian. However, there were no statistically significant models in the dominant, recessive, homozygote and heterozygote genetic models.

Conclusion: The MMP-9-C1562T polymorphism was associated with the susceptibility to middle-aged and elderly COPD patients. Compared with T allele, C allele increased the risk of disease, especially in Caucasian, but not found in Asian.

Abbreviations: α1-AT = α1-antitrypsin, COPD = chronic obstructive pulmonary disease, MMP-9 = matrix metalloproteinase-9, MMPs = matrix metalloproteinases, NOS = Newcastle-Ottawa scale, TIMPs = tissue inhibitor of metalloproteinases.

Keywords: chronic obstructive pulmonary disease, meta-analysis, matrix metalloproteinase-9, polymorphism, rs3918242

1. Introduction
Chronic obstructive pulmonary disease (COPD) is a chronic airway disease involving chronic local and systemic inflammatory changes, clinically characterized by continuous and progressive airflow obstruction with airway remodeling and lung parenchyma destruction as pathological basis.[1] It is one of diseases that seriously affect human health and life quality worldwide. In 2013, COPD has become the third leading cause of death for residents in China, resulting in >900 thousand deaths.[2] A nationwide cross-sectional study based on 10 provinces (or autonomous regions) in mainland China that was recently published in Lancet magazine, have shown that the prevalence of COPD is 8.6% in people over 20 years old while 13.7% in people aged 40 and over.[3] To find out the pathogenesis of COPD and its corresponding treatment has become an important research direction. The existing research results indicate that COPD is the result of a combination of environmental and genetic factors. The former is mainly cigarette smoking, in addition to occupational dust exposure and indoor air pollution represented by fumes from kitchen and fuel combustion. 85% of COPD patients are long-term smokers, but only 10% to 20% will develop into symptomatic COPD patients, indicating that individuals have different susceptibility to COPD, which is the phenotype of gene polymorphism.[4] The genetic factor has been proved to be the α1-antitrypsin (α1-AT) deficiency, which is closely associated with early-onset emphysema and smoking increases the risk of its incidence. Researchers at home and abroad attach great importance to the role genetic factor plays in the occurrence and development of COPD, and many genes have been selected as candidate genes for COPC molecular genetic research. Matrix metalloproteinases (MMPs) is a family of calcium- and zinc-dependent proteinase. There are currently at least 26 subtypes that can degrade almost all extracellular matrix and basement...
membrane components. Matrix metalloproteinase-9 (MMP-9) is one of the key members in the MMP family, mainly secreted by macrophages, neutrophils, and eosinophils in the alveoli and can also be produced by lung tissue cells under certain conditions. The MMP-9 gene is located on human chromosome 16, including 13 exons and 12 introns and its regulation mainly occurs at the transcriptional level. In the pathogenesis of COPD, MMP-9 mainly degrades the extracellular matrix and basement membrane of alveolar wall, destroying the normal structure of lung tissue. At the same time, MMP-9 also repairs the extracellular matrix and participates in respiratory tract reconstruction. In addition, MMP-9 can also participate in inflammatory response, causing inflammatory cells to accumulate in the airway, thus increasing airway responsiveness. Study found that MMP-9 is highly expressed in the lung tissues of COPD patients and leads to generation of sputum. Therefore, the analysis of MMP-9 gene polymorphism is an important starting point to explore the susceptibility to COPD. It has been found that there is a mutation from C to T at site 1562 of promoter MMP-9, which may affect the expression level of MMP-9 gene. The MMP-9-C1562T polymorphism is an important reason for the abnormal increase of MMP-9 expression level. In the past few years, several studies have reported the relationship between MMP-9-C1562T and susceptibility to COPD in middle-aged and elderly patients, but the conclusions are very different and inconclusive. Therefore, it is necessary to apply Meta-analysis to collect and analyze the data of each independent study to quantitatively understand the correlation between the both. In this study, we further analyzed the relationship between MMP-9-C1562T polymorphism and susceptibility to COPD in middle-aged and elderly patients, so as to provide theoretical basis and corresponding evidence-based medical basis for clinical medicine and other professional researches.

2. Materials and methods

2.1. Literature retrieval

PubMed, EMBASE, CNKI, Wanfang, VIP, and other databases were searched by computer in the inception to August 2019, with “matrix metalloproteinase,” “matrix metalloproteinase 9,” “MMP-9,” “gene polymorphism,” “rs3918242,” “SNP,” “chronic obstructive pulmonary disease,” “COPD” as keywords. All the case-control studies that met the inclusion criteria were collected. The retrieval papers were limited to English and Chinese, and the unpublished papers were not included.

2.2. Inclusion and exclusion criteria

2.2.1. Inclusion criteria. Studies on the association between MMP-9-C1562T polymorphism and COPD susceptibility; case-control studies with the average age over 40 years old in the cases; complete data or statistical indicators OR (95% CI) provided directly or indirectly.

2.2.2. Exclusion criteria. Simple case studies, case reports, reviews, and comments; repeatedly published literature with incomplete information; controls were not in accordance with Hardy-Weinberg (H-W) genetic balance.

2.3. Data extraction

Two researchers screened and extracted data independently, and disagreement was resolved by discussion or assistance of the third researcher. The extracted information was included as following: first author, year of publication, country, race, sample size of cases and controls, age of cases, number of genotypes, sequencing method, source of controls. For the H-W balance test of the controls, cases with \( P < 0.05 \) were considered to be not in accordance with H-W balance.

2.4. Literature quality evaluation

Read the full text. According to the Newcastle-Ottawa scale (NOS), the literature quality was evaluated. Literature awarded below 6 stars are low-quality literature, while 6 stars and above are high-quality ones. Only the latter ones were included in this study. According to the uniform quality standard, two assessors evaluated independently to extract data, and then performed cross-check. Discrepancies were resolved by discussion or assistance of the third researcher.

2.5. Statistical method

Meta-analysis was performed by Stata 15.0 statistical software (Texas, USA). \( Q \) test was used to test the heterogeneity of each research result. If \( I^2 \geq 50\% \) or \( P < 0.05 \) suggested heterogeneity, the random effect model (REM) can be used for data merging. If \( I^2 < 50\% \) and \( P > 0.05 \) suggested no heterogeneity, the fixed effect model (REM) can be used. \( Z \) test was used to test the significance of the pooled OR, and subgroup analysis of ethnicity was performed. In this Meta-analysis, funnel plot and Egger test were used to evaluate publication bias. Funnel plot was drawn by using the standard error of log (OR) and OR. The asymmetric funnel plot indicates publication bias.

3. Results

3.1. Literature retrieval basic information

According to the criteria, a total of 13 literature was included, including 7 in Europe and the United States and 7 in Asia. There were 2512 patients in COPD group and 2716 in the control group. The specific screening process is shown in Fig. 1. The study characteristics and genotype frequencies are shown in Table 1.

3.2. Meta-analysis results

3.2.1. Allele comparison. The result of Meta-analysis is shown in Table 2 and Fig. 2. T allele was compared with C allele, there was statistically significant heterogeneity among studies \( (I^2 = 93.8\%, P < 0.05) \), so the REM was used. The final result showed that there was statistically significant difference (pooled OR = 0.35, 95% CI: 0.23–0.52, \( P < .01 \)). According to the subgroup analysis of ethnicity, the results showed that there was statistically significant heterogeneity in Caucasian \( (OR = 0.24, 95\% CI: 0.17–0.34, P < .01) \) but not in Asian \( (OR = 0.62, 95\% CI: 0.23–1.70, P > .05) \). Forest plot is shown in Fig. 2A. This suggested that MMP-9-C1562T polymorphism was associated with susceptibility to COPD in middle-aged and elderly patients, and allele C is a risk factor for COPD patients. The symmetry deviation of funnel plot (Fig. 3A) and Egger test indicated that there was a certain publication bias \( (P < .05) \).

3.2.2. Dominant genetic model. TT+TC genotype was compared with CC genotype. There was statistically significant heterogeneity among studies \( (I^2 = 66.8\%, P < .05) \), so the REM was used. The result showed that there was no statistically significant difference (pooled OR = 1.18, 95% CI: 0.93–1.50, \( P = .27 \)).
The subgroup analysis showed the same results (Fig. 2B). The Caucasian and Asian dominant genetic models were not statistically significant. This suggested that MMP-9-C1562T polymorphism could not be associated with COPD in middle-aged and elderly patients. The funnel plot was basically symmetrical (Fig. 3B), and Egger test showed that the publication bias was well controlled ($P > .05$).

### 3.2.3. Recessive genetic model

TT genotype was compared with TC+CC genotype. There was no statistically significant heterogeneity among studies ($I^2 = 31.0\%$, $P > .05$), so the FEM was used. The result showed that there was no statistically significant difference (pooled OR = 1.11, 95% CI: 0.79–1.58, $P > .05$). The result of subgroup analysis was the same (Fig. 2C). The Caucasian and Asian dominant genetic models were not statistically significant. This suggested that MMP-9-C1562T polymorphism could not be considered to be associated with susceptibility to COPD in middle-aged and elderly patients. The funnel plot was basically symmetrical (Fig. 3C) and Egger test showed that the publication bias was well controlled ($P > .05$).

### 3.2.4. Homozygote model

TT genotype was compared with CC genotype. There was no statistically significant heterogeneity among studies ($I^2 = 41.8\%$, $P > .05$), so the FEM was used. The result showed that there was no statistically significant difference (pooled OR = 1.19, 95% CI: 0.84–1.69, $P > .05$). The result of subgroup analysis was the same (Fig. 2D). The Caucasian and Asian dominant genetic models were not statistically significant. This suggested that MMP-9-C1562T polymorphism could not be considered to be associated with susceptibility to COPD in middle-aged and elderly patients. The funnel plot was basically symmetrical (Fig. 3D) and Egger test showed that the publication bias was well controlled ($P > .05$).

### 3.2.5. Heterozygote model

TC genotype was compared with CC genotype. There was statistically significant heterogeneity among studies ($I^2 = 62.0\%$, $P < .05$), so the REM was used. The result showed that there was no statistically significant difference (pooled OR = 1.18, 95% CI: 0.94–1.48, $P > .05$). The result of subgroup analysis was the same (Fig. 3E). The Caucasian and Asian dominant genetic models were not statistically significant. This suggested that MMP-9-C1562T polymorphism could not be considered to be associated with susceptibility to COPD in middle-aged and elderly patients. The funnel plot was basically symmetrical (Fig. 3E) and Egger test showed that the publication bias was well controlled ($P > .05$).
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| Genetic models | N   | OR   | 95% CI | P    | I² (%) | P for heterogeneity | Model | P for publication bias |
|----------------|-----|------|--------|------|--------|---------------------|-------|------------------------|
| Allelic model  |     |      |        |      |        |                     |       |                        |
| Asian          | 13  | 0.35 | 0.23–0.52 | 0.00 | 93.8  | .000                | REM   | .000                   |
| Caucasian      | 6   | 0.62 | 0.22–1.70 | 3.50 | 96.6  | .000                | REM   | .021                   |
| Dominant model | 13  | 1.18 | 0.93–1.50 | 0.163| 66.8  | .000                | REM   | .011                   |
| Asian          | 6   | 1.57 | 0.87–2.84 | 0.137| 82.9  | .000                | REM   | .093                   |
| Caucasian      | 7   | 1.02 | 0.87–1.19 | 0.808| 0.0   | .595                | FEM   | .307                   |
| Recessive model| 12  | 1.11 | 0.79–1.58 | 0.548| 31.0  | .143                | FEM   | .516                   |
| Asian          | 5   | 1.21 | 0.73–2.01 | 0.469| 63.1  | .029                | REM   | .425                   |
| Caucasian      | 7   | 1.04 | 0.64–1.67 | 0.965| 0.0   | .908                | FEM   | .463                   |
| Homozygous model | 12  | 1.19 | 0.84–1.69 | 0.320| 41.8  | .063                | FEM   | .396                   |
| Asian          | 5   | 1.38 | 0.83–2.32 | 0.218| 69.1  | .012                | REM   | .357                   |
| Caucasian      | 7   | 1.05 | 0.65–1.69 | 0.851| 0.0   | .610                | FEM   | .486                   |
| Heterozygous model | 13  | 1.18 | 0.94–1.48 | 0.159| 62.0  | .002                | REM   | .007                   |
| Asian          | 6   | 1.57 | 0.89–2.76 | 0.118| 79.8  | .000                | REM   | .064                   |
| Caucasian      | 7   | 1.02 | 0.87–1.19 | 0.843| 0.0   | .596                | FEM   | .329                   |

COPD = chronic obstructive pulmonary disease.

3.3. Sensitivity analysis

The result of sensitivity analysis is shown in Fig. 4. Each study was excluded one by one and Meta-analysis was performed. The result showed that in dominant genetic model, the OR value was statistically significant after excluding the result of Lee et al.[24] indicating that it has a great influence on the result of dominant genetic model. The combined effect size of other genetic models did not change significantly, indicating that 14 included literature were stable.

4. Discussion

COPD is a common and frequently occurring disease of human respiratory system. It is recognized as one of the main diseases threatening human health, clinically characterized by cough, expectoration, shortness of breath, and decreased activity endurance, and complicated with progressive airflow obstruction and decreased lung function. COPD now is an important public health problem.[25] Its important pathogenesis includes oxidative imbalance, but lung tissue destruction has been fully studied in cellular and molecular level. MMPs are a part of a large metalloendopeptidase superfamily. They typically consist of a pro-domain and a catalytic domain. The latter contains a zinc ion in the active site, as well as a characteristic methionine residue downstream of the zinc binding site.[26] MMP-9 is a member of metalloproteinases family, which can be synthesized and released by various cells including alveolar macrophages. MMP-9 is 1 of the 2 gelatinases and the other is MMP-2. Gelatinase not only contains the original structure of MMPs, but fibronectin type II-like repeats in its catalytic region, leading to its higher binding affinity to gelatin and elastin.[27] MMP-9 contains collagen fiber type V-like region with high glycosylation, which may affect the specificity and anti-degradation of the substrate. MMP-9 is released by cells in the form of zymogens. In vitro, MMP-9 can be activated by organic mercury while in vivo, by a variety of proteases,[28] including MMP-3 (matrix degrading enzyme), MMP-2, or hypochloric acid.[29] Among them, MMP-3 may be the most effective activator.[30] The most effective inhibitor in the cycle is the α 2-macroglobulin. The activity of MMP-9 is closely related to the regulation of specific endogenous inhibitors: tissue inhibitor of metalloproteinases (TIMPs). TIMP1,2,3,4 are now known has affinity to MMP-9.[31,32] Although environmental factors, including smoking, are the main risk factors for COPD, genetic factors are also closely related to its incidence.[33,34] The combination of these 2 factors leads to COPD, which involves multiple pathogenesis, such as inflammatory response, protease-antiprotease, and oxidation–antioxidation imbalances. Each pathogenesis is interrelated and interact with each other.[35,36]

Previous studies on the pathogenesis of COPD have found many COPD susceptible genes and their single nucleotide polymorphisms (SNP). At present, there are many studies related to the relationship between MMP-9-C1562T and susceptibility to COPD, but the conclusions are inconsistent. Minematsu et al.[12] have suggested that people who carry the C allele in MMP-9-C1562T polymorphism have a lower risk of COPD than those with T allele, indicating that TT genotype may be a high risk genetic factor for COPD. However, some studies have come to the opposite conclusion. Gilowska et al.[23] have believed that people who carry the T allele in MMP-9-C1562T polymorphism have a lower risk of COPD than those with C allele, which is consistent with the result of Lee et al.[24] Therefore, in order to comprehensively evaluate the relationship between MMP-9-C1562T polymorphism and the risk of COPD, this study conducted a Meta-analysis of MMP-9-C1562T polymorphism and susceptibility to COPD in middle-aged and elderly patients.

A total of 13 literature[22–24] were included in this study, among which 6 from Asia and 7 from Europe and America. The results showed that there was a strong correlation between MMP-9-C1562T gene polymorphism and susceptibility to COPD in middle-aged and elderly patients. According to the subgroup analysis of ethnicity, compared with C allele, the T allele showed that there was statistical significance in Caucasian
(OR = 0.24, 95% CI: 0.17–0.34, P < .01) but not in Asian (OR = 0.62, 95% CI: 0.23–1.70, P > .05). It suggested that MMP-9-C1562T gene polymorphism was associated with susceptibility to COPD in middle-aged and elderly patients, especially in Caucasian, but not in Asian. The results of funnel plot of allele model and Egger test indicated that there was a certain publication bias (P < .05). The results heterogeneity test showed that there was heterogeneity among the studies (I² > 50%). The result of sensitivity analysis after excluding a single literature showed that there was no significant change in the combined

Figure 2. Forest plot for the association between MMP-9-C1562T and COPD (A: Allelic model; B: Dominant model; C: Recessive model; D: Homozygous model; E: Heterozygote model).
effect size of allele model, indicating that the included literature have good stability. Therefore, it could be considered that there was a correlation between MMP-9-C1562T polymorphism and susceptibility to COPD in middle-aged and elderly patients, and those with C alleles were more susceptible to COPD than those with T alleles. In the dominant genetic model, there was no significant difference (pooled OR = 1.18, 95% CI: 0.93–1.50, P > .05). The same result was found in subgroup analysis of ethnicity, and there was no statistical significance in Caucasian and Asian in dominant genetic models. However, the result of sensitivity showed that the result of Lee et al[24] has a great influence on the result in dominant genetic model. The difference

Figure 3. Funnel plot for the assessment of publication bias (A: Allelic model; B: Dominant model; C: Recessive model; D: Homozygous model; E: Heterozygote model).
was statistically significant after excluding it. But in the recessive, homozygote and heterozygote genetic model, there was no statistical significance, indicating that publication bias was well controlled and the result of sensitivity analysis was stable. A previous Meta-analysis of MMP-9 gene polymorphism and susceptibility to COPD in middle-aged and elderly patients by Chen et al.[37] has showed that compared with C allele, T allele was a high risk factor for COPD patients. The dominant genetic model in their Meta-analysis has statistical significance, and TT genotype is one of the risk genotypes. The results of the dominant genetic model are related to the results of the sensitivity analysis in this study. Therefore, the conclusion of the dominant genetic model should be careful. However, their conclusion of alleles is contrary to our study, the reason of which laying on the inclusion of the literature. The latest studies were included in this study, and the control groups that were not in accordance with Hardy Weinberg Equilibrium (HWE) were excluded. Therefore, to a certain extent, the conclusion of this study is reliable.

Certainly, this study also has some limitations: there was great heterogeneity among alleles for both Asian and Caucasian; the
results of sensitivity analysis of dominant genetic model showed that the conclusion was unstable to some extent; there was a certain publication bias in allele model and dominant genetic model; the effects of gene linkage and gene–environment interaction in middle-aged and elderly COPD patients were not analyzed.

To sum up, MMP-9/C1562T polymorphism was associated with susceptibility to COPD in middle-aged and elderly patients, and the risk of C allele was higher than that of T allele, especially with susceptibility to COPD in middle-aged and elderly patients, the manuscript for important intellectual content: All authors; Drafting of the manuscript: All authors; Critical revision of the manuscript for important intellectual content: All authors; Drafting of the manuscript: All authors; Critical revision of the manuscript: All authors; Analysis and interpretation of data: All authors; Author contributions

Author contributions

Study concept and design: All authors; Acquisition of data: All authors; Analysis and interpretation of data: All authors; Drafting of the manuscript: All authors; Critical revision of the manuscript for important intellectual content: All authors; Statistical analysis: All authors; Administrative, technical, and material support: All authors; Study supervision: All authors; all authors have read and approved the manuscript.

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