Association of Matrix Metalloproteinase 9 C-1562T Polymorphism with Genetic Susceptibility to Myocardial Infarction: A Meta-Analysis

Zhang Juan, MD, Zhang Wei-Guo, PhD*, Song Heng-Liang, MD, Wan Da-Guo, MD

Department of Cardiovascular Medicine, The Second Affiliated Hospital of Zheng Zhou University, Zhengzhou, China

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ABSTRACT

Background: Myocardial infarction (MI) is the major cause of death by disease in the world. Many studies have identified the associations between matrix metalloproteinase 9 (MMP9) C-1562T polymorphisms and MI. However, the results remain inconclusive. To clarify the role of MMP9 C-1562T polymorphism in MI risk, we conducted a systematic review and large-scale meta-analysis.

Methods: Studies published between January 2005 and March 2014 were obtained from the electronic databases PubMed, Medline, and Embase. The odds ratios (ORs) with 95% CIs were calculated for comparisons of the alleles and genotypes in the overall population and in ethnicity subgroups to measure the strength of genetic associations.

Results: A total of 7 related studies, including 3952 MI cases and 4977 healthy control subjects were included in our meta-analysis. Our results show a statistically significant association between T allele and MI in the overall population (OR = 1.23; 95% CI, 1.02–1.48; P = 0.03). The risk of MI was also significantly higher in patients carrying the T allele (TC + TT genotypes) than in those with the CC genotype (P < 0.05). In stratified analysis by ethnicity, we found the T allele was strongly associated with MI in white populations, whereas in Asian populations there appeared no significant association.

Conclusions: Our data show that the MMP9 C-1562T polymorphism is a risk factor associated with increased MI susceptibility in the total population and white populations, although no significant association was observed in Asians populations. Further studies with larger sample sizes and assessing gene–gene and gene–environment interactions are required.

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Introduction

Myocardial infarction (MI) is a leading cause of morbidity and mortality worldwide. It is an extreme manifestation of coronary artery disease. Approximately 90% of all cases of MI are the result of acute occlusive thrombus due to a ruptured coronary artery atheromatous plaque.1 New myocardial ischemia occurs in 54% of patients within the first year after MI and 1-year risk of reinfarction presents in 17.4% of patients with MI.2,3 The American Heart Association reports that approximately 600,000 new patients sustain an MI and 320,000 patients have an episode of recurrent MI each year in the United States.4 Despite the available therapeutic approaches, MI is still associated with high rates of acute death and long-term complications such as heart failure. Its pathophysiology is complex, and several inherited and acquired risk factors that predispose to the development of atherosclerotic lesions and plaque rupture are involved.

Matrix metalloproteases (MMPs) are a family of Zn^{2+}-depend-ent enzymes with proteolytic activity against connective tissue proteins that play important roles in the development and progression of atherosclerotic lesions.5–7 They are involved in degradation of the extracellular matrix, weakening of the fibrous cap, and subsequently destabilization of atherosclerotic lesions.8,9 MMP-9, also known as gelatinase B or 92-kDa type IV collagenase, is 1 member of the MMP family and is an enzyme in human beings that is encoded by the MMP9 gene. It plays an important role in the distant metastatic potential of cancer cells for its collagenolytic activity that is known to be associated with the disruption of basement membrane.10 MMP9 is highly expressed in the vulnerable regions of atherosclerotic plaques and has been suggested to be causally involved in plaque rupture.11 Dysregulation of MMP9 expression is a characteristic of several pathologic conditions, including metastasis, vascular and cardiac remodeling, atherosclerotic plaque rupture, and acute coronary syndrome.12 Studies have indicated that higher plasma concentrations of MMP9 may be a
predictor of cardiovascular disease risk, and plasma MMP9 concentrations are elevated in patients with acute MI. The MMP9 gene C-1562T (rs3918242) polymorphism (C-T) in the promoter region abolishes the DNA-protein interaction, resulting in higher activity of the T-allelic promoter. This polymorphic site has a close association with susceptibility to coronary atherosclerosis and subsequent acute coronary syndromes, and influences critical steps in the binding of transcription factors or the overall efficiency of transcription.

Although numerous reports have identified the association of MMP9 C-1562T polymorphism and MI risk, inconsistency has been presented for different allele frequencies among study populations. The purpose of conducting this meta-analysis was to clarify and quantify the overall risk of MMP9 C-1562T polymorphism on developing MI.

Materials and Methods

Identification and eligibility of relevant studies

A comprehensive literature search was conducted using the electronic databases PubMed, Medline, and Embase for relevant articles published between January 2005 and March 2014. The key words matrix metalloproteinase 9 or MMP9 or gelatinase B, polymorphism or mutation or variant, and myocardial infarction or ischemic cardiovascular disease as well as their combinations were used. All studies matching the eligibility criteria were retrieved, and references were checked for other relevant publications. The language was limited to English only. The search was focused on studies that had been conducted in human beings. Only full-text articles and the most recent studies were included in our meta-analysis.

Criteria for inclusion

Studies eligible for inclusion in our meta-analysis must have met the following criteria: case-control or cohort study; MI events were confirmed by reviewing symptoms, cardiac enzymes, and ECG findings according to the guidelines and the controls should be the healthy age- and sex-matched persons; only MI as the study outcome and the polymorphism site should be C-1562T; the results were expressed as odds ratio (OR) and corresponding 95% CI; the distribution of genotype in MI and control groups were available; and genotype distribution of control must be in Hardy-Weinberg equilibrium.

Quality assessment and data extraction

Two investigators independently extracted data and reached consensus on all of the items. Any disagreement was resolved by discussing with the third expert. Data retrieved from the reports included first author, publication year, demographics, total numbers of cases and controls, and genotype distribution of MMP9 C-1562T polymorphism in cases and controls.

Statistical analysis

The overall association between MMP9 C-1562T polymorphism and MI was measured by ORs and 95% CI, which were calculated according to the method of Woolf. The significance of the pooled ORs was determined by the Z test, and a P value < 0.05 was considered statistically significant. The allelic model (T vs C) and genetic models (dominant effect: TT + TC vs CC and CC vs TT + TC) were examined to evaluate the C-1562T allele and the risk of MI. The I² test was used to assess the proportion of statistical heterogeneity and the Q statistic test was used to define the degree of heterogeneity. A P value < 0.10 for the Q test and I² < 50% was considered significant among the studies. Data were combined using both a fixed-effects model (the inverse variance-weighted method) and a random effects model (DerSimonian and Laird method). The fixed-effects model is used when the effects are assumed to be homogenous, whereas the random effects model is used when they are heterogenous. The evidence of publication bias was assessed by visual funnel plot inspection.

To assess if our results were substantially influenced by the presence of any individual study, we conducted a sensitivity analysis by systematically removing each study and recalculating the significance of the result. Statistical analyses were conducted in Review Manager (version 5.2, The Cochrane Collaboration, Oxford, United Kingdom). All tests were 2-sided.

Results

Study selection and characteristics

The literature search identified 97 articles. Of those, 27 records were excluded after title review and 70 articles were left for further review. After abstracts were screened for relevance, 53...
Association between MMP9 polymorphism and MI among the total population

Table III shows the meta-analysis of the MMP9 C-1562T polymorphism under various genetic models. The heterogeneity between studies was assessed and the fixed-effect model or the random-effect model was employed for calculating the pooled OR. Overall, our results showed that the frequency of the T allele is higher in persons with a history of MI than in healthy controls (14.3% vs 12.9%), and demonstrated statistically significant positive association between the risk factor T allele and MI in the overall population (OR = 1.23; 95% CI, 1.02–1.48; P = 0.03) in a random-effect model, as shown in Figure 2. The risk of MI was also significantly higher in patients carrying the T allele (TC + TT genotypes) than in those with the CC genotype (OR = 1.24; 95% CI, 1.01–1.51; P = 0.04). Furthermore, as shown in Figure 3, the CC genotype significantly increased the risk of MI (CC vs TC + TT: OR = 0.81; 95% CI, 0.66–0.99; P = 0.04).

Stratified analysis by ethnicity

Various genetic models were examined under stratified analysis by ethnicity, as shown in Table III. In white populations, the frequency of the T allele is higher in persons with a history of MI than in healthy controls (26.8% vs 24.6%), and our results showed a statistically significant association between T allele and MI risk (OR = 1.14; 95% CI, 1.03–1.26; P = 0.01) in a fixed-effect model. Moreover, presence of the T allele (TC + TT) had a more significant relationship with MI risk than in controls when comparing with the CC genotype (TC + TT vs CC: OR = 1.17; 95% CI, 1.05–1.31; P = 0.005). The CC genotype also significantly increased the risk of MI (CC vs TC + TT: OR = 0.85; 95% CI, 0.76–0.95; P = 0.005) in a fixed-effect model. However, in Asian populations, no significant association was found between allelic model and genetic models (T vs C: OR = 1.33; 95% CI, 0.86–2.03; P = 0.20; TT + TC vs CC: OR = 1.28; 95% CI, 0.78–2.11; P = 0.33; CC vs TC + TT: OR = 0.78; 95% CI, 0.47–1.28; P = 0.33) in a random-effect model. There was no heterogeneity between these 2 subgroups (P = 0.75; I² = 0%), as shown in Figure 4.

Sensitivity analyses and publication bias

A single study included in the meta-analysis was deleted each time to reflect the influence of the individual data set to the pooled ORs, and the corresponding pooled ORs were not materially changed. This procedure confirmed the stability of our overall result.

Begg's funnel plot was conducted to assess the publication bias of the literature. The shape of funnel plots did not reveal any evidence of funnel plot asymmetry (as shown in Figure 5). Thus there was no possibility of publication bias risk in the meta-analysis.

Discussion

We found that the frequency of T alleles is higher in patients with a history of MI than in healthy controls and demonstrated a statistically significant strong association between the risk factor T allele and MI risk in the overall human population and in white populations (P < 0.05). The risk of MI was also significantly higher in patients carrying the T allele (TC + TT genotypes) than in those with the CC genotype. However, in Asian populations, no significant association appeared. Overall, we found a significant
association between MMP9 C-1562T polymorphism and MI in the overall human population and white populations. Our results were consistent with a previous meta-analysis conducted by Wang et al.\textsuperscript{33} Their analysis included 7 articles published between 1999 and 2010, including 4473 cases and 3343 healthy controls, and the result demonstrated significant associations between this polymorphism and MI risk. However a subsequent meta-analysis identified that MMP9 C-1562T was significantly associated with MI in East Asians, whereas no significant association was observed in Western populations.\textsuperscript{29} Our study is an update analysis of MMP C-1562T polymorphism with the risk of MI.

The human MMP9 gene is located on chromosome 20q12.2-13.1, and is functionally implicated in the process of infarct healing. A number of MMP9 single nucleotide polymorphisms in the promoter, coding, and untranslated regions have been reported.\textsuperscript{30} Among them, promoter C-1562T polymorphism with a cytosine to thymidine transition is the most studied and functional studies indicate that this polymorphism has an allele-specific effect on MMP-9 transcription.\textsuperscript{13} The variant T allele of MMP-9 C-1562T polymorphism has been associated with an increase in expression of the gene and higher MMP9 levels, due to preferential binding of the transcriptional repressor protein to the C allele (binding weaker to the T allele), and overexpression of MMP9 was found in human atherosclerotic plaques and involved in rupture of the plaques.\textsuperscript{31,32} Zhang et al.\textsuperscript{33} revealed that the sequence between nucleotide position –1567 and –1559 relative to the transcription start site of the MMP9 gene, which encompasses the –1562 polymorphic site, can interact with a nuclear protein whose entity is still unknown. Clinical studies have also established a relationship between MMP9 and post-MI remodeling and mortality, making MMP-9 a viable candidate to add to the multiple biomarker list.\textsuperscript{33} It is therefore possible that the expression level associated with this polymorphism affects the activity of this enzyme.\textsuperscript{34}

Few epidemiologic studies have investigated associations between MMP9 and MI onset. Setianoto et al.\textsuperscript{35} demonstrated that the MMP9 C-1562T polymorphism is associated with high serum MMP9 levels in patients with segment elevation MI. Hansson et al.\textsuperscript{36} found that serum MMP9 and tissue inhibitor of metalloproteinases-1 levels were related to mortality risk of MI. Jefferis et al.\textsuperscript{37} identified that serum MMP9 is univariately associated with risk of MI and stroke. Rodius et al.\textsuperscript{38} indicate that the MMP9 polymorphism does not seem to be associated with clinical outcome and in particular with the development of left ventricular dysfunction and heart failure.

Thrombosis is generally accepted as the most common pathogenetic pathway of MI. It has been reported that MMP9 brings about destabilizing structural changes in vulnerable atherosclerotic plaques.\textsuperscript{39} MMP9 also potentiates the chemokine interleukin-8 and modifies the local chemokine profile,\textsuperscript{40} and hence may promote cellular infiltration of plaques, weakening the fibrous cap of the atherosclerotic plaque, and increasing the size of the lipid core. These processes render the plaque susceptible to rupture due to reduced mechanical strength and hence increase the probability of atherothrombotic ischemia. It has been identified that MMP9 is present in the early stages of atherosclerotic plaque formation,\textsuperscript{41} having a protective role against its rupture.\textsuperscript{42} Neutrophils, macrophages, and lymphocytes are cell sources of MMP-9. MMP-9 expressed in post-MI cardiac remodeling, and clinical studies showed that MMP-9 is a biomarker for cardiac remodeling and predicted adverse left ventricular remodeling after MI.\textsuperscript{43} Structural details about the flexibility of MMP-9 monomers show that it may be viewed as a multidomain enzyme in

**Figure 2.** Forest plot of association between C-1562T polymorphism and myocardial infarction (T vs C allele). M-H = Mantel-Haenszel.

**Figure 3.** Forest plot of association between the C-1562T polymorphism and myocardial infarction in dominant model CC versus TC-TT genotype. M-H = Mantel-Haenszel.
which the hemopexin, the O-glycosylation, and the catalytic domains yield support for attachment, articulation, and catalysis, respectively.

Several limitations are present in our meta-analysis. First, the MMP9 polymorphism may interact with other known and unknown risk factors relating to MI that should be considered, such as hypertension, diabetes, dyslipidemia, and prior coronary artery disease. Second, 3 relevant articles were excluded because we could not obtain sufficient information to calculate the genotype distribution. Third, sample sizes of several studies were too small, and studies in other languages were not retrieved. Fourth, additional analysis taking into account sex and information of MI should be conducted to give a new insight into the role of the studied polymorphism in the development of MI in various populations.

### Conclusions

Our meta-analysis suggests a role of MMP9 C-1562T polymorphism in the occurrence of MI. This polymorphism is significantly associated with MI in white populations, although no significant association was observed in Asians populations. Further studies with larger sample size and assessing gene–gene and gene–environment interactions are required. Meanwhile, race selection should be paid more attention because the pathogenesis of a disease might have a different basis in different racial populations.

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### References

1. Sakowicz A, et al. Genetic variability and the risk of myocardial infarction in Poles under 45 years of age. Arch Med Sci. 2010;6(2):160–167.
2. Goldstein JA, et al. Multiple complex coronary plaques in patients with acute myocardial infarction. N Engl J Med. 2000;343(13):915–922.
3. Molonas C, et al. Effect of Angiotensin-converting enzyme inhibition on one-year mortality and frequency of repeat acute myocardial infarction in patients with acute myocardial infarction. Am J Cardiol. 2010;105(9):1229–1234.
4. Go AS, et al. Heart disease and stroke statistics—2013 update: a report from the American Heart Association. Circulation. 2013;127(1):e6.
5. Williams KJ, Tabas I. Atherosclerosis and inflammation. Science. 2002;297(5581):521–522.
6. Brauer PR. MMPs–role in cardiovascular development and disease. Front Biosci. 2006;11:447–478.
7. Dutta P, et al. Myocardial infarction accelerates atherosclerosis. Nature. 2012;487(7407):325–329.
8. Koenig W, Khuseyinova N. Biomarkers of atherosclerotic plaque instability and rupture. Arterioscler Thromb Vasc Biol. 2007;27(1):15–26.
9. Ye S. Influence of matrix metalloproteinase genotype on cardiovascular disease susceptibility and outcome. Cardiovascular research. 2006;69(3):636–645.
10. Jones J, Walker R. Control of matrix metalloproteinase activity in cancer. The Journal of pathology. 1997;183(4):377–379.

**Table 1:**

| Study or Subgroup | Experimental Events | Control Events | Weight | Odds Ratio M-H, Random, 95% CI | Year |
|-------------------|---------------------|----------------|--------|--------------------------------|------|
| **1.1 Caucasian** |                     |                |        |                                |      |
| Haberbosch        | 314                 | 1280           | 121    | 535                            | 21.7%| 2005 |
| Nuzzo             | 42                  | 115            | 37     | 123                            | 9.7% | 2006 |
| Horne             | 474                 | 1693           | 864    | 3455                           | 27.5%| 2007 |
| Sewelam           | 8                   | 40             | 40     | 0                              | 0%   | 2013 |
| **Subtotal (95% CI)** | 3128               | 4153           | 539%   | 1.18 [0.98, 1.41]               |      |
| **1.2 Asian**    |                     |                |        |                                |      |
| Koh               | 55                  | 206            | 31     | 173                            | 10.8%| 2008 |
| Ghaderian         | 57                  | 234            | 59     | 200                            | 13.1%| 2010 |
| Wang              | 98                  | 384            | 78     | 451                            | 16.8%| 2012 |
| **Subtotal (95% CI)** | 824                | 824           | 40.7%  | 1.28 [0.78, 2.11]               |      |
| **Total (95% CI)** | 3952               | 4977           | 100.0% | 1.24 [1.01, 1.51]               |      |

**Figure 4:** Odds ratios of myocardial infarction (with 95% CIs) in T allele carriers (CT and TT genotype) of matrix metalloproteinase 9 C-1562T polymorphism versus CC genotype carriers examined in 7 studies, by ethnicity analysis. M-H = Mantel-Haenszel.

**Figure 5:** Begg’s funnel plot analysis of publication bias for myocardial infarction. OR = odds ratio.
