Author’s response to reviews

Title: Association of matrix metalloprotease 1, 3, and 12 polymorphisms with rheumatic heart disease in a Chinese Han population

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Author’s response to reviews:

Dear Matteo Pasini,

Thank you for your letter and for the reviewers’ comments concerning our manuscript entitled “Association of matrix metalloprotease 1, 3, and 12 polymorphisms with rheumatic heart disease in a Chinese Han population” (MGTC-D-16-00278).

Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. And here we listed the changes in “Appendix” below and marked with "Revision" Tool of Microsoft Word in revised paper. The main corrections in the paper and the responds to the reviewer’s comments are as flowing.
We appreciate for your warm work earnestly, and hope that the correction will meet with approval. Once again, thank you very much for your comments and suggestions.

Sincerely,

Zhaohui Meng,
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Reviewer 1 comments:

1. The manuscript contains new information to justify publication.
2. Title and Abstract describe clearly the content of the article.
3. The interpretations and conclusions were justified by the results.
4. Adequate references were made to other work in the field.

Decision: Accept.

Answer to Reviewer 1 comments:

Dear Prof. Sacide Pehlivan,

We appreciate very much your favorable comments concerning our manuscript. We hope this manuscript will bring new information to readers as you said. Thank you again for your precious time and hard work devoted for reviewing the manuscript.

Best wishes,

Zhaohui Meng,
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E-mail: zhhmeng@aliyun.com

Reviewer 2 comments:
1. In the abstract: "genetic factors" is too generic. Why it is important to investigate matrix metalloproteinases and no other genes from the immune response, for example (as those cited in the introduction)? Why only those three polymorphisms?

2. Introduction: The authors mention that MMP promoter polymorphisms alter gene expression, but fail to indicate the location of the investigated SNPs (in the promoter region). The relevance of these three polymorphisms is not clear: why not others? Why not polymorphisms of MMP2, whose expression increases with periostin (Hakuno et al. 2010)? Why not MMP9, whose levels increase in RHD patients with more than 30 years of age (Lee et al. 2006)? In conclusion, gene and polymorphism description is rather poor and fail to set the basis for interpreting the results. Most of the description found in the discussion should be moved to the introduction, in order to clarify these points. The possible impact of other MMPs must be included in the discussion.

3. Material and Methods: The authors tried to "pair" controls and patients for sex and age, but how was their socioeconomic status, known to be highly influential in the susceptibility to RHD? Were all participants from the same ethnic group (Han, Yunnan)? Regarding age, p value mentioned in the first Results section was close to significance. Please correct all associations for this factor (and possibly others), using logistic regression. Results would be highly enriched with real-time RT-PCR quantification of mRNA expression (since the original hypothesis for the inclusion of these SNPs is that they alter gene expression!), ELISA and/or immunohistochemical analysis.

4. Results: first section would be more appropriate in the Mat & Meth description of study participants. It is not clear what is meant with the allele nomenclature (1G?, 2G?). There are other, less stringent ways to correct for multiple comparisons (why Bonferroni and not FDR?). Although the association with the 2G/2G genotype ends up without significance, the authors still maintain the association statement for this genotype. Without age correction, the results are not reliable.

5. Table 1: use Courier new font for DNA sequences.

6. Discussion: as mentioned before, most sentences must be move to the introduction, in order to understand the rationale of the study.

Answer to Reviewer 2:

Dear Prof. Angelica Beate Winter Boldt,

I quite appreciate your favorite consideration and insightful comments. Now I have revised the MGTC-D-16-00278 exactly according to your comments, and found these comments are very helpful. I hope this revision can make my paper more acceptable. The revisions were addressed point by point below.

Best wishes,
Comments and Reply

1. In the abstract: "genetic factors" is too generic. Why it is important to investigate matrix metalloproteinases and no other genes from the immune response, for example (as those cited in the introduction)? Why only those three polymorphisms?

Response:

a. The statements of “genetic factors” were corrected as “genetic susceptibility” in the abstract.

b. Why it is important to investigate matrix metalloproteinases (MMPs) is that they can modulate immune responses by processing chemokines and cytokines in various diseases [1]. McQuibban GA et al [2] demonstrated that the N-terminus of monocyte chemoattractant protein 1 (MCP1), MCP2 and MCP4 was cleaved by MMP 1 and 3 to produce antagonist factors which dampen inflammatory processes. Further study has shown that MMP3 has a dual role in biphasic modulation of inflammatory mediator activity by cleaving Interleukin 1β precursor into active form and degrading the biologically active cytokine [3].


c. There are two reasons why we chosen the 3 polymorphisms to investigate:

First, studies have shown that MMP1 and MMP3 are related to rheumatic heart disease (RHD) [1, 2], but the relationship between MMP12 and RHD has not been reported before. We can speculated that MMP12 may play a role in RHD due to its involvement in rheumatic diseases [3].
Second, MMP1, 3, and 12 are known to be adjacently localized on the same chromosome 11q22.3. Studies have shown a combined effect of MMP1 -1607 1G/2G, MMP3 -1612 6A/5A and MMP12 -82A/G polymorphisms associated with esophageal adenocarcinoma (EA) risk [4]. However, the relationship between the 3 gene polymorphism and RHD has not been reported before.


2. Introduction: The authors mention that MMP promoter polymorphisms alter gene expression, but fail to indicate the location of the investigated SNPs (in the promoter region). The relevance of these three polymorphisms is not clear: why not others? Why not polymorphisms of MMP2, whose expression increases with periostin (Hakuno et al. 2010)? Why not MMP9, whose levels increase in RHD patients with more than 30 years of age (Lee et al. 2006)? In conclusion, gene and polymorphism description is rather poor and fail to set the basis for interpreting the results. Most of the description found in the discussion should be moved to the introduction, in order to clarify these points. The possible impact of other MMPs must be included in the discussion.

Response:

a. According to your suggestion, we have rearranged this part and indicated the location of the investigated SNPs in the introduction section (for details see the appendix below and revised edition).

b. Although the association between MMP1 -1607 1G/2G, MMP3 -1612 6A/5A and MMP12 -82A/G polymorphisms is not yet clear, studies have shown a combined effect of the three polymorphisms. Bradbury PA et al [1] investigated the relationship between matrix metalloproteinase 1, 3 and 12 polymorphisms and esophageal adenocarcinoma (EA) risk, they demonstrated by haplotype analysis that MMP1 (-1607)–MMP3 (-1612)–MMP12 (-82) 2G-5A-A (adjusted odds ratio 1.36, 95% confidence interval 1.0–1.8; P 5 0.03) and 2G-5A-G (adjusted odds ratio 1.70, 95% confidence interval 1.1–2.6; P 5 0.01) were associated with
increased EA risk. Another study showed that when MMP1 (-1607) 2G/2G and MMP3 (-1612) 6A/6A were combined, the risk factor for internal carotid artery stenosis was 3-fold higher (OR, 3.31; 95% CI, 1.48 to 7.42; P=0.004) [2].


c. It is really true as you suggested that other MMP members should be enrolled in this study due to the special characteristics of each family members in different diseases. However, there are two reasons why we did not select other MMP family members in the study:

First, Considering that this is the first time to investigate the relationship between MMP polymorphism and rheumatic heart disease (RHD), we just take MMP1, 3 and 12 not others as study objects which localized on the same chromosome and has the combined effect;

Second, In case a significant difference was found, future study will be performed to observe the association between RHD and other MMP members such as MMP2, MMP9.

d. Special thanks to you for your good comments, we have rearranged the manuscript and some descriptions in the discussion were move to the introduction. The possible impact of other MMPs has been included in the discussion (for details see the appendix below and revised edition).

3. Material and Methods: The authors tried to "pair" controls and patients for sex and age, but how was their socioeconomic status, known to be highly influential in the susceptibility to RHD? Were all participants from the same ethnic group (Han, Yunnan)?

Regarding age, p value mentioned in the first Results section was close to significance. Please correct all associations for this factor (and possibly others), using logistic regression. Results would be highly enriched with real-time RT-PCR quantification of mRNA expression (since the original hypothesis for the inclusion of these SNPs is that they alter gene expression!), ELISA and/or immunohistochemical analysis.

Response:

a. In our study, the majority of the population came from the remote rural areas in Southern China with poor economic conditions; Although a small number of people are in good economic condition, they have been poor in their early childhood.

In this study, we only enrolled the Chinese Han population in Yunnan Province.
b. We are very sorry for our negligence of mistaking “Mean Difference value (0.08)” for “P-value (0.921)” in Table 2. We have modified the mistake in Table 2. We have corrected all associations through logistic regression according to your comments (for details see the appendix below and revised edition).

c. Special thanks to your good suggestion that we can use other methods to enrich the results. However, we are currently faced with the problems below:

First, the samples cryopreserved for more than 1 years will affect the accuracy of the experimental results for RT-PCR and ELISA research;

Second, it is difficult for us to collect the normal valve samples as control for immunohistochemical study due to the lack of cases in our institute.

In the future study, we will adopt various methods to validate the experiment according to your suggestion.

4. Results: first section would be more appropriate in the Mat & Meth description of study participants. It is not clear what is meant with the allele nomenclature (1G?, 2G?). There are other, less stringent ways to correct for multiple comparisons (why Bonferroni and not FDR?). Although the association with the 2G/2G genotype ends up without significance, the authors still maintain the association statement for this genotype. Without age correction, the results are not reliable.

Response:

a. We have revise first section about description of study participants in Results according to your suggestion. The descriptive sections are included in the Mat & Meth and the statistical results are included in the Results (for details see the appendix below and revised edition).

b. Concerning the allele nomenclature (1G?, 2G?): The polymorphism is caused by a variation in the number of guanine located at position -1607 in the promoter region, resulting in one allele having two guanines (GG) and the other allele having one guanine (G).

c. The Bonferroni correction is a safeguard against multiple tests of statistical significance on the same data, where 1 out of every 20 hypothesis-tests will appear to be significant at the $\alpha = 0.05$ level purely due to chance. It was developed by Carlo Emilio Bonferroni. The False Discovery Rate (FDR) of a set of predictions is the expected percent of false predictions in the set of predictions. The FDR is very different from a p-value, and as such a much higher FDR can be tolerated than with a p-value.

According to your suggestion, a statistical analysis was conducted using unconditional logistic regression models (for details see the appendix below and revised edition). Compared to
genotype 1G/1G, genotype 2G/2G was found have a significantly higher frequency in the cases than in the controls after adjusting for age and gender (for details see the appendix below and revised edition).

5. Table 1: use Courier new font for DNA sequences

Response: We have made correction according to your comments.

6. Discussion: as mentioned before, most sentences must be move to the introduction, in order to understand the rationale of the study.

Response: We have rearranged this part according to your suggestion (for details see the appendix below and revised edition).

appendix

1. Abstract Part:

Line 17, the statements of “factors” were corrected as “susceptibility”.

Line 24-25, “a” was deleted, “Unconditional logistic regression models and ” was added.

Line 25-26, “or Fisher’s exact test. Multiple comparisons were corrected using the Bonferroni method.” was deleted.

Line 28-29, “OR = 3.31; 95% CI: 1.15–9.50; p = 0.022” were corrected as “OR = 3.227; 95% CI: 1.118–9.31; p = 0.03”.

Line 30, “p = 0.037” were corrected as “p = 0.048”.

Line 30-31, “but these associations did not survive the Bonferroni correction for multiple comparisons (pc = 0.26 and 0.44, respectively).” was deleted

Line 33, “p > 0.37” were corrected as “p > 0.05”.

2. Background Part:

Line 62-70, “Although MMP polymorphisms have been associated with various diseases, such as oligodendroglioma, coronary artery disease, osteoarthritis, lumbar disc disease, lung cancer, myocardial infarction, rheumatoid arthritis, systemic sclerosis, ovarian carcinoma, and ischemic stroke [17, 19–29], the role of genetic polymorphisms in MMPs has not yet been evaluated in patients with RHD. In addition, MMP1, 3, and 12 are known to be adjacentally localized on chromosome 11q22.3 [15] and these 3 loci are considered to act in cooperation with each other [30]. In the present study, we evaluated the associations of 3 MMP polymorphisms, rs1799750 in MMP1, rs3025058 in MMP3, and rs2276109 in MMP12, with RHD in a Han population in Southern China.” was deleted.
In the promoter of the MMP1 gene, an insertion (2G)/deletion (1G) polymorphism was detected at position -1607 (rs1799750). It has been demonstrated that the 2G promoter processes higher transcriptional activity than the 1G promoter by binding more Ets-1 transcription factor [16]. This MMP1 promoter polymorphism has been reported to be associated with oligodendroglioma [19], coronary artery disease [20], osteoarthritis [21], and lumbar disc disease [22].

Another insertion (6A)/deletion (5A) polymorphism has been reported at position -1612 (rs3025058) of the MMP3 promoter. The 6A promoter has a reduced transcription level due to its higher affinity to the repressor binding site [17]. This MMP3 promoter polymorphism has been associated with osteoarthritis [23], lung cancer [24], and myocardial infarction [25].

A single nucleotide polymorphism (SNP) in the MMP12 promoter region has been reported to influence transcriptional activity [18]. This A to G substitution polymorphism is located at position -82 (rs2276109) adjacent to the transcription factor activator protein-1 (AP-1). It has been suggested that this SNP may be a risk factor for rheumatoid arthritis [26], systemic sclerosis [27], ovarian carcinoma [28], and ischemic stroke [29].

Although MMP polymorphisms have been associated with various diseases, the role of genetic polymorphisms in MMPs has not yet been evaluated in patients with RHD. In addition, MMP1, 3, and 12 are known to be adjacenty localized on chromosome 11q22.3 [15] and these 3 loci are considered to act in cooperation with each other [30]. In the present study, we evaluated the associations of 3 MMP polymorphisms, rs1799750 in MMP1, rs3025058 in MMP3, and rs2276109 in MMP12, with RHD in a Han population in Southern China.

3. Methods Part:

Unconditional logistic regression models were applied to compare the differences in the allele and genotype frequencies of the polymorphisms between the cases and controls.
the controls, adjusting for age, gender. P < 0.05 was considered statistically significant.” was added.

4. Results Part:

Line 147, “p = 0.08” were corrected as “p =0.921”.

Line 148-150, “Carditis was found in 100% of the patients with RHD. No arthritis, subcutaneous nodules, chorea, or erythema marginatum was found in the patients (Table 2).” was deleted.

Line 157, “p = 0.07” were corrected as “p = 0.072”.

Line 159, “p = 0.022; OR = 3.31, 95% CI: 1.15–9.50” were corrected as “p = 0.03; OR = 3.227, 95% CI:1.118-9.31”.

Line 159-160, “but this difference did not survive the Bonferroni correction for multiple comparisons (pc = 0.26; Table 3).” was deleted.

Line 162, “p = 0.08” were corrected as “p = 0.09”.

Line 163-164, “p = 0.037; OR = 1.59, 95% CI: 1.03–2.45” were corrected as “p = 0.048; OR = 0.644, 95% CI:0.416-0.996”.

Line 164-165, “but this difference did not survive the Bonferroni correction for multiple comparisons (pc = 0.44; Table 3).” was deleted.

Line 167, “p = 0.51 and 0.46” were corrected as “p = 0.509 and 0.473”.

Line 169, “p = 0.55 and 0.56” were corrected as “p = 0.767 and 0.576”.

4. Discussion part:

Line 178-179, “OR = 3.31; 95% CI: 1.15–9.5; p = 0.0220” were corrected as “OR = 3.227; 95% CI:1.118-9.31; p = 0.03”.

Line 180, “p = 0.037” were corrected as “p = 0.048”.

Line 180-186, “This MMP1 polymorphism is an insertion (2G)/deletion (1G) polymorphism detected at position -1607 in the promoter of the MMP1 gene. It has been demonstrated that the 2G promoter processes higher transcriptional activity than the 1G promoter by binding more Ets-1 transcription factor [16]. This MMP1 promoter polymorphism has been reported to be associated with oligodendroglioma [19], coronary artery disease [20], osteoarthritis [21], and lumbar disc disease [23].” was deleted.

Line 202-204, “Another insertion (6A)/deletion (5A) polymorphism has been reported at position -1612 (rs3025058) of the MMP3 promoter. The 6A promoter has a reduced transcription level due to its higher affinity to the repressor binding site [17].” was deleted.
A single nucleotide polymorphism (SNP) in the MMP12 promoter region (rs2276109) has been reported to influence transcriptional activity [18]. This A to G substitution polymorphism is located at position -82 adjacent to the transcription factor activator protein-1 (AP-1). It has been suggested that this SNP may be a risk factor for rheumatoid arthritis [26], systemic sclerosis [27], ovarian carcinoma [28], and ischemic stroke [29].

Although our study did not reveal correlation between MMP3 -1612 6A/5A, MMP12 -82A/G polymorphisms and RHD in Han population, studies have shown a combined effect of MMP1 -1607 1G/2G, MMP3 -1612 6A/5A and MMP12 -82A/G polymorphisms associated with esophageal adenocarcinoma (EA) risk in Caucasian [45]. Therefore, different ethnic groups and diseases may lead to diversity in polymorphism research.

In addition, the expression level of other MMP members were found elevated in patients with RHD, such as MMP2 and MMP9 [46, 47]. Elevated MMP2 levels in patients with RHD may be involved in atrial remodeling and atrial fibrosis by modulating the balance between B-cell lymphoma 2 (Bcl-2) and Bcl-2-associated X protein (BAX). Increased MMP-9 may facilitate cardiac remodeling in RHD by playing a compensatory role for the decreased insulin-like growth factor (IGF)-1 levels. Thus other MMP members should be included in the polymorphism study due to their different pathogenic mechanisms in RHD.

5. References part:

References part:

6. Table 1

“Times New Roman font” were corrected as “Courier new font” for DNA sequences.

7. Table 2

Line “Age”, p value “0.08” were corrected as “0.921”.

8. Table 3

“pc column” was deleted.

“p value column” was recalculated:

MMP1:

Line “Genotype”, “0.07” were corrected as “0.072”.

Line “1G/2G”, “0.08” were corrected as “0.09”.

Line “2G/2G”, “0.022” were corrected as “0.03”.

Line “2G”, “0.037” were corrected as “0.048”.

MMP3:

Line “Genotype”, “0.51” were corrected as “0.509”.

Line “5A/6A”, “0.96” were corrected as “0.983”.

Line “5A/5A”, “0.37” were corrected as “0.230”.

Line “5A”, “0.46” were corrected as “0.473”.

MMP12:

Line “Genotype”, “0.55” were corrected as “0.767”.

Line “A/G”, “0.55” were corrected as “0.569”.
Line “G”, “0.56” were corrected as “0.576”.

“OR (95% CI)” was recalculated:

MMP1:

Line “1G/2G”, “2.44 (0.87–6.88)” were corrected as “2.455 (0.87-6.926)”.
Line “2G/2G”, “3.31 (1.15–9.50)” were corrected as “3.227 (1.118-9.31)”.
Line “2G”, “1.59 (1.03–2.45)” were corrected as “0.644(0.416-0.996)”.

MMP3:

Line “5A/6A”, “0.98 (0.49–1.96)” were corrected as “0.993 (0.498-1.981)”.
Line “5A/5A”, “4.12 (0.45–37.88)” were corrected as “3.908(0.421-36.242)”.
Line “5A”, “1.25 (0.70–2.24)” were corrected as “1.24(0.689-2.231)”.

MMP12:

Line “A/G”, “0.70 (0.22–2.29)” were corrected as “0.708(0.215-2.324)”.
Line “G”, “0.71 (0.22–2.27)” were corrected as “0.717(0.223-2.307)”.

Line 454: “pc: Bonferroni-corrected p value;” was deleted.