Author's response to reviews

Title: Impact of Matrix Metalloproteinase 9 rs3918242 Genetic Variant on Lipid-lowering Efficacy of Simvastatin Therapy in Chinese Patients with Coronary Heart Disease

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Author's response to reviews:

We would like to take this opportunity to thank the reviewer for his/her expert evaluation and appropriate suggestions for our manuscript.

Reviewer #1: In present study, the authors correlated the MMP9 -1562 C>T SNP genotype with lipid-lowering efficacy of simvastatin in Chinese CHD patients. They found that MMP9, as well as LDL-C, were increased in serums from CHD patient carrying TT genotype compared with whom carrying CC genotype. After simvastatin treatment, the authors observed more significant decrease in LDL-C in serums from CHD patient with TT genotype when compared with patient with CC genotype.

Major problems:

1. Based on the observations (the correlation of MMP9 -1562 C>T SNP genotype with % change in LDL-C after simvastatin treatment), the authors stated that " In this study, we showed that MMP-9 1562C>T polymorphism significantly regulated circulating MMP-9, TG and LDL levels, and modulated LDL-C lowering response to simvastatin in Chinese patients with CHD". However, there is no evidence that this SNP can regulate LDL-C or modulate simvastatin LDL-C lowering efficiency. It is just correlation, probably because of LDL-C level was correlated with MMP9 level and MMP9 level was correlated with the SNP genotype. It will not be convincing to claim that a SNP, in which gene is not related to

simvastatin PK/PD, can modulate simvastatin LDL-C lowering efficiency without consideration of functional SNPs in the genes (such as SLCO1B1) that involved in the simvastatin PK/PD.

Thank you for this important comment. We consulted the medical research statisticians, and carefully reanalyzed the data using the appropriate statistical methods in CHD patients with different rs3918242 genotypes (see modified "statistical analysis"). We found that MMP9 in serums did not differ among three groups of CAD patients, but the baseline LDL-C and TG in patients carrying TT genotype were increased compared with those carrying CC genotype (New Table 4). Further, we did a correlation analysis on MMP9 and LDL-C, and we found no correlation between serum MMP9 level and LDL-C in this cohort (see the supplemental information). Thus, current data did not support that LDL-C level was correlated with MMP9.

We agreed with the reviewer that with our current data, we could not conclude that this SNP modulate simvastatin LDL-C lower efficiency. Based on the current observations, we modified our conclusion as "In this study, we showed that CAD patient carrying MMP9 rs3918242 TT genotype had significantly increased plasma TG and LDL-C levels than patients carrying CC genotype, and their LDL-C lowering response to simvastatin treatment were more robust than patients with CC genotype."

2. It is not surprising that after simvastatin treatment, LDL-C was decreased. Based on the SNP genotype, there is no significant difference in absolute LDL-C levels after simvastatin treatment. The significant larger % change in LDL-C in patients with TT genotype may merely due to a significant higher baseline LDL-C level. However, there is no evidence that MMP9 -1562 C>T variant genotype is a cause of higher baseline LDL-C level, or regulates LDL-C levels.

Thank you. We apologize that the data were presented in the confusing way. After carefully reanalyzing the data, we found that at baseline, the LCL-C level was significantly increased in CAD patients with TT genotype compared to CC genotype. After statin treatment, the LCL-C level was indeed decreased in CAD patients with TT genotype compared to CC genotype. Thus, the absolute LDL-C concentration change was significantly greater in patients with TT genotype than those with CC genotype (1.28mmol/L for TT genotype vs. 0.74mmol/L for CC genotype). To present these information in a more clear way, we followed the suggestion by Reviewer #1 and made the dot plot of serum LDL-C concentration of all the CAD subjects with 3 genotypes before and after statin treatment (New Figure 2)

3. Some of the data are not convincing or not visualized properly. In Table 4, MMP9 levels at week 0 for CT genotype seems more significant than TT genotype since the amount is

higher and the n = 69, which is much larger than 7 for TT genotype. The % change for TT genotype may also significant different from other genotypes. These data, at least for MMP9 and LDL-C, should be showed in figure with x-axle as genotype and y-axle as concentrations, each patient should be dotted and the 95% confidence interval should be showed.

Thank you. We agree that the number of patients with TT genotypes were very limited (n = 7) in this cohort, which was due to the low frequency of T allele. In view of this important point, we are making effort to recruit more CHD patients into this study, so that we will be able to validate this initial finding in a new cohort of patients.

To present the data in a more clear and straightforward manner, we plotted the LDL-C and MMP9 concentration of each patient with 90% CI indicated (new Figure 2). Thank you for the excellent suggestion.

4. Some sentences are not clear and confusing.

Thank you. We significantly modified the sentences and languages in the revised manuscript.

Other problems:

1. Many important backgrounds that the authors state in the Introduction has no references;

Thank you. We added references to the background statement in the introduction section in the revised manuscript.

2. MMP9 1562 (should be "-1562") C>T is not a professional term to refer the SNP. The "rsID" for the SNP should be used;

Thank you. We substituted the MMP9 -1562C>T with MMP9 rs3918242 in the revised manuscript.

3. Page 6, line 53. What is the mean for "hand"? hr and

Thank you. The typo was corrected.

4. Page 7, line 9. What is the mean for "gene frequencies"? That is "minor allele frequency".

Thank you. We used “allele frequency” instead.

5. Table 2 showed that smoking is strongly associated with CHD. It was known that smoking could induce MMP9 expression in lung. Is that also happen in blood? Is smoking a cause of variations in MMP9 level rather than the SNP genotype?

Excellent point. Thank you. We found that the percentage of smokers in the CHD was significantly higher than that in the control group (41.23% vs. 24.19%). This was associated with elevated MMP9 level in the blood in CHD patients. When adjusted for smoking, MMP9 level remained significantly elevated in CHD patients than controls, suggesting that other factors besides smoking also contributed to the increased MMP9 level in CHD patients.

After reanalyzing the data, we found that MMP9 levels were comparable in CHD patients with 3 genotypes. Due to the limited sample size, particularly for the TT genotype, we could not do further stratification for smoking. However, we agreed that it would be important to assess the role of smoking in MMP9 level in the blood with in CHD patients with three genotypes.

6. Page 9, line 1. Need to specify what is the mean of "improved", plasma lipid increased or decreased? Decreased.

Thank you. We clearly pointed out that the plasma lipid level was “decreased” in the revised manuscript.

7. Page 10, line 1. Not clear.

Thank you. We modified the sentence to emphasize that the serum TG, TC and LDL-C were reduced and HDL-C level was increased after statin treatment in CHD patients.

8. Page 11, line 38. The authors cited their previous study (as claimed "our previous….") and labelled [17]. However, reference 17 and present study has no author or affiliation overlaps.

Thank you for pointing out this typo. We modified the sentence as “The previous study also revealed that MMP-9 rs3918242 polymorphism….”

9. Page 12, line 12. "….simvastatin on MMP9 level,…" is not clear.

This sentence is modified as “Simvastatin treatment lead to similar reduction of MMP9 level in the blood, independent of MMP9 rs3918242 polymorphism”

10. Page 13, line 6. Not appropriate.

This sentence is modified as “, and thus provided new evidences that genetic variation of MMP8 was associated with the lipid-lowering efficacy of simvastatin”

11. The quality and resolution of Figure 1 is low.

Thank you. We provided the higher resolution image for the new Figure 1.

Reviewer #2: This is a timely study, with only some limitations that should be addressed in the discussion.

1) The fact that only a single dose of simvastatin (the one that is usually prescribed to start with) was used is certainly a limitation, but the authors should at least address whether or not a dose-dependent effect is to be expected;

Excellent suggestion. We discussed this limitation in the revised manuscript as follows: “Patients in this cohort were uniformly treated with the medium dose of simvastatin (20mg/day) for 12 weeks, and their serums LDL-C was significantly reduced to the level comparable to the control subjects. In this context, we found that patients carrying MMP9 rs3918242 TT genotype had more robust LDL-C-lowering response to statin treatment compared to patients carrying CC genotype. It would be interesting to examine whether the association of genetic variation of MMP9 rs3918242 with simvastatin’s LDL-C lowering response was dose-dependent, and we still expected improved LDL-C-lowering response of MMP9 rs3918242 T-allele to simvastatin even at lower dose. “

2) The fact that diet was modified may partly bias the results: also this aspect should be addressed.

Thank you. We discussed this limitation in the revised manuscript as follows: “All the CHD patients were given simvastatin in combination with low-fat diet intervention. Therefore, the LDL-C lowering effect could possibly be biased by the diet intervention. Future studies without diet intervention would be helpful to address this problem. ”

3) There are some minor English style issues to be checked.

Thank you. We carefully checked the English style in the revised manuscript.