Author's response to reviews

Title: A Functional Polymorphism T309G in MDM2 Gene Promoter, Intensified by Helicobacter pylori Lipopolysaccharide, Is Associated with Both an Increased Susceptibility and Poor Prognosis of Gastric Carcinoma in Chinese Patients

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Author's response to reviews: see over
Dear Editor,

Thank you very much for your advice. We have revised the paper for your consideration. We have addressed each of the comments, and amendments are highlighted in red in the revised manuscript. We have also provided detailed point-by-point responses to each comment below.

We hope that the revised manuscript is acceptable for publication in the journal, and we look forward to hearing from you soon.

With best wishes,

Yours sincerely,

Guoxin Zhang
Reviewer: Bing Xia

1. What method authors used to confirm genotyping.
   Answer: Thank you for your suggestion, and we have added the method we confirmed genotyping in “Genotype analysis” of the “Methods” (Line 12, Page 9). First, the gel images were read independently by two research assistants. If a consensus was not reached on the tested genotypes, then the genotyping was repeated independently until a consensus was reached. In addition, to validate the RFLP method, 100 (50 from cases and 50 from controls) PCR products were selected randomly for direct sequencing with ABI 3700 sequencer, and the concurrence rate of these two methods was 99%.

2. Authors should give P value for Association between MDM2 SNP309 and gastric carcinoma in relation to location, metastasis and TNM stage in Table 4.
   Answer: We have revised the Table 4 according to your suggestion.
1. In subtitle "Genotype analysis" of "Material and Methods" section, they should describe the RFLP method briefly, including what kind of restriction enzyme they used.

   Answer: We have revised the "Genotype analysis" of "Methods" section according to your suggestion (Line 12, Page 9).

2. The authors found that MDM2 SNP309 polymorphism is closely associated with the risk of gastric carcinoma and overall survival. However, the polymorphism was not associated with vascular invasion, lymph node metastasis, liver metastasis, peritoneal metastasis and the TNM stage. Thus, they need to explain why the polymorphism is associated with overall survival or poor prognosis.

   Answer: Although the risk gastric carcinoma was significantly increased in MDM2 SNP309G/G homozygotes, compared with T carriers, regardless of the presence or absence of vascular invasion, lymph node metastasis, liver metastasis, peritoneal metastasis and the TNM stage, however, the increased risks were great in lymph node metastasis present carcinoma, liver metastasis present carcinoma, peritoneal dissemination present carcinoma, and advanced stages carcinoma (Table 4), which are all associated with poor prognosis. Therefore, this may also explain why the polymorphism is associated with overall survival or poor prognosis. Thank you very much for your question, and we have rewritten the results section for table 4 (Line 7, Page 16).

3. In in vitro system, the authors found that H. pylori elevated the transcriptional activity of the MDM2, in both SNP309T and SNP309G alleles, and that the expression MDM2 protein was induced by H. pylori LPS in a dose and time-dependent manner in both AGS and MKN45 cells. In Table 1, there is...
no significant difference in *H. pylori* infection rate between cases (71.1%) and healthy controls (67.9%). I wonder whether it is possible to conclude that there is a joint effect of MDM2 SNP309G/G allele and *H. pylori* infection.

Answer: Although there is no significant difference in *H. pylori* infection rate between cases and controls from our study, however, the trend is that *H. pylori* infection could elevate the gastric carcinoma risk among T carriers (adjusted OR=1.14). *H. pylori* is identified as a major cause of gastric carcinoma development already, why we failed to demonstrate a positive association, the reason may be because of the loss of *H. pylori* from the stomach or the reduced immune response to *H. pylori* infection during gastric carcinogenesis or degradations of specific IgG antibody in the serum [1, 2]. It has been shown that the level of specific IgG antibodies falls with age, and thus elderly people infected with *H. pylori* may not produce a sufficiently high specific IgG antibody level [3]. Therefore, it could conclude that there is a joint effect of *MDM2 SNP309G/G* allele and *H. pylori* infection, in our opinion.


4. page 20, line 5; stomac should be stomach.

Answer: Sorry for the mistake, and we have revised it according to your suggestion (Line 3, Page 21).

5. They should correct mis-typing in Table 1 (n=???).
Answer: Sorry for the mistake, and we have revised it according to your suggestion.