Author's response to reviews

Title: The MLH1 2101C>A (Q701K) variation is related to risk of gastric cancer in Chinese males

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Author's response to reviews: see over
Dear Dr. Chap,

Thank you very much for your e-mail on Aug 30 in which you sent us your suggestions together with the referees' reports on our paper MS-1756834127438044 Version 2 entitled “The MLH1 2101C>A (Q701K) variation is related to risk of gastric cancer in Chinese males”. We would also like to thank the referees for their valuable comments and suggestions. We have revised the manuscript according to your and the referees’ suggestions and your journal’s format. The modified parts are emphasized in blue in the text.

The response to revision request or comment and major modifications include:

To REVIEWER 1:

1. About the added photography of MLH1 staining: Thank you for the reminding. This photography of MLH1 staining is the IHC assay for sample 905522 (Patient G150). As you see, it shows not stained both in cancer cells and normal cells, perhaps suggesting the loss of MLH1 expression already occurred in germline due to the mutation. We examine the samples carried out by IHC assay simultaneous with this sample, and found significant MLH1 staining in them (One example is sample 935082, see supplement figure 1). We hope this can deplete the possibility of failure staining.

To REVIEWER 2:

1. About the loss of clinical classification of the 74 cases: The 74 cases were diagnosed to be gastric cancer by gastroscope. However, they did not carry out operation in the hospital. So only the blood are available and not the tumor tissues for exact pathologic diagnosis.

2. Thank you for the reminding. We have modified the presentation in Page 7, Results: MLH1 genotypes and risk of gastric cancer and Page 11, Discussion: paragraph 7.

To REVIEWER 3:

1. For the gastric cancer patients, the status of familial recurrence for gastric cancer cases is obtained, however we are not available for this data on the controls, though we now realized that it might be important for confusion assessment.

2. Anhui and Jiangsu provinces are located just nearby and all belonged to East District of China, the ethnicity of the cases and control selected is the same, which is Han of Chinese. We collected samples from the two provinces simultaneously to get enough samples in a short time. Now we have changed the expression from ‘Anhui and Jiangsu provinces’ into ‘East District of China’ in order not to cause misapprehension as if they were two distinct districts. This modification is in Methods: Clinical samples.

3. We realize that it’s not proper to indicate the 236 cases and 240 controls are matched for age and gender, we now indicate that as ‘there is no significant difference in the cases and controls for age and gender (p = 0.997 and p = 0.915 for age and gender, respectively)’ in Results: Characteristics of the study population. The status of smoking and alcohol consumption for GC cases is obtained, but we are not available for this data on the controls selected, and we now realize that this might cause bias.

Thank you for the instruction of the three forms of statistical analysis for case-control study. We now have used ‘non-conditional logistic regression’ adjusted by age and gender (using the polymorphism, age, and gender as covariates) to present the ORs, CI and p value in Table 2.

4-6. When using non-conditional logistic regression (using the genotype of MLH1 2101C>A, age, and gender as covariates), the OR for genotype, gender, and age is 2.77 (0.73 -10.57), 1.02(0.67-1.55), and 1.00(0.81-1.22), respectively. The $P$ value is 0.136, 0.940 and 0.961, respectively. We comprehend the results show that, taken the 236 cases and 240 controls as a whole, when adjusted by age and gender, there is not significant difference between MLH1 2101C
and 2101A; when adjusted by genotype and age, there is not significant difference between male and female; when adjusted by genotype and gender, there is not significant difference among the four age grades.

But the results can not elucidate the point of view we try to show, that is ‘what’s the status in males’? As shown in Table 1, female GC patients only occupy one quarter of the total GC patients and are far less than male GC patients in East District of China. The number of only 58 female GC patients might be too small for correct statistical evaluation and might interfere the analysis of male GC patients. So, though stratified analysis might lose some statistical power, we still carry out it to try to demonstrate the effect of the genotype in males, as well as its effect in different age groups (Table 3). Though we realize the sample size might limit the study, especially in females.

7. Yes, haplotype analysis will be better to elucidate the association for SNPs, however in our data the mutation rate of -28A>G is only 1.7%, not effective for haplotype analysis. So we used pairwise joint association assay to proximately demonstrate the association for the two SNPs as shown in Table 4.

8. Thank you for the reminder. We now have evaluated the Hardy-Weinberg equilibrium in controls for SNPs, shown in Results: MLH1 variations identified in gastric cancer patients.

9. We have modified the structure of the discussion, in particularly emphasizing the limitations of the study.

Besides the major modifications, some minor changes have also been made in this revised version. They are as follows:

1. Following your instruction, normal has been changed into controls in Methods Clinical samples, Results Characteristics of the study population, and Discussions.
2. PCR conditions and primers sequences have been supplied in Methods (Mutation screening) and Supplement Table 1.
3. The discussion in the penultimate paragraph: The presentation has changed as: However, it was not significantly associated with the risk of gastric cancer (P=0.069, Table 3), and only A trend was observed.
4. Following your suggestion, we have emphasized the limitations of the study in Discussion.
5. At the end of the conclusions: The presentation is modified as: It was found that the MLH1 2101 C>A mutation may contribute to an increased risk of gastric cancer in males.

I hope we have addressed your request clearly and these modifications will make the manuscript meet the criteria of BMC GASTROENTEROL. Thank you very much for your time and effort that goes into the publications of this paper.

Sincerely yours,
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