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 email on Dec 6 in which you sent us your suggestions together with the referees’ reports on our paper MS-1756834127438044 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.

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

To Dr Schulmann:

1. We have added the introduction of ESEfinder in ‘Method’ section—Bioinformatics analysis of MLH1 variants according to your suggestion.
2. About the replication of this findings regarding 2101C>A: In another independent samples, we first detected this mutation in 2/105 gastric cancer patients with low familial recurrence, and not in 82 CRC patients nor 122 normal controls.

Yes. As you mentioned, RNA transcripts and protein expression analysis is important information for impaired splicing. As fresh blood of the patients is not available till now, we used bioinformatics analysis to predict exon splicing. IHC is added to demonstrate expression of MMR proteins and another important protein E-cadherin related to GC. IHC of index G150 showed loss of MLH1 protein, and normal MSH2 and CDH1 expression. We hope the results, together with the the case-control study, are adequate to demonstrate the importance of this mutation.

3. Following your direction, we noticed the polymorphism c. -28 A>G has been reported in other ethnics as well. Sorry for the error, and this has been corrected in the discussion part, the fourth paragraph.

4. The language has been modified by a professional copyediting service (www.biomedes.co.uk).

To Dr Chan:

1. About the selection of MLH1 gene and immunohistochemistry/MSI test: Most cases of HNPCC are caused by germline mutations of mismatch repair genes, most often in MLH1. Gastric cancer, mainly of the intestinal type, has been
identified as a common extracolonic tumor of HNPCC in East Asians. We suggest there might be association between mutations of $MLH1$ and gastric cancer, so we select $MLH1$ gene. This elucidation is added in ‘Background’ section, the first paragraph.

Yes it would be better to carry out a comprehensive immunohistochemistry/MSI test. In this paper we suggest to give a comprehensive report of $MLH1$ variations in Chinese GC patients, not just some obvious pathogenic mutation. There might be some polymorphisms, even some mutations, will not cause the loss of MLH1 protein, so immunohistochemistry/MSI test was not carried out as a preliminary screening strategy. Another reason for it is not all the tumors are available for IHC analysis.

2. In another task, we carried out germline methylation analysis, the eight patients with $MLH1$ 2101 C>A mutation did not show evidence of $MLH1$, $P16$ or $CDH1$ methylation.

3. One of the eight patients carrying $MLH1$ 2101 C>A mutation has low cancer family history. His brother had gastric cancer at the age of 52, but we are not available for his blood.

The language has been modified by a professional copyediting service (www.biomedes.co.uk). All major changes made have been highlight with blue coloured words in the revised manuscript. I hope these modifications will make the manuscript qualified to be published on BMC Gastroenterology. Thank you very much for your time and effort that goes into the publications of this paper.

Sincerely yours,
Yimei Fan, Ph.D
Associate professor of the Department of Medical Genetics, Medical School, Nanjing University