Author's response to reviews

**Title:** Association between H-RAS T81C genetic polymorphism and gastrointestinal cancer risk: a population based case-control study in China

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**Author's response to reviews:** see over
Dear Dr. or Prof. Iratxe Puebla,

Thank you for your letter and for the opportunity to resubmit the manuscript MS: 1388742149189765 entitled “Association between H-RAS T81C genetic polymorphism and gastrointestinal cancer risk: a population based case-control study in China”.

We are also thankful to the three reviewers and we agree with their comments. The manuscript was extensively revised according to their suggestions and all concerns raised by the reviewers are addressed in the revised version of the manuscript. Changes are in red font in the revised version of the manuscript.

Sincerely,

Yongjing Zhang
Reviewers’ comments:

Reviewer Hongbing Shen

1. Page 3, line 2, the authors described that the CC genotype was with more risk, which was not appropriate, because the adjusted ORs were 3.67 and 3.29 for TC and CC genotype, respectively.
   The risk of gastric cancer was described according to the adjusted ORs.(Page 3)

2. In this study, the cases and controls were not matched by age and gender and they were significantly different between gastric cancer cases and healthy controls. The gastric cancer cases were older and more men than their controls. Therefore, the authors need to describe more details of their study design and exclude the confounding effects of age and gender on the association between genotypes and gastric cancer risk. It was better to perform stratified analysis according to variables shown in Table 1 (age, gender, smoking, drinking and family history) though the sample size was small. In addition, the interaction effect was not directly estimated from stratified analysis (see Page 9, paragraph 2).
   More details of the study population and controls sampling were added in the study subjects paragraph (Page 5). Taking into account the potential confounding effects, we have already carried out the stratified analysis according to age, gender, smoking, drinking and family history. However, neither confounding effects nor interactions were observed (data not shown in the paper).

3. The authors needed to present the definitions of smoking, drinking and family history.
   The definitions of cigarette smoking, alcohol drinking and family history of
cancer were presented at Page 6.

4. Please add the note for adjusting factors in Table 2. In addition, family history should be included.
   The requested note for adjusting factors was added in Table 2. (Page 18)

5. In the meta analysis, another 254 controls from hospital in Johne’s study were cancer-free and needed to be included in the meta analysis. Furthermore, the authors should not divide their study into two comparisons because those two case groups shared one control group.
   The data included in the meta-analysis was revised according to the suggestion.

6. The risk effect of variant allele found in gastric cancer was not detected in colorectal cancer, so the eligible explanation was needed in the discussion.
   The probably mechanism by which H-RAS T81C polymorphism modifies risk of different kinds of cancer was presented in the discussion section. (Page 10)

7. Add the rs no. of T81S in the article.
   The Reference SNP number of H-ras T81C is rs12628 which was added in page 4, paragraph 2.

Reviewer Laura Ottini

Methods
1. More details should be given about sampling procedure within the cohort. Why the Authors considered only cases alive at the end of follow up and not all incident cases? If the polymorphism examined is associated to survival the
prevalence of H-RAS T81C polymorphism in cases could be underestimated (or overestimated). This could explain the negative result for colorectal cancer. Why the controls were selected at the end of follow up and not with a nested design? Again if the polymorphism examined is associated to other cancer(s), the prevalence of H-RAS T81C polymorphism in controls could be underestimated.

More details of the study population and controls sampling were added in the study subjects paragraph (Page 5). Initially, a cohort study of colorectal cancer was conducted in this study population, however, the gastric cancer patients were not included. Therefore, we could not perform a nested case-control study design. The questionnaire investigation and blood sample collection of cancers were carried out regularly after the cancer surveillance and registry system reporting, other than at the end of follow up. And the information of controls was collected during the same periods. But it was still possible to underestimate the effects of H-RAS T81C polymorphism on cancers. Further study on the association between this polymorphism and cancer survival is needed.

2. The Authors investigated the effect of H-RAS T81C polymorphism also by stratification of family history, cigarette smoking and alcohol drinking status and did not find any interactions. More details should be given about the questionnaire and the methods used to measure alcohol consumption and dietary history. Moreover, what do the Author mean by “family history of cancer”? (family history of gastric cancer, colon cancer or cancer in general?) This is an important issue since a subset of hereditary gastrointestinal cancers can be linked to specific molecular phenotype (e.g. microsatellite instability). The definitions of cigarette smoking, alcohol drinking and family history of cancer were presented at Page 6. And no significant difference was observed.

3. The genotyping results obtained by PCR-RFLP should be confirmed by
sequencing analysis (e.g. a subset samples with each of the three possible genotypes for the H-RAS T81C polymorphism should be confirmed by sequencing and included on each genotyping assays as positive controls).

According to Johne’s report, when they sequenced the coding region of H-ras derived from urinary bladder cancer tissue, they found this polymorphism H-ras T81C, and then they performed the PCR-RFLP analysis for genotyping in large sample size study. The same method was selected by Sanyal et al and the results were confirmed by direct sequencing. And we randomly selected 10% of the samples to be identified repeatedly for accuracy, and the results were 100% concordant. Therefore, it is reasonable to believe that our genotyping results are corrected. We could perform the sequencing analysis if it is strongly suggested, however, we need some more time.


4. The description of the unconditional regression analysis should be better clarified indicating how the three genotypes were included in the model, what was the reference and that models with dominant inheritance were also tested. The confounders used should be indicated with more details (in continuous or as categorical variables?).

The detailed information of unconditional logistic regression analysis was added in statistical analysis paragraph (Page 7).

5. It’s not clear how interaction has been tested. I am convinced that interaction should be better tested using likelihood ratio test comparing models
with and without interaction terms.
Stratified analyses were used to detect the potential gene-environment interactions in this paper.

6. Meta-analysis methods should be described with more details, e.g. which specific test of heterogeneity was used?
Requested information about the meta-analysis was added in the statistical paragraph. (Page 7)

Results
1. In the first paragraph: I suppose that “significantly” mean p<0.05, please specify.
A p value of less than 0.05 was considered statistically significant in this article and we defined it in the statistical analysis paragraph (Page 7).

2. I have some concern about the rationale to perform a meta-analysis in relation to different cancer sites. Although no heterogeneity was observed, this could be due to the low power of this kind of test. A descriptive table is enough.
To date, some meta-analyses have carried out to investigate on a variant for risk in multiple cancer types. TGFBR1*6A, STK15 T91A and MDM2 SNP309 variant were assessed for cancer risk in multiple cancer types. It is likely that some variants reported in the literature to be associated with a specific type of cancer risk will be general cancer susceptibility factors and that meta-analysis of multiple cancer types will lead to a better understanding of overall risk. So we performed this meta-analysis to explore the potential effect of H-RAS for all kinds of cancer, at least we could get a clue for further studies.

-Minor Essential Revisions
1. Two-side is unnecessary in the first sentence of the statistical analysis paragraph.
2. Results section: page 10, line 10: p=0.000 became p<0.0001.
The manuscript has been revised according to these two suggestions (Page 7 and 8).

Reviewer Jae Yong Park

1. A major concern is the selection of cases and controls. 1) In the selection of cases: this study included patients who had survived up to May 2005. Therefore, there is a possibility that only the patients with good prognosis or early stage of cancer may be selected (selection bias). That is, the case population is not truly representative of whole gastrointestinal cancer in the population studied. The HRAS SNP may have an influence on disease progression and/or prognosis, thus the genotype distributions between cancer patients with early stage of disease and those with late stage disease, as well as patients with good prognosis and those with poor prognosis. 2) In selection of controls: Author should explain eligible criteria for the selection of controls. It should be answered to the following concerns: how to match the controls to the cases; and is the prevalence of smoker among the controls (40.0%) comparable to that of authors’ country. - I am impressed with low prevalence of smoking particularly in the control after consideration of the low prevalence of smoking in Chinese women). Would you present the prevalence of smoking (or the trends in smoking habit) in China? 3) It is uncertain why the demographics of stomach cancer cases are statistically different from other cancers and controls (age and smoking rates) –Are these true in the authors’ population. In order to understand these points, the authors would provide the epidemiologic characteristics of gastrointestinal cancers in authors’ population.

More details of the study population and controls sampling were added in the study subjects paragraph (Page 5). Some explanations have to be addressed here.

1) The questionnaire investigation and blood sample collection of cancers
were carried out regularly after the cancer surveillance and registry system reporting, other than at the end of follow up. And the information of controls was collected during the same periods. But it was still possible to underestimate the effects of H-RAS T81C polymorphism on cancers. Further study on the associations between this polymorphism and cancer progression or prognosis are needed.

2) Although the production and consumption of cigarettes are very large in China, the prevalence of smoking in the south of China is relatively lower than that in other parts of China. Consistent with the results reporting by Zhu et al, the prevalence of smoking is almost the same in this area of China around 30%~40%.

3) In our study, the results of investigation indicated that the average age of gastric cancer patients was significantly older than that of controls. The risk of cancer increased with the age, and probably because the healthy controls were the represent sample of the study population, the average age of the cases was a little older.

2. The authors should be described the potential mechanism(s) for the tumor origin-specific association (The HRAS SNP was associated with gastric cancer; however, it was not significantly associated with colorectal cancer) in the Discussion section.

The probably mechanism by which H-RAS T81C polymorphism modifies risk of different kinds of cancer was presented in the discussion section.(Page 10)

3. It would be explained why the colon cancer was divided into colon and rectum cancers. Are there differences in the etiology and pathogenesis between colon and rectum cancers?

Colon cancer and rectal cancer share many features and are often referred to as 'colorectal cancer'. The issue whether colon and rectal cancer should be considered as a single or two distinct entities is still debated. The colon and
rectum have different biological functions, morphologic features and histochemical reactions. Some studies revealed the differences in the clinicopathological features between the two cancers. Therefore, we divided colorectal cancer into colon and rectum cancers in order to investigate whether there is any distribution difference of RAS gene between the two kinds of cancers.

4. P-values should be provided in Tables.
Because there was not enough space to show all of the P value in the Tables, we added the P value in the text if necessary.

5. The rs number and reference sequence of the polymorphism should be provided.
The Reference SNP number of H-ras T81C is rs12628 which was added in page 4, paragraph 2.

6. The results would be shortened.
As suggested, the result section of this paper has been simplified.