Author's response to reviews

Title: Polymorphism in COX-2 modifies the inverse association between Helicobacter pylori seropositivity and esophageal squamous cell carcinoma risk in Taiwan: a case control study

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

First of all, I would like to say thank you for the valuable and constructive commands for the manuscript “Polymorphism in COX-2 modifies the inverse association between Helicobacter pylori seropositivity and esophageal squamous cell carcinoma risk in Taiwan: a case control study” from you and reviewers. According the comments and suggestions, we have revised the content of the manuscript. Thanks for the suggestions from reviewer Sara Raimondi, we changed the reference category in Table 4 and found that the protective effect to lower third ESCC risk provided by H. pylori seropositivity may be mediated by influencing COX-2 AA genotype expression. The interaction between COX-2 polymorphism and H. pylori infection in ESCC is easier to understand. Here is what we have modified.

Approval of an appropriate ethics committee

Answer: We have added the information about the approval of ethics committee of the study at the first paragraph of Methods section. Please see page 10.

“Competing interest”

Answer: The “competing interest” has been added. Please see page 22.

Advices from reviewer Sara Raimondi

MAJOR COMPULSORY REVISIONS:

1) As previously stated, I think there is a main problem concerning the study design. On line 3 from the bottom in the Background section the authors refer to a
“prevalence study”. Then, in the Methods section, they did not further specify whether all the cases are prevalent or how many are incident and how many prevalent cases. The main problem with prevalent cases is that the information on risk factors may not be collected at the time of diagnosis, but even several years after diagnosis. It is possible that patients changed their life-style habits, as alcohol, smoking and betel quid consumption, as a result of the disease; therefore these data may be biased. Moreover, prevalent cases may be selected according with the severity of ESCC: incident cases with severe ESCC have lower probability to be included in the study due to the shortness of disease in comparison with incident cases with less severe ESCC. This may affect the exposures under study (including H. pylori infection and COX-2), since any risk factor associated with longer survival will also become linked with the disease outcome, even if it may be protective against the development of severe disease. I suggest performing all the analysis on incident cases only. If necessary, the authors could recruit new incident cases in order to reach an appropriate number of patients.

Answer: Many thanks. We use the wrong term to describe the study design. This is an incident case-control study. All the esophageal cancer cases were collected within one month after diagnosis, and the controls were also selected at the same period. The related statement has been revised. Please see the last paragraph of the Background at page 9. More information about the recruitment of cases and controls are described in first paragraph of Methods section. Please see page 10.

2) Both the presentation and the discussion of the results on H. pylori and COX-2 interaction are confusing. Basing on the title, Table 4 should be modified so that the reference category is “H. pylori-negative with GG+AG polymorphism”. This way authors may observe how the association between H. pylori seropositivity with ESCC development varies according with COX-2 polymorphism. The discussion of results should be changed accordingly with the new analysis.

Answer: Many thanks for the valuable comments. We have modified the presentation of Table 4 with *H. pylori*-negative with COX-2 GG+AG genotypes as the reference category. We also revised the related statement in Abstract (page 5), the last paragraph of Results (page 16). We also modify the Discuss and Conclusions section according the new results. Please see the third paragraph of Discuss section (page 18) and Conclusions (page 20).

3) A description and discussion of the strengths and limitations of the study is completely lacking. First of all, the sample size is relatively small. How does this affect the statistical power of the study? Especially for interaction analysis, the sample
size should be very large to reach a good statistical power. Authors should also discuss the limitation of study design, collection of information on risk factors, possible misclassification,…

Answer: The strength and limitation of this study has been described at the last paragraph of Discuss section. Please see page 19 and 20. About the influence by sample size, typically, a small sample size leads to a relatively lower statistical power. However, power of the statistical testing is also dependent of the effect size (in this study, the strength of the association). We used the stratified data for *H. pylori*-negative patients as a subgroup to approximately calculate the statistical power. With conditions as $\alpha = 0.05$, 0.28 of the probability of exposure in controls, 38 of the number of case patients, 2 matched controls per case patients and 6.9 of OR (the observed risk), the estimated power is 99.8%. The results reveal that the large effect size in the defined subgroup confers a large enough power to detect the significance of the hypothesis testing. However, the limited sample size in certain study subgroups is a reality. We have added awareness with regard to limited sample size to the revised paper, please see last sentence of the Result section (page 16) and last paragraph of the Discussion (page 20).

MINOR ESSENTIAL REVISIONS:
1) ABSTRACT, Background: When listing the purposes of the study the authors never refer to interaction analysis between *H. pylory* infection and COX-2, which seems to be the main result of the study.
Answer: Many thanks. The background in Abstract section has been revised. Please see page 4

2) ABSTRACT, Results: Please replace “0.5-fold risk” with something like “*H.pylori* seropositivity was found to be inversely associated with ESCC development (OR; 95%CI: 0.5; 0.3-0.9).”
Answer: We have replaced the statement as reviewer’s suggestion. Please see page 5.

3) ABSTRACT, Results + Results, last line + CONCLUSIONS: “COX-2-1195 AA homozygous was associated with an increasing risk of contracting ESCC in comparison with the non-AA group, especially AMONG PATIENTS WITHOUT *H. PYLORI* SEROPOSITIVITY”. Please replace with “AMONG PATIENTS WITH *H. PYLORI* SERONEGATIVITY”, which seems clearest.
Answer: We have revised the statement as reviewer’s suggestion in Abstract (page 5). In order to describe the meaning more clearly, we rewrote the sentence. Please see
In Conclusions, we also modify the sentence, so it was deleted. Please see page 20.

4) BACKGROUND, last paragraph: “However, very few studies…”. Please add references.  
Answer: In fact, we did not find the related paper from Pubmed website. To our knowledge, this might be the first manuscript for evaluating the interaction between \textit{H. pylori} and COX-2 polymorphism in risk of ESCC. We have changed the sentence. Please see the last paragraph of Background section (page 9).

5) METHODS: The authors should specify what the main diseases of the control group were.  
Answer: All the controls were healthy and cancer-free subjects. They were collected from the group receiving routine health examination. We have added the description about the controls in first paragraph of Methods section. Please see page 10.

Answer: We have revised the statement according reviewer’s suggestion (page 13).

7) METHODS, Statistical analysis: Please add the name of the statistical software used to carry out the analyses.  
Answer: The statistical software used in this study has been added in the Statistical section. Please see page 14, the last sentence in statistic analysis section and reference 35.

8) TABLE 1: to be complete, please add the adjusted ORs also for age, gender, and ethnicity, even if they are not significant.  
9) TABLE 1: add the p-value for trend for education level, tobacco smoking, alcohol drinking, betel quid chewing.  
Answer: The adjusted ORs for age, gender and ethnicity have been reported in the revised Table1. Also the p-value for trend has been presented accordingly.

10) RESULTS, last paragraph: “To understand the influence of COX-2….we performed a stratification analysis in Table 4”. Please replace with: “…we presented a stratified and interaction analysis in Table 4”.  
Answer: It has been revised according reviewer’s suggestion. Please see the last paragraph of Results section (last sentence in page 15).
11) DISCUSSION, first paragraph: “the trend was the same in the lower third ESCC (...), although the DIFFERENCE was not...”. What difference? Do you mean the OR for lower third ESCC?

Answer: As reviewer’s speculation, the “difference” is used to describe the OR of lower third ESCC in H. pylori seropositive subgroup (Table 2). However, we decided to delete the description after rewriting the discussion in order to focus on the interaction of *H. pylori* and COX-2.

12) DISCUSSION, second paragraph: “On the contrary, there are different VIEWS”. More than “VIEWS” they seem to me “RESULTS/FINDINGS”.

Answer: We have replaced the statement according reviewer’s suggestion. Please see the fourth line in second paragraph of Discussion section (page 17).

13) DISCUSSION, third paragraph, last line: I could not understand this sentence. Please re-write it.

Answer: The original sentence described the interaction between *H. pylori* and COX-2 in risk of ESCC was notably enhanced in lower third esophagus, and we used the sentence to link the relationship to acid exposure described at the next paragraph. In combination to the newly statistic data, we have added several sentences to describe the information at the end of third paragraph in Discussion. Please see page 18 and 19.

14) DISCUSSION, last paragraph: “These findings provide a clue.....by COX-2 EXPRESSION...”. I think it should be “POLYMORPHISM” rather than “EXPRESSION”.

Answer: In order to let the sentence easy to understand and to focus on the relationship between acid exposure and ESCC, we have rewritten this sentence. Please see the fifth line from the bottom of the fourth paragraph in Discuss section (page 19).

DISCRETIONARY REVISIONS
1) ABSTRACT, Conclusion: I suggest rewriting the conclusive sentence as: “H. pylori seropositivity was inversely associated with the risk of ESCC in Taiwan,...”.

Answer: We have revised the sentence according reviewer’s suggestion. Please see page 5

2) METHODS, Statistical analysis + RESULTS, third paragraph + TABLE 3 +
DISCUSSION, first paragraph: I suggest calculating and reporting p-value for H-W equilibrium only in controls. H-W equilibrium in cases is not of interest since cases may not be in H-W equilibrium if there is indeed an association between genotype and disease outcome, as it happened in your study (see also Takkinstian A, Statistics in Medicine 2005; 24:1291-1306).

Answers: We have modified the presentation of Table 3 to indicate the p-value for H-W equilibrium only for the controls. The related sentences in statistic analysis section of Methods (page 13) and third paragraph of Results (page 15) have also been revised. We have deleted the description about ESCC group in Discussion section.

3) The first four sentences of the discussion are more similar to an introduction; I would delete them or at least move them to the Introduction section.

Answers: The first four sentences of the discussion were moved to the second paragraph of Background section. Please see page 8.

4) DISCUSSION, last sentence: it seems to me that the first three sentences of this paragraph are out of theme. I would delete them.

Answer: we agree the suggestion of reviewer and it has been deleted. Please see page 19.

Advices from reviewer Farhad Islami

1. In the first sentence of the Background section, other high incidence areas should be mentioned (such as central Asia and southern and eastern Africa: Parkin et al 2005). Otherwise, the sentence should be changed to something like: the incidence is high in some geographical areas, such as certain parts of China. A more recent reference should also be used for this sentence.

Answer: Thanks for the suggestion. We have modified the sentence at first paragraph of Background (page 7), and add new references (1 and 3).

2. I wonder if the controls were healthy people who underwent routine physical examinations or they were outpatients who referred to a clinic because a health problem. Although polymorphism in COX-2 is not likely to be related to control selection method, the authors should add a few sentences to the Methods to describe the control selection process.

Answer: All the controls were healthy and cancer-free subjects. They were collected
from the group receiving routine health examination. We have added the description about the controls in first paragraph of Methods section. Please see page 10.

3. In “polytomous logistics regression was applied in the multivariate analysis to determine the risk of contacting the two defined cancer groups”, the word “contacting” was not clear to me, as well as “contracting” in “an increased risk of contracting ESCC” (Abstract).  
Answer: Many thanks. That is one of the typos in this manuscript. This has been corrected. Please see page 13.

4. Were the P-values one-sided or two-sided? This should be mentioned in the last sentence of the Methods.  
Answer: All the p-values in our study have been calculated for the two-sided tests. This has been corrected. Please see the second line on page 14.

3. One row in Table 4 is redundant. In addition, p for H-W3 should be changed to p for H-W2.  
Answer: These have been revised. Please see Table 3 and 4.

4. Numbers beginning a sentence must be spelled.  
Answer: This has also been revised. Please see methods in abstract section (page 4), Methods (the third line on page 10) and the second paragraph of Results section (third line from bottom on page 14).

5. There are some typos in the text, for example: low-third ESCC, once time per week, remain blood cells.  
Answer: Many thanks. We have tried our effort to correct the typos.

6. Some words should also be changed, for example: pathologically to histopathologically, computer tomography to computed tomography or computerized tomography, gastroendoscope to gastroscopy or upper gastrointestinal endoscopy or another relevant term.  
Answer: Those terms have been modified according reviewer’s suggestion. Please see page 10 and 11, and last paragraph of Discussion section (page 20).

7. In the following sentence “It means that H. pylori seropositivity has an inverse association with ESCC risk and it was modified by COX-2 polymorphism, especially in the lower third of the esophagus, the anatomy close to the stomach where H. pylori
presented”, the first phrase seems to be a little bit strong; I would suggest changing “means” to “suggests”. In addition, “was” should be changes to “is”. The importance of anatomical proximity to the source of H. Pylori infection is not clear to me. The fact that the lower third of esophagus, compared to upper esophagus, is more exposed to acid (in case of acid reflux) may be more important.

Answer: In combination to the newly statistic data, we have added several sentences to describe the information clearly at the end of third paragraph in Discussion. Please see page 18.

8. Finally, in footnotes of Tables 2 to 4, the authors should provide some information about the covariates (if there were continuous, or describe the categories). If categories in Table 1 were used, the authors could only state this in each footnote.

Answer: We have modified the footnote of Table 2 to 4.

We would appreciate your kindness if the manuscript could be accepted.

Sincerely

Huang-Ming Hu