Reviewer's report

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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Reviewer: Sara Raimondi

Reviewer's report:

This case-control study on 180 ESCC patients and 194 controls investigated 1) the association between H. pylori infection and ESCC development, 2) the association between COX-2 polymorphism and ESCC development, 3) the interaction between H. pylori infection and COX-2 polymorphism in ESCC development.

The paper is generally well written and the methods of analysis are appropriate and well described. However the study design is not clear: it seems a prevalence study, but the authors did not provide any further information on how the patients were recruited (i.e: years after diagnosis) and when patients answered the questionnaire on life-style habits. Inclusion of prevalent cases would significantly bias the results and may lead to wrong conclusions. For example, if H. pylori seropositivity was associated with a more severe ESCC, then the inverse association found in this study would be explained with the fact that prevalent cases had less severe disease, and therefore were more probably H. pylori-negative. Finally, a description and discussion of the possible limitations of the study is completely lacking.

Detailed comments follow:

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.

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

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,…

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.

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).”

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.

4) BACKGROUND, last paragraph: “However, very few studies…”. Please add references.

5) METHODS: The authors should specify what the main diseases of the control group were.


7) METHODS, Statistical analysis: Please add the name of the statistical software used to carry out the analyses.

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.

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”.

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?

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

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

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

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,...”.

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).

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.

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

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests