**Author's response to reviews**

**Title:** Etiological study of esophageal squamous cell carcinoma in an endemic region: a population-based case control study in Huaian, China

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

Enclosed, please find a revised version of our manuscript entitled “Etiological study of esophageal squamous cell carcinoma in an endemic region: a population-based case control study in Huaian, China” (Manuscript ID# 2141159512106379). As you will recall, the reviewers diverged significantly in their opinion about this manuscript. Although we feel that both reviewers had very good suggestions for improving the overall value of the manuscript, we strongly disagree with Reviewer 2 on two main points. First, we do feel that our manuscript is “scientifically sound.” Second, we feel that Reviewer 2 may not have understood the rationale behind some of our statistical analysis and, as a result, misinterpreted some of our results (i.e., p-values associated with esophageal lesions) as being inconsistent. Nevertheless, we have tried to address all comments in this revision.

Responses to Comments

Reviewer 1

1-The food frequency questionnaire was validated?

The food frequency questionnaire (FFQ) used in the present study is well suited for dietary assessment and investigation of the relationship between diet and cancer. Currently, there is a debate about the utility of FFQs in studies of diet and cancer (Kristal AR, et al., Cancer Epidemiol Biomarkers Prev. 2005, 14: 2826-2828. Willett WC, et al., Cancer Epidemiol Biomarkers Prev. 2006, 15:1757-1758. Kristal AR, et al., Cancer Epidemiol Biomarkers Prev. 2006, 1759-1760), however FFQs are still highly informative in epidemiologic studies, especially in light of the lack of suitable alternatives.

2-The FFQ was tested for reproducibility?

The FFQ has been used in several studies in China, for instance the breast cancer cohort study conducted in Shanghai (Fowke JH, et al., Cancer Epidemiol Biomarkers Prev. 2003, 12:1536-
3-The reader (and the reviewer) would like to know the results of temperature of tea.

The temperature of tea (hot, warm, and cold) was part of questionnaire. The temperature of tea during consumption is 60~80 °C in the study area. This has been added into the Results in Page 9.

4-The tea was green or black?

Green tea was the only tea source consumed by the local residents. This has been changed accordingly.

5-What test the authors employed to determine the introvert personality?

The personality was part of the questionnaire and no specific test was used to determine the personality of participants. The personality was classified into 3 categories including optimistic (or extrovert), relative optimistic (intermediate extrovert), and introvert. Extrovert was defined as sociable, active, assertive, energetic, animated, enthusiastic outgoing, impulsive, emotionally expressive, talkative, act before thinking, and broad experience. Introvert was defined as shy, unsociable, often appear reserved, quiet and thoughtful, usually do not have many friends, and have difficulties in making new contacts, like concentration and quiet. The changes have been made in study procedure at Page 6 accordingly.

6-Could be also of interest to know the percentage of extrovert personality, psychotic personality, depressive personality, authoritative personality, etc.

As demonstrated above, in our study, the personality was classified into 3 categories including optimistic (or extrovert), relative optimistic, and introvert, and the percentages for optimistic, relative optimistic, and introvert personality were 49, 41, and 10 respectively. This has been added into the Results at Page 9.

7-Please clarify why passive smoking was more risky than active smoking. I suggest to examine to role of active smoking regarding intensity, years smoked, cessation, type of tobacco, type of cigarette.

It has been suggested that passive smoking (or Environmental tobacco smoke, ETS), 85% of which consists by sidestream smoke due to burning cigarette, was more risky than active smoking, and concentrations of some carcinogens are higher in sidestream than in mainstream smoke, which is exhaled by the smoker (CDC, 1986). ETS has been classified as a Group A carcinogen which means it is known to cause cancer in humans (US EPA, 1992). The number of known carcinogens in ETS has been reported to be 69 in the year 2000 (IARC, 2004). The role of active smoking has been examined in our manuscript by age started, smoking intensity, years smoked, cessation, type of tobacco, and type of cigarette. No association between active smoking
and esophageal squamous cell carcinoma (ESCC) was found (p>0.05). Changes have been made in Discussion at Page 12 accordingly.

8-What means esophageal lesion?

Esophageal lesion in our study was defined as including one or more of the following clinically diagnosed esophagus diseases or changes: 1) chronic esophagitis, 2) reflux esophagitis, 3) esophageal epithelium regeneration, 4) esophageal epithelium metaplasia, 5) esophageal polyps, and 6) esophageal erosion. Changes have been made accordingly in the study procedure part.

9-The readers could be interested about the effect of other food group or items like dairy foods, red meat, fried meat, etc.

Food items like dairy foods, red meat, fried meat, etc were included in the questionnaire among the 8 food groups in this manuscript including 1) staple diet; 2) fried food; 3) pickled and salty food; 4) animal meat, egg and milk; 5) bean and bean product; 6) vegetables; 7) fresh fruits; and 8) nuts and dried fruits. The effects of these food group or items like daily foods, red meat, and fried meat on ESCC were also investigated, but no significant associations were found.

Reviewer 2

General comments:

In this manuscript, authors try to evaluate the association between environmental carcinogens, genetic polymorphisms and ESCC in Huaian, however, 107 newly diagnosed cases and 107 controls were involved in this study and the number of cases and controls is too small to get meaningful and powerful results, which could represent the real situation of this area.

We partially agree with the reviewer’s comment on the sample size of this study. Although the sample size of 107 cases and 107 controls was relatively small for a traditional epidemiological study, our study was conducted in an endemic area of esophageal cancer with an ESCC incidence rate of 80/100,000. Nevertheless, enrollment of 107 cases was completed within one year. Furthermore, having 1:1 matched case-controls increases the power of our study. One to one matched 107 cases and 107 controls were large enough to attempt to evaluate the effects of lifestyle factors on ESCC, especially in the context of this endemic area which rarely has been studied. On the other hand, the sample size of our study is comparable to the case-control study published from the reviewer’s group, which included 135 ESCC cases and 152 normal controls (Yu HP, et al., Cancer Genet Cytogenet. 2004, 154:10-15; Yu HP, et al., Cancer Detect Prev. 2004, 28:194-199).

All the risk factors determined in this manuscript are well known as risk factors to carcinoma and no novel risk factors and hypothesis were reported.

We disagree with the comments of the reviewer. Some new viewpoints have been stated in our manuscript, such as “introverted personality type is a risk factor of ESCC”, while “clean up of
food storage utensils, and alcohol abstinence are protective factors for ESCC” were first reported. Apart from these, even the well known risk factors rarely have been studied in relation to ESCC in this endemic area. We do not mean to imply that our results are generally applicable to other areas. Data presented in our study will be beneficial for this specific area, and the findings from this study will be important for future intervention studies in this high risk population.

More study about the co-effect of risk factors and gene polymorphism to the generation of carcinoma should be developed.

Co-effects of risk factors and gene polymorphism to the generation of carcinoma have been analyzed to estimate the interactions between genetic polymorphisms and other common risk factors, as well as gene-gene interactions. However, no significant interactions were found in our study. This has been addressed in the last paragraph of the Results.

Moreover, the statistic results of different part are conflict and confusing the ideas they developed. Different results were drawn by Univariate conditional logistic regression analysis and multivariate conditional logistic regression analysis about esophageal lesion.

We feel that Reviewer 2 may not have understood the rationale behind some of our statistical analysis and, as a result, misinterpreted some of our results (i.e., p-values associated with esophageal lesions) as being inconsistent. The p-value in the univariate regression assesses the significance of a variable’s coefficient (e.g., esophageal lesions), whereas the p-value in a multivariate model assesses the significance of a variable’s partial regression coefficient (i.e., holding all other variables in the model constant). Thus we expect that results for individual variables may differ between the two approaches.

1. Methods: Only 107 cases and 107 controls were involved and it is hard to get convincible results.

As previously stated, we partially agree with the reviewer’s comment on the sample size of this study. Although the sample size of 107 cases and 107 controls was relatively small for a traditional epidemiological study, our study was conducted in an endemic area of esophageal cancer with an ESCC incidence rate of 80/100,000. Nevertheless, enrollment of 107 cases completed within one year in 5 townships should be representable for this endemic area. We have no means to represent other areas. Furthermore, one to one matched 107 cases and 107 controls were large enough to attempt to evaluate the effects of lifestyle factors on ESCC, especially in the context of this endemic area which rarely has been studied.

On the other hand, the sample size of our study was comparable to the case-control study published from the reviewer’s group, which included 135 ESCC cases and 152 normal controls (Yu HP, et al., Cancer Genet Cytogenet. 2004, 154:10-15; Yu HP, et al., Cancer Detect Prev. 2004, 28:194-199).

2. Methods: was the Hardy-Weinberg equilibrium test preformed in controls?
The genotype frequencies among cases and controls were compared with values predicted by the Hardy-Weinberg equilibrium using $\chi^2$ analysis. All the genotype frequencies in this manuscript were under the Hardy-Weinberg equilibrium (P>0.05). This has been described in the revised Methods and Results accordingly.

3. Methods: Stratified analysis is recommended to be employed to estimate the interaction between genetic polymorphisms and other common risk factors, such as cigarette smoking, eating fatty food and alcohol drinking.

In our study, stratified analyses have been employed to estimate the interactions between genetic polymorphisms and other common risk factors, such as cigarette smoking, eating fatty food and alcohol drinking, as well as gene-gene interactions. However, no significant interactions were found in our study (P>0.05). This has been addressed in the last paragraph of the Results.

4. Results: Analyzed by multivariate conditional logistic regression analysis, the P value of esophageal lesion is 0.04, while by univariate conditional logistic regression analysis, the P value is 0.05.

This is absolutely correct, and is one of the primary reasons for conducting the multivariate analyses! The p-value in the univariate regression assesses the significance of a variable’s coefficient (e.g., esophageal lesions), whereas the p-value in a multivariate model assesses the significance of a variable’s partial regression coefficient (i.e., holding all other variables in the model constant). Thus – we expect that results for individual variables may differ between the two approaches.

5. Discussion: The authors do not present any new viewpoint. All the risk factors are well known and the co-effect or interact of gene and environmental factors are not developed.

As also previously mentioned, we disagree with the comments of the reviewer. Some new viewpoints have been stated in our manuscript, such as “introverted personality type is a risk factor of ESCC”, while “clean up of food storage utensils, and alcohol abstinence are protective factors for ESCC” were first reported. Apart from these, even the well known risk factors rarely have been studied in relation to ESCC in this endemic area. We do not mean to imply that our results are generally applicable to other areas. Data presented in our study will be beneficial for this specific area, and the findings from this study will be important for future intervention studies in this high risk population.

As has been addressed in question 3, co-effects or interactions of gene and environmental factors have been analyzed, but no significance was found.

As a case-control study, it is very essential that this manuscript is seen by an expert statistician.

Although we are not opposed to such a comment, we are concerned that this will unnecessarily increase the amount of time that this manuscript will be in review. Two authors (Drs. Wei-Min Gao and Stephen B. Cox) on this manuscript have substantial experience in biostatistics, including teaching introductory and advanced graduate courses in statistics, and we are confident
that the statistical methodology is sound. Therefore, we respectfully request that the review process not be prolonged if at all possible.

In summary, we believe that we have adequately responded to the comments of the reviewers, and we anticipate that the changes will be satisfactory. We appreciate the opportunity to revise our manuscript and look forward to your final decision.

I will be happy to provide you with any further information. Please feel free to contact me at:
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Sincerely,

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