Author's response to reviews

Title: The Pstl/Rsal and Dral polymorphisms of CYP2E1 and head and neck cancer risk: a meta-analysis based on 21 case-control studies

Authors:

- Kefu Tang (tkf2006@163.com)
- Yang Li (yangli185@hotmail.com)
- Zhao Zhang (zuogouquan@hotmail.com)
- Yunmin Gu (guyunmin2@yahoo.com.cn)
- Yuyu Xiong (gracexyy84@yahoo.com.cn)
- Guoyin Feng (guoyingfeng@online.sh.cn)
- Lin He (helinhelin@gmail.com)
- Shengying Qin (chinsir@sjtu.edu.cn)

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Author's response to reviews: see over
Dear Dr. Gabriella Anderson

Thank you very much for your most quick edition on our manuscript “The PstI/RsaI and DraI polymorphisms of CYP2E1 and head & neck cancer risk: a meta-analysis based on 21 case-control studies” (MS: 2052541857316477). According to your suggestions, we have responded to the comments of reviewers on the one to one basis as follows.

For Reviewer Comments:

Reviewer: Devendra Parmar

Comments to the Author

This is a meta-analysis involving different populations. However, differences amongst different populations particularly with respect to the genotypes present in controls needs to be addressed.

Answer: Yes, we have redraw the table and added the number of controls in each genotype in to it.

Authors appear to take Asians as one unit which may not be the case. This is the major drawback of the study basically because it fails to stratify the data based on ethnicity. Indians are very different than Chinese and Japanese. SNPs in CYP1A1 and 1B1 follow a different pattern when compared to the oriental populations. This needs to be addressed in detail.

Answer: Yes, Dr. Devendra Parmar is right. Indians are very different than Asian and Caucasian. Since there are at least 6 studies concerning the Caucasian or Asian population, which means there is a big sample size of these two ethnicities to perform subgroup analysis. But there are just three studies concerning the Indians, thus we did not conduct a further subgroup analysis. In addition, the genotype distribution of controls of CYP2E1 are presented in the revised table I, and it seems that the genotype distribution of CYP2E1 in Indians are more similar as Caucasian other than Asian. However, we still performed a subgroup analysis in which Caucasian and Indian population were analyzed separately. Unfortunately, we did not find any positive result (data not shown) for these polymorphisms in Indians and Caucasians.

So, to pool Indians and Caucasian population together seems not to inference the
overall results. If there are more and more association studies concerning Indians in the future, it would be very helpful to make a further analysis.

Stratification with respect to risk factors appears to be weak link though it can be strengthened. This should be addressed with caution as the exposure to environmental risk factors is to very different levels in Asians when compared to the Caucasians. 

**Answer:** Yes, we agree with Dr. Devendra Parmar. In fact, the definition of alcohol and cigarette consumption of different research varies greatly, thus making it is impossible for us to make a further analysis according to the levels of alcohol and cigarette consumption. To the best of our knowledge, a single common gene variant or environment is rarely sufficient to explain a complex disease, such as head and neck cancer. In order to identify if these environmental risk factors combined with CYP2E1 polymorphisms have any effect on HNSCC susceptibility, we have no other choice but to pool all available data to increase the statistic power to find a result. Unfortunately, even the pooled analysis failed to find any significant result. It seems that we still need more studies to reach a conclusion.

**Reviewer: Silvia Rogatto**

Comments to the Author

In this manuscript, Tang and collaborators performed a comprehensive meta-analysis in order to investigate the possible association of single nucleotide polymorphisms of CYP2E1 gene and the risk of development of head and neck carcinomas. The highly polymorphic P450 enzyme superfamily is the most important system involved in the biotransformation of many endogenous and exogenous substances. In the last decades, several reports showed evidences of association between gene polymorphism in several genes of this superfamily and cancer susceptibility. CYP2E1 gene is among of the most responsible for the biotransformation of chemicals, especially for the metabolic activation of pro-carcinogens as alcohol. Pathways of carcinogen metabolism are complex, and are mediated by activities of multiple genes, while single genes have a limited impact on cancer risk. Although the focus of this meta-analysis is the two most frequently studied SNPs of the CYP2E1 gene, the data just confirm the association of these polymorphisms in the Asiatic population and do not reveals new relevant data.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

The authors presented clearly the objectives of the present study and the methods were appropriated and well described. However, several confounders bias were not considered such as sex, age and tumor heterogeneity. In this sense, some points should be considered as:

1. Although apparently the number of literature reports is still so little to reach a homogeneous group of cancer patient’s, head and neck cancer as a single group may have obscured important biological differences of cancers at different locations (oral cavity, pharynx and larynx).

**Answer:** Yes, we agree with Dr. Silvia Rogatto. Head and neck cancer itself is a heterogeneous (oral cavity, pharynx and larynx) and complex disease. Thus, it would be more reasonable to conduct a subgroup analysis according to tumor site. However,
almost all studies included in our meta-analysis failed to report genotype distribution of patients according to tumor site, which make further subgroup analysis impossible. In addition, even if we could make a subgroup analysis according to tumor location, it would be more likely not to find any useful information since the sample size of each tumor site is limited, thus making the power of such analysis is too small to detect any effect. In order to increase the power of meta-analysis, we did not discriminate different tumor site of head and neck cancer and pooled them together. But, if there is more and more research concerning the relationship between CYP2E1 polymorphism and head and neck cancer risk in the future, it would be possible for us to make a detail analysis. That is why in the end of our manuscript, we have suggested that future studies should use homogeneous cancer patients and well-matched controls.

2, Ethnicity: The meta-analysis failed to confirm any association between the polymorphisms and head and neck cancer among Caucasians. However, the authors should consider that in this group were included three papers from Brazilian population (totalizing 487 cases and 459 controls). Historically, Brazilian population always experienced large degrees of ethnic and racial interbreeding, including Amerindians, Europeans, Africans and Asians. Since Brazilian population is known to be highly miscegenated, the authors could review their data considering the impact of the inclusion of Brazilian data among the Caucasian group. 

**Answer:** Yes, we agree with Silvia Rogatto. Brazilians are very different than Asian and Caucasian. Because there are just three studies concerning the Brazilian population, thus we did not conduct a further subgroup analysis in Brazilian population. However, the genotype distribution of controls of CYP2E1 are presented in the revised table I, and it seems that the genotype distribution of CYP2E1 in Brazilians are more similar as Caucasian other than Asian. In addition, we have performed a subgroup analysis in which Caucasian and Brazilian population were analyzed separately. Unfortunately, we did not find any significant result (data not shown) for these polymorphisms and head and neck cancer risk in the two populations. Thus, to pool Brazilians to Caucasian population seems not to inference the overall results.

3, The criteria for study classification in high or low quality is very subjective.

**Answer:** Yes, we agree with Dr. Silvia Rogatto and that is why we discussed this problem in discussion of the manuscript. It is known that a validated quality assessment system does not currently exist [1], but making quality assessment to the paper included in meta-analysis is very important and it would help us to minimize the potential for selection bias and thus help us to get a more reliable result.

Main points included in our quality assessment system are listed as follows:

1. Diagnostic criteria
2. Ethnicity (subjects were composed of the same ethnicity or different populations were analysis separately)
3. Hardy-Weinberg equilibrium (genotype distribution in controls)
4. Sex (matching between cases and controls)
5. Age (matching between cases and controls)
6. Experimental method
7. Bias in data processing (raw data rechecked or use “blind” during experimental and statistical periods)
Because these points are relatively objective and have been used by another published paper [2], thus we adopt the quality assessment system.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Although historically single nucleotide polymorphisms were identified by the corresponding restriction enzyme used in the genotyping by PCR-RFLP, the authors should be encouraged to update allele/genotype nomenclature recommended by the Human Cytochrome P450 (CYP) Allele Nomenclature Committee (available at http://www.cypalleles.ki.se/). Also, the SNP identifier of PstI/RsaI polymorphism should be given as described to the DraI polymorphism, a T7632A transversion corresponding to the SNP ID rs 6413432 (see page 4, introduction section, second paragraph, line 11).

**Answer:** Yes, the SNP were represented by corresponding restriction enzyme at first. Because the PstI polymorphism and the RsaI polymorphism are in complete linkage disequilibrium, thus we pooled these two sites and present it as PstI/RsaI according to a previously published meta-analysis [3].

Page 2, Abstract, first line: This phrase should be corrected for "CYP2E1 encodes a member of the cytochrome P450 superfamily of enzymes which play.". Besides, the authors should follow the recommendations of Guidelines for Human Gene Nomenclature (http://www.genenames.org). Thus, CYP2E1 should appear in italic.

**Answer:** Yes, we have corrected this sentence and CYP2E1 are presented using italic form.

Pages 2 (abstract) and 3 (introduction): head & neck cancer or head and neck cancer? The tumor type should be described in the same way throughout the text.

**Answer:** Yes, we have corrected the mistake using head and neck cancer in stand of head & neck cancer.

Page 3, Introduction section, first paragraph: A bibliographic reference should be included in the end of this paragraph.

**Answer:** Yes, we have added reference in the end of this paragraph.

Page 3, Introduction section, second paragraph, lines 1-2: "The CYP2E1 gene, located..catalysis of xenobiotic. It specifically activates." should be changed for "The CYP2E1 gene, located.. The protein encoded by this gene activates."

**Answer:** Yes, we agree with Dr. Silvia Rogatto and we have corrected this sentence.

Page 5, Materials and Methods section, line 2: The acronym HCSCC should be changed to the correct form HNSCC. The same observation is valid to page 6 (line 3) where appears NHSCC.
**Answer:** Yes, all the abbreviation for Squamous cell carcinoma of head and neck has been rechecked and those wrong forms have been corrected.

Page 11, Discussion section, line 2: ".and it is therefore probable that the the observed ethnic differences".should be corrected for "..and it is therefore probable that the observed ethnic differences"

**Answer:** Yes, sorry for the mistake and we have deleted the redundant “the”.

Legends of Figures 1, 2 and Begg’s funnel plots should be corrected for head and neck cancer instead lung cancer.

**Answer:** Yes, we have corrected the mistake in revised manuscript.

The reference section should be completely reviewed and standardized according the BMC recommendations for authors.

**Answer:** Yes, we have corrected the form of reference according to the BMC recommendations.

**Discretionary Revisions (which the author can choose to ignore)**

Table 1, Since RFLP was used by 20 studies and just one was based on real time PCR (RT-PCR). Thus, the column of methods should be deleted.

**Answer:** Yes, we have deleted the genotyping method column from the table.

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**Reviewer: Volker Harth**

Comments to the Author

In the present publication, Tang and colleagues conducted a meta-analysis of case-control studies on the role of CYP2E1 gene polymorphisms in association of ethnicity, smoking and alcohol with HNSCC. The meta-analysis is based on 21 eligible case-control studies. To date, some studies have examined the interaction of these polymorphisms with HNSCC. Nevertheless, we have to deal with a large number of putative pathways, genes and sequence variations in the metabolism of carcinogenic agents. A single common gene variant is rarely sufficient to explain a complex disease. However, the hypothesis that variations in genes coding for metabolic enzymes determine an individual susceptibility to an exogenous exposure still is basically correct.

This is the first meta-analysis dealing with the association between CYP2E1 polymorphisms and head neck cancer. The specified inclusion and exclusion criteria as well as the statistical methods were clearly described. The limitations of the summary and a possible publication bias were extensively discussed.

**Major Compulsory Revisions:**

1. Further editing for grammar and style is necessary

**Answer:** Yes, the manuscript was written by a Chinese but it has been revised by an English native speaker.
2. PstI/RsaI, DraI and HNSCC have to be revised for consistent notation throughout the manuscript.

**Answer:** Because the PstI polymorphism and the RsaI polymorphism are in complete linkage disequilibrium, thus we pooled these two sites and present it as PstI/RsaI according to a previously published meta-analysis [2]. In addition, HNSCC has been rechecked and the wrong form has been corrected.

3. The titles of figures have to be revised (e.g. Fig. 1: Forest plot of LUNG cancer risk ….. The diamond represents the overall summary estimate, WITH CI REPRESENTED BY ITS WIDTH).

**Answer:** Sorry for the mistake, we have corrected it in the revised manuscript.

Thanks a lot for your attention to our paper. We look forward to hearing from you soon.

Yours sincerely,

Kefu Tang

**Reference**

