Author's response to reviews

Title: Association between novel PLCE1 variants identified in published esophageal cancer genome-wide association studies and risk of squamous cell carcinoma of the head and neck

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Author's response to reviews: see over
April 08, 2011

Dear Dr. Renske Steenbergen:

Thank you for your decision email of Mar. 23, 2011, in which you encouraged us to revise our manuscript (MS: 8472926144986010) entitled "Association between novel PLCE1 variants identified in published esophageal cancer genome-wide association studies and risk of squamous cell carcinoma of the head and neck."

Here we submit the revision of our manuscript that was revised, point by point, according to reviewers’ comments and suggestions. We would like to thank the editors and the reviewers for their valuable comments and recommendations that have greatly improved the quality of this paper. We hope our responses are satisfactory.

Sincerely,

Qingyi Wei, M.D., Ph.D.
Professor of Epidemiology

Enclosures
Response to the reviewers’ comments and suggestions on “Association between novel PLCE1 variants identified in published esophageal cancer genome-wide association studies and risk of squamous cell carcinoma of the head and neck” (MS: 8472926144986010) submitted to BMC Cancer by Ma et al.

Reviewer 1 (Dongping Li):
Increasing evidence has demonstrated a key role of PLCE1 in pathologic processes, including human malignancies. However, nothing is known about the association between functional SNPs in PLCE1 and susceptibility to SCCHN. Using genotyping, the authors in this manuscript clearly showed that the novel PLCE1 polymorphisms may confer susceptibility to SCCHN associated with tobacco and alcohol consumers. This manuscript is the first report that PLCE1 gene polymorphisms associated with SCCHN case-control study in Caucasian and it is going to act an important and potential affects to this field study.

I believe the paper should be published, if the following minor essential revision is addressed.
1. Page 4, line 7, put a space before "PLCE1 is involved in cell....."
2. Page 4, line 13, add a comma after GCA,
3. page 5, line 21, add a space after "...SNPinfo"
4. Page 6, line 18, subscript MgCl2 "2"
5. Page 7, line 17, "were" instead of "are"
6. Page 8, line 8, "were" instead of "are"
7. Page 9, line 6, add a space after "....SCCHN"
8. Page 9, line 15, add a space after "...only -oropharyns sites"
9. Page 10, line 14, change the sentence to"...smoking status, alcohol use and disease stage..."
10. Page 11, line 1 and 2, add a space after "...smokers and drinkers, respectively"
11. Page 11, line 6 and 7, change the sentence as "...by alcohol status (P=0.005), but interestingly we did...
12. Page 12, line 1, change the sentence as" about 30% of all human cancers. Several studies have..."
13. Page 12, Line 16, change the sentence as" the published GWA studies [8,9]. Our results..."
14. Page 22, Table 1, make the columns of "cases, controls and P" in straight lines.
15. Page 23, Table 2. add spaces between PLCE1 rs3203713 and Non-oropharynx Case lines.
16. Page 25, Table 4. add spaces between the numbers and parentheses if they are necessary.

Response: Thanks for the careful checking, and we appreciate the reviewer’s positive comments and encouragement. We have corrected the above-mentioned errors in the revision according to the suggestions.
Reviewer 2 (Lidong Wang):
The authors here reported that three PLCE1 SNPs may have a joint effect on the risk of SCCHN from non-Hispanic white population. They also stratified the significance of these variant SNPs by smoking and alcohol exposure. The manuscript should be acceptable after minor revision.

Response: Thanks for the encouraging comments.

The major concerns are as follows:

1. It is not appropriate to imply the HPV infection and PLCE1 variants with SCCHN without any supporting data. The author should rewritten the conclusions, just focus on the important findings.

Response: We have changed the conclusions in the Abstract as following:

“Our findings suggest that PLCE1 variants may have an effect on risk of SCCHN associated with tobacco and alcohol exposure, particularly for those tumors arising at non-oropharyngeal sites. …” (Page 2)

2. In the sections of “Abstract” and “Background”, the author described that esophageal squamous cell carcinoma and gastric cardia adenocarcinoma may share some similar risk factors with SCCHN, which should be described more clearly with literature findings.

Response: As suggested, we added some information in the revised manuscript to describe the question more clearly. The sentence was described as following:

“Studies have reported that SCCHN shares some similar risk factors with ESCC and GCA, such as tobacco smoke and alcohol consumption [17-19].” (Page 4)

3. Obviously, non-Hispanic white population may be very different with Chinese people in many aspects, especially on the etiology of esophageal and gastric cardia cancers and SCCHN. It would be better to describe these differences more in detail in the “Background” or “Discussion” sections.

Response: Thanks for the suggestions. In the revision, we have discussed the question in the Discussion as following:

“Furthermore, different carcinogenic mechanisms between esophageal and gastric cardia cancers and SCCHN or genetic difference in different populations may result in the discrepancy for the main effect of rs2274223 between our study on SCCHN and two GWASs of esophageal and gastric cancers.” (Page 13)

4. The P value for rs2274223 and rs11599672 should be added.

Response: We have added the P values for significant results of rs2274223 and rs11599672 in the revised manuscript. The related sentences were revised as following:
“After adjustment for age, sex, smoking and alcohol status, the group of “2-3” risk alleles was borderline associated with risk of SCCHN (adjusted OR=1.20, 95% CI= 0.97-1.50, adjusted \(P=0.096\)) and the “4-6” risk allele group was significantly associated with risk of SCCHN (adjusted OR=1.31, 95% CI= 1.00-1.73, adjusted \(P=0.049\)). When “2-3” and “4-6” groups were combined for a larger number in the same stratum, the association between a larger number of risk alleles and risk of SCCHN remained statistically significant (adjusted OR=1.23, 95% CI=1.00-1.52, adjusted \(P=0.048\)).” (Page 9-10)

“As shown in Table 2, rs2274223 variant genotypes were associated with a significantly increased risk of SCCHN only for non-oropharyngeal sites (AG vs. AA: adjusted OR=1.29, 95% CI=1.01-1.64, adjusted \(P=0.042\); AG/GG vs. AA: adjusted OR=1.30, 95% CI=1.03-1.64; adjusted \(P=0.025\)), while rs11599672 variant genotypes were associated with a significantly decreased risk of SCCHN for this group of patients (GG vs. TT: adjusted OR=0.54, 95% CI=0.34-0.86, adjusted \(P=0.009\); TG/GG vs. TT: adjusted OR=0.76, 95% CI=0.61-0.95, adjusted \(P=0.018\)). No association was observed for rs3203713 variant genotypes and risk of SCCHN in both subgroups. In addition, the locus-dose effect of combined risk alleles was also seen in SCCHN arising at non-oropharyngeal sites (\(P_{\text{trend}}=0.017\)). When the group of “0-1” risk allele was used as the reference, the group with “2-6” risk alleles had a significantly higher risk of SCCHN arising at non-oropharyngeal sites (adjusted OR=1.35, 95% CI=1.03-1.78; adjusted \(P=0.033\)).” (Page 10)

Reviewer 3 (Balraj Mittal):

The manuscript entitled “Association between novel PLCE1 variants identified in published esophageal cancer genome-wide association studies and risk of squamous cell carcinoma of the head and neck” by Ma et al had observed the association of three potentially functional SNPs (rs2274223A/G selected from previously published genome wide association studies and two SNPs rs3203713A/G and rs11599672T/G selected on the basis of predictive functional value) of PLCE1 in 1,098 SCCHN patients and 1,090 controls in a non-Hispanic white population. None of three SNPs was alone significantly associated with overall risk of SCCHN, their combined effects of risk alleles were found to be associated with risk of SCCHN in a locus-dose effect manner, particularly for non-oropharyngeal tumors. However, manuscript has some limitation enlisted below:

Major:
1) Authors should mention the recent Genome wide association (GWA) studies conducted in the head and neck cancer and should discuss the results of replication study of GWA study in Head and Neck cancer in the introduction, if any?

Response: When we submitted the manuscript, no head and neck GWAS had been published yet. In this March, a GWAS of upper aerodigestive tract cancers (UATC) including head and neck cancer (McKay et al., 2011) was just published, which identified five variants at 4q21, 12q24 and ADH gene cluster, significantly associated with risk of UTAC. Among 3 SNPs investigated in our study, only rs2274223 was included in the Illumina Sentrix HumanHap300 BeadChip used by McKay et al., but no association of this SNP at the GWAS significance level was found with risk of UATC. Thus, we discussed this GWAS in the Introduction as following:
“However, only one recent GWAS focused on SCCHN risk and identified five variants at 4q21, 12q24 and ADH gene cluster, significantly associated with risk of upper aerodigestive tract cancers (UATC) including SCCHN [8]. It may be a very small proportion of SNPs associated with SCCHN risk because of the strict criteria for the GWAS significance level \((P=10^{-7} \text{ or } P=10^{-8})\). Thus, further exploration for the genetic variants that did not reach the GWAS significance level in the development of SCCHN is needed.”(Page 3-4)

2) Is there any Molecular/functional study on the role of PLCE1 in head and neck carcinogenesis? If yes it should be included in the Introduction and Discussion of the manuscript.

**Response:** In fact, few studies have focused on the role of *PLCE1* in head and neck carcinogenesis until now. Only one study by Bunney *et al.* showed that *PLCE1* has an oncogenic role in head and neck cancer progression by binding Ras family small GTPase [Ref.15]. We have mentioned this study in the Introduction of our original manuscript (Page 4 Line 18).

3) The exclusion and inclusion criteria of case and control should be clearly indicated in the material and methods section.

**Response:** As suggested, we have added more information about the exclusion and inclusion criteria of case and control in the Study populations as following:

“All cases were diagnosed with histologically confirmed SCCHN, and there were no age, sex or stage restrictions... Additionally, patients who had received prior surgery (other than diagnostic biopsies), chemotherapy or radiation therapy before recruitment, and any blood transfusion during the preceding 6 months were also excluded.” (Page 5)

“The inclusion criterion for controls was defined as the self-reported absence of prior history of cancer.” (Page 5)

4) Genome wide association study by Abnet *et al.* showed 5 SNPs at locus 10q23 of PLCE1 to be significantly associated with ESCC and GCA including two nonsynonymous variants rs2274223 and rs3765524. However most notable was association with rs2274223. Why authors have not included other SNPs of PLCE1 from GWA study in their study? It will be interesting to replicate the others SNPs as well to look their role in head and neck cancer.

**Response:** We understood the reviewer’s concern. With *PLCE1* as playing a functional role in the etiology of cancer in our mind, the strategy of SNPs selection in this study was to select all potentially functional SNPs located in the 5’ near gene, 5’- and 3’-untranslated regions of *PLCE1* instead of selecting all SNPs identified by GWAS, for which we would have to identify the functional SNPs that are in LD with them. Although rs3765524 is a nonsynonymous variants, it is in high LD with rs2274223 in the CEU population \((D'=1, r^2=0.828, \text{Hapmap Rel 27})\) and thus we only selected rs2274223 for replication, which was the most notable SNP identified by both of two GWASs.

5) What are the criteria of selection of SNPs rs3203713 and rs11599672, which were not explored in the earlier published genome wide association study?
**Response:** Rs3203713 is located in the miRNA binding site (3' UTR) and rs11599672 is located in the transcription factor binding site (TFBS in the 5' near gene), both of which are potentially functional SNPs in PLCE1 as predicted by SNPinfo. As mentioned in the SNPs selection (Page 6 Line 12-15) and the answers to Question 4, we finally included these two SNPs in our study besides the GWAS identified rs2274223.

6) None of the three PLCE1 SNPs selected was found to be associated with overall risk of head and neck cancer either at genotype or allelic level. However, when subjects were trichotomized according to risk alleles, subjects carrying 3-6 alleles were at increased risk of SCCHN. What could be possible reason of the discrepancy?

**Response:** The possible reason of the discrepancy may be due to joint effects of these three SNPs, because they are not in tight LD, meaning there was some independent effect from each of the SNPs. As we know, single SNP with low-penetrance only has a modest effect on the risk of cancer, which may not be detected by a relative small sample size. In our study, we conducted the combination analysis and found that the joint effect of these SNPs on SCCHN risk increased with the number of risk alleles ($P_{\text{trend}}=0.046$). The findings suggest that these three SNPs may jointly contribute to the risk of SCCHN. We have discussed this question more clearly in the revision as following:

“Given only a modest effect of each SNP individually, evaluating their combined effects may help us better understand any role of PLCE1 SNPs in cancer etiology.” (Page 13)

7) Authors should also mention the P value at least of the significant results in the text and tables.

**Response:** As suggested, we have added the $P$ values of significant results in the revised manuscript. However, we did not add the $P$ values in Table 2 because it contained too much data. The related sentences were revised as following:

“After adjustment for age, sex, smoking and alcohol status, the group of “2-3” risk alleles was borderline associated with risk of SCCHN (adjusted OR=1.20, 95% CI= 0.97-1.50, adjusted $P=0.096$) and the “4-6” risk allele group was significantly associated with risk of SCCHN (adjusted OR=1.31, 95% CI= 1.00-1.73, adjusted $P=0.049$). When “2-3” and “4-6” groups were combined for a larger number in the same stratum, the association between a larger number of risk alleles and risk of SCCHN remained statistically significant (adjusted OR=1.23, 95% CI=1.00-1.52, adjusted $P=0.048$).”(Page 9-10)

“As shown in Table 2, rs2274223 variant genotypes were associated with a significantly increased risk of SCCHN only for non-oropharyngeal sites (AG vs. AA: adjusted OR=1.29, 95% CI=1.01-1.64, adjusted $P=0.042$; AG/GG vs. AA: adjusted OR=1.30, 95% CI=1.03-1.64; adjusted $P=0.025$), while rs11599672 variant genotypes were associated with a significantly decreased risk of SCCHN for this group of patients (GG vs. TT: adjusted OR=0.54, 95% CI=0.34-0.86, adjusted $P=0.009$; TG/GG vs. TT: adjusted OR=0.76, 95% CI=0.61-0.95, adjusted $P=0.018$). No association was observed for rs3203713 variant genotypes and risk of SCCHN in both subgroups. In addition, the locus-dose effect of combined risk alleles was also seen in SCCHN arising at non-oropharyngeal sites ($P_{\text{trend}}=..."
When the group of “0-1” risk allele was used as the reference, the group with “2-6” risk alleles had a significantly higher risk of SCCHN arising at non- oropharyngeal sites (adjusted OR=1.35, 95% CI=1.03-1.78; adjusted $P$=0.033).” (Page 10)

8) Author should apply multiple testing corrections like Bonferroni’s correction in the subgroup analyses to avoid the false positive associations.

**Response:** As suggested, we conducted the Bonferroni’s correction in the subgroup and found that only $P$ values for the effect of rs11599672 variant genotypes in tumors arising at non-oropharynx remained significant after Bonferroni corrections (adjusted $P$<0.05). In the revision, we have added some sentences in the Discussion as following:

“All 3 SNPs were included in this study and we cannot rule out the possibility of false-positive associations because of multiple tests. Actually, only $P$ values for the effect of rs11599672 variant genotypes on tumors arising at non-oropharyngeal sites remained significant after Bonferroni corrections.” (Page 15)

**Minor**

1) Show the gel picture of restriction digestions of PCR product

**Response:** We have provided the gel picture of restriction digestions of PCR product for rs11599672 in the revision (Fig.1).

2) Author should correct the grammatical and spelling errors throughout the manuscript.

**Response:** We have checked the manuscript and corrected the errors in the revision.