Author’s response to reviews

Title: TNFSF15 promoter polymorphisms increase the susceptibility to small cell lung cancer: a case-control study

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Author’s response to reviews:

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Re: MGTC-D-18-00363

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Dear Editor,

We highly appreciated for your efforts in review of our manuscript. We have carefully revised the manuscript as requested. Our responses to the comments are detailed in

“Response to referees” in this cover letter. The amendments made are highlighted in red in the revised version.
Thank you very much for your and the reviewers’ constructive comments, which are very important for improving our manuscript. We hope that these changes will make the manuscript acceptable for publication in BMC Medical Genetics. If there are additional changes that we should make, please let us know.

Yours sincerely

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Response to Referees’ Comments

Angela T. Alleyne, Ph.D. (Reviewer 1)

The authors provide a well written population study on the relationship between between rs7848647 and rs6478109 of TNFS15 and small lung cell carcinoma (SCLC) and non-small lung cell carcinoma (NSCLC) in a Chinese population. The theoretical and methodological approaches are acceptable and soundly analysed. I would suggest that the authors include a sample size calculation to show that the sample size is appropriate for their conclusions.

Response: Thank you for your suggestion. Done as your request (See page5, line1 and page5, line27).

In a large population such as the China how significant is a sample of 549 cases and 460 controls to conclude that there is a statistically significant risk of SCLC and the rs7848647 GG polymorphism among males and rs6478109 CC polymorphism among females?

Response: Thank you for your comment. I agree with you. After stratified by risk selected risk factors, this sample size is small, so we should carefully conclude that there is a statistically significant risk of SCLC and the rs7848647 GG polymorphism among males and rs6478109 CC polymorphism among females. We have discussed this part as limitations of this study in our revised manuscript (See page8, line27).

There are also a few minor grammatical errors outlined below. Please accept with minor corrections.
Minor corrections:

1. The correct use of abbreviations should be checked throughout the manuscript. Page 4, line 25. The first mention of the term TNF needs to be properly abbreviated. It needs to be written out fully here, abbreviated and then the abbreviation used throughout the manuscript.

Response: Thank you for your comments. Done as your suggestion.

2. The use of italics for gene name symbols to distinguish these from protein symbols is not consistent throughout the manuscript. For example, page 5 line 31; TNFSF15 should be in italics, so too on page 6 lines 40. Please check the entire manuscript carefully and correct this oversight.

Response: As your comment, we have revised all symbols in our revised manuscript.

3. Also, please check your use of punctuation throughout. See line 35 page 4, there is an extra square bracket around eth reference for example and page 8 line 9, and extra full stop is seen before the citation.

Response: We are truly sorry for the careless. Corrected as your suggestion.

4. Page 5, line 44, replace "premier" with primer.

Response: We are very sorry for the careless, we have replaced "premier" with primer.

5. Include a sample size calculation to show the statistically significant nature of the study.

Response: We have added the sample size calculation in method section (See page5, line1) and in result section (See page5, line27).

Milena Cavic (Reviewer 2)

This study investigates the distribution of TNFSF15 promoter polymorphisms among lung cancer patients and healthy controls and aims at assessing whether two SNPs are associated with the occurrence of lung cancer subtypes in the Chinese population.

The work has been performed to an adequate standard, the topic is interesting, and the main scientific question is well defined. Although the influence of these SNPs has already been evaluated as a cancer risk in other populations and cancer types, it is one of the largest reported studies so far for lung cancer risk and might be useful for future meta-analyses.
Major comments:

1. Methodology section: to ensure the quality control of genotyping, random selection of samples for re-genotyping is not enough. Please confirm the validity of your PCR-RFLP genotyping method by sequencing a random selection of samples (homozygotes- and heterozygotes) and state that in the Methodology section if the data matches.

Response: Thank you for your suggestion. We also added the sequence of each genotype (See Fig 1) and state that in the Methodology section (See page4, line27).

Minor comments:

1. There are grammatical and typographical errors throughout the Manuscript, please use a spell checking program, and apply a/an/the appropriately. There are verbs missing in many sentences. It would be helpful if a native speaker polished the general language, especially in the Introduction and Discussion sections.

Response: We have checked and modified the grammatical and typographical error carefully. We also asked Dr. Miao, Xiaoping to review the manuscript. He is the professor of epidemiology at Tongji Medical College, Huazhong University of Science and Technology (HUST) in China. He has published many scientific papers including Nature Genetics. He is perfectly capable of high-quality academic writing.

2. Keywords section contain "Susceptibility" which is not specific enough, I recommend amending it to "Cancer susceptibility".

Response: Thank you for your comments. Done as your suggestion.

3. Abstract: I recommend writing the abstract in the past tense "this study aimed to,…included…" etc. Also in the sentence "This case-control study includes 209 small cell lung cancer patients (SCLC), 340 non-small cell lung cancer patients (SCLC) and…", the abbreviation for non-small cell lung cancer patients should be changed to NSCLC.

Response: In our revised manuscript, we have corrected all the tense and changed SCLC to NSCLC.

4. Introduction section: "Lung cancer remains one of the most common malignant tumors worldwide and the first leading cause of cancer-related mortality in China [1, 2]." Please use the updated GLOBOCAN data available since September 2018 and change Reference 1 accordingly (https://onlinelibrary.wiley.com/doi/full/10.3322/caac.21492)
Response: We have made correction according to Reviewer’s comments.

5. Introduction section: "According to World Health Organization, lung cancer is divided into two main types: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), of which NSCLC accounts for almost 85 percent and SCLC accounts for about 15 percent [3]. Reference 3 (Lazzari C, et al. Combination of immunotherapy with chemotherapy and radiotherapy in lung cancer: is this the beginning of the end for cancer? Therapeutic advances in medical oncology 2018, 10:1758835918762094) is not the original source of these data. Please change Reference 3 accordingly.

Response: Thank you for your comment. We have changed the Reference 3.

6. Introduction section: I did not understand the meaning of this sentence: "The occurrence of lung cancer involved in polygenes and multiple factors". Please rephrase or delete.

Response: We have deleted this sentence.

7. In the Introduction section, some phrases should be changed

"In fact, not all individuals exposed to..." to "However, not all individuals exposed to...

"By stimulating T cell activation, TNFSF15 involved in the modulation of inflammation" to "By stimulating T cell activation, TNFSF15 is involved in the modulation of inflammation"

"…Single nucleotide polymorphisms (SNPs) in gene regulatory region can effect on the expression of gene…” to "…Single nucleotide polymorphisms (SNPs) in gene regulatory regions can affect the expression of genes…”

"…changed on the transcriptional activity..." to "…changed the transcriptional activity..."

"…that over-expression of TNFSF15 can inhibit the growth of various tumors and reduced VEGI is associated with…” to "…that over-expression of TNFSF15 can inhibit the growth of various tumors and its reduced expression is associated with…”.

Response: Thank you for your comments. We have changed all.

8. Introduction section: "Tumor necrosis factor superfamily 15 (TNFSF15), also known as vascular endothelial growth inhibitor (VEGI), belongs to the tumor necrosis factor (TNF) ligand family...". Please amend the labeling of these proteins to non-Italic (and genes to Italic in other sentences where it applies) throughout the Manuscript.

Response: We have amended the labeling of all proteins to non-Italic in our revised Manuscript.
9. Introduction section: Please use TNFSF15 instead of VEGI throughout the Manuscript if that is the chosen primary label.

Response: Thank you. We have checked throughout the manuscript and used TNFSF 15 instead of VEGI.

10. Introduction section: "...In the present study, we tested if these two promoter variants of TNFSF15". Please state the specific investigated variants here as they have not been introduced so far. From this point on, please use either their rs labels or -638 A>G/-358 T>C labels consistently throughout the Manuscript.

Response: We have introduced these two specific investigated variants in introduction section in our revised manuscript (See page3, line21). We also checked and amended the -638A>G/-358T>C labels throughout the manuscript.

11. Methodology section: the Study population was not described in enough detail. Please add the age range and median age for each group, and provide information why 57 was chosen as a cut off for age and why 25 was chosen as a cut off for smoking pack year. I suggest using 30 as a cut off pack year value if the Authors don't have a strong rational for using 25 (https://journals.sagepub.com/doi/pdf/10.1177/0969141315579119).

Response: Thank you for your suggestion. We have inserted age range and median age for each in Table1 and added the references in Methodology section (See page5, line10). We also used the 60 as a cut off for age and 30 as a cut off for pack-year and made corresponding change in Results section (See Table 4, 5).

12. In the Methodology section, some phrases should be changed:

"health controls" to "healthy controls";

"All lung cancer patients did not receive any..." to "None of the patients were treated with..."

"premier" to "primer";

Response: Thank you for your comments. We have corrected all.

13. In the Methodology section it is stated: "All subjects had no genetic correlation." Please rephrase this sentence

Response: We have rewritten this sentence to “All subjects were unrelated ethnic Han Chinese”.
14. Please amend μl to μL throughout the Manuscript, as L is the IUPAC accepted label for volume

Response: Thank you. Done as your suggestion.

15. In the Results section, please add the percentage of smokers in the control group at the end of this sentence: "The proportion of heavy smokers in lung cancer patients (62.1% for NSCLC and 63.3% SCLC) was significantly higher than that in healthy controls."

Response: Thank you. Done as your suggestion.

16. In the Results section, it is stated "For rs6478109 variant, our data showed that CC genotype and TC genotype were associated with the higher risk of SCLC (OR=2.18, 95%CI=1.31-3.36…"

As Table 3 shows an OR of = 1.31-3.63, please amend the numbers so the data matches.

Response: We are truly sorry for the careless. We have carefully checked throughout our manuscript and amend the numbers.

17. In the Results section, it is stated "However, we didn't find that rs6478109 polymorphism was associated with the susceptibility to NSCLC with OR (95% CI) of 1.18 (0.82-1.68) and 1.13 (0.87-1.95)," As Table 3 shows an OR of 1.30 please amend the numbers so the data matches.

Response: We are truly sorry for the careless. We have carefully checked throughout our manuscript and amend the numbers.

18. In the Results section, it is stated: "To evaluate the effect of environmental factors on the association of TNFSF15 rs7848647 with the risk of SCLC". Please amend this sentence as age and gender are not environmental factors.

Response: We changed “environmental factors” to “smoking status and non-modifiable risk factors (age and gender)“

19. In the Discussion section some sentences need to be rephrased:

"Small-cell lung cancer (SCLC) is a deadly tumor, originates from neuroendocrine-cell with high-grade malignancy and poor prognosis" to "Small-cell lung cancer (SCLC) is a deadly tumor with poor prognosis, which originates from high-grade malignant neuroendocrine cells"

"…and there is an urgent need for biomarkers can act as a potential therapeutic target in SCLC" to "and there is an urgent need for biomarkers that can act as a potential therapeutic target in SCLC"
"...and are used for clinical biological treatment or clinical trial." to "...and are used for clinical biological treatment or in clinical trials."

"...effected on the expression level of TNFSF15..." to "...affected the expression level of TNFSF15..."

"Studies have shown strongly association between tobacco exposure and the development of SCLC, which further support our results." to "Studies have shown strong association between tobacco exposure and the development of SCLC, which further supports our results."

"It has reported that TNFSF15 can..." to "It has been reported that TNFSF15 can..."

"The distribution of age and gender is considered to be a risk factor for tumor development and progression" to "Age and gender are considered to be risk factors for tumor development and progression"

"...with the risk of SCLC among females and individuals among those aged younger than 57 years" to "...with the risk of SCLC among females and individuals younger than 57"

"Consistently, our results were in accordance with the study that women with lung cancer were younger than men" to "Our results are in accordance with the study that showed that women with lung cancer were younger than men"

"Due to the small size of several subgroups, we need carefully explain these findings. In the future, further study with larger samples still need to be conducted." to "Due to the small size of several subgroups, a further larger-scale study needs to be conducted in order to carefully evaluate these.

Response: As your comments, we have rephrased all these sentences in our revised manuscript.

20. Discussion section: "The mechanism of tobacco for lung cancer is multiple." "Cigarette smoking may result in gene mutation and pro-inflammatory in squamous cell carcinomas arising in the lung" "Collectively, these changes may lead to cells lose growth control and eventually form tumors." The meaning of these sentences was lost due to writing errors and omissions, please amend accordingly.

Response: Thank you! We have rewritten the sentences.

21. Discussion section: "The distribution of age and gender is considered to be a risk factor for tumor development and progression [36, 37]." References 36 and 37 are not the original source of these data. Please change them accordingly.

Response: Thank you for your comment. We have changed the references 36 and 37.
22. The Discussion section can be strengthened if a clinically-driven explanation for the need for studies of this type is included beside the biological one. For example, the Authors can elaborate on the usefulness of this test as a low cost SCLC screening tool in China which has recently been proposed for lung adenocarcinoma in other populations (https://jcp.bmj.com/content/72/1/75) and/or as an initiative to provide data for the construction of a future genetic panel for the prediction of lung cancer risk in China.

Response: Thank you so much for your suggestion. We have added this part in our revised manuscript (See page9, line1).

23. In the Conclusions section: "Taken together, our results indicated that TNFSF15 promoter polymorphisms involved in the development of SCLC" should be changed to "Taken together, our results indicated that TNFSF15 promoter polymorphisms might be involved in the development of SCLC".

Response: Thank you for your comment. Done as your suggestion.

24. Table 1. legend "Frequency distribution of select characteristics..." should be changed to "Frequency distribution of selected characteristics..."

Response: Thank you for your comment. Done as your suggestion.