Reviewer’s report

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

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Reviewer: Milena Cavic

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

COMMENTS TO THE AUTHORS

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 (homo- and heterozygotes) and state that in the Methodology section if the data matches

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

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

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.

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)

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.

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.

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...".

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.

9. Introduction section: Please use TNFSF15 instead of VEGI throughout the Manuscript if that is the chosen primary label.
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.

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). Please state the study inclusion criteria, and whether genotyping was carried out blind to case-control status.

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";

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

14. Please amend μl to μL throughout the Manuscript, as L is the IUPAC accepted label for volume.

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."

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.

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.

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.

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."

…and 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"

…and 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 findings".

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.

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.
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.

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"

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

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

Quality of written English
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

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