Author's response to reviews

Title: P53 genetic polymorphisms, interactions with lifestyle factors and lung cancer risk: a case control study in a Chinese population

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

Thank you very much for giving us the opportunity to revise our manuscript, entitled “P53 genetic polymorphisms, interactions with lifestyle factors and lung cancer risk: a case control study in a Chinese population”. We thank you and the reviewers for the comments and suggestions that are very helpful in improving our manuscript.

We have revised our manuscript in response to the comments and suggestions, and all changes are highlighted in the revised manuscript. Our point-by-point responses to the critiques and comments are listed below. We believe that our revised manuscript addresses all of the issues and concerns, but we will be glad to make further changes should it is necessary.

Sincerely yours,

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Reviewer 1

The manuscript of Li et al entitled “p53 genetic polymorphisms, interactions with lifestyle factors and lung cancer risk: a case control study in a Chinese population” reported for the first time an association between a novel p53 SNP, rs2078486, and lung cancer risk in specific Chinese sub-populations (smokers, alcohol drinkers and individuals with high indoor air pollution exposure). In contrast, the most studied p53 SNP at codon 72 is not associated with heterogeneity of lung cancer risk but rather with an overall increase in lung cancer risk. This study is well conducted, well written and most of the conclusions are supported by the data. Only few points need to be taken into account to improve the manuscript.

Major points

1- In the study of the interaction between p53 SNP rs2078486 and lifestyle factors (Table 3), the authors used a dominant model in contrast to p53 SNP rs1042522 for which a recessive model has been used. While it is clear why for p53 SNP rs1042522, the authors should add sentence to explain why they use a dominant model in the case of rs2078486.

Response: The major reason to use dominant model for rs2078486 is based on the sample size consideration, since only 33 lung cancer cases and 31 controls had homozygous variant alleles of rs2078486 in the current study population and sample sizes get even smaller in the stratified analyses and interaction analyses.

2- In the Discussion section, authors claim that “this study is among the first to report an increased lung cancer risk associated with variant genotype of p53 SNP rs2078486 in an Asian population. Moreover, we found synergistic effects of smoking and indoor air pollution exposure with p53 SNP rs2078486 on lung cancer risk” (page 9 lines 2-4). While the second sentence fits with the data, the first one is not supported by the statistics since only a tendency is observed between this p53 SNP and increased lung cancer risk in the overall population (as said by the authors page 8 lines 7-9). This sentence should be re-written accordingly.
Revision: We have changed the sentence into ‘this study is among the first to report a tendency of increased lung cancer risk associated with variant genotype of TP53 SNP rs2078486 in an Asian population (page 9, paragraph 3).

3- Authors suggested in the Discussion section that p53 SNP rs2078486 can be a functional SNP that affects p53 function. Can the authors give a hypothesis whether this SNP affects p53 function based on p53 SNP location within p53 gene (impact on p53 expression and/or activity)? When sentences are related to p53 functional SNP, the authors should cite the review of Whibley et al (reference 5), the best one reporting the different impact of p53 SNP on p53 expression and activity.

Response: We added a paragraph to discuss the possible mechanisms through which TP53 rs2078486 polymorphism modulates cancer risk.

Revision: We have added the following sentences in the Discussion section (page 10, paragraph 3):

TP53 rs2078486 is located in intron 1 and thus is not likely to be a direct disease-causing polymorphism. However previous studies suggest that TP53 rs2078486 is in a large linkage disequilibrium block extending from upstream of exon 1 to the first half of intron 1[17]. Therefore it is possible that TP53 rs2078486 might be in linkage disequilibrium with some functional polymorphisms, which in turn alter susceptibility to human diseases. Some other intronic variations in TP53 were reported to affect disease risk previously and the most widely studied one is TP53 intron 3 duplication polymorphism (rs17878362) [5, 25]. The underlying mechanism by which TP53 rs2078486 modulates cancer risk is not fully understood and warrants further investigations. However prior studies provided some initial evidence that TP53 rs2078486 is in perfect linkage disequilibrium with TP53 rs2287498, which is predicted to affect function at a splice site and TP53 rs2078486 is also in weak linkage disequilibrium with TP53 rs12951953, which might affect a transcription factor binding site [13].

4- There are few inconstancy between p-value given in the text and Table (i.e. page 8 line 4, p < 0.001 while in Table 1 p < .0001).
Revision: We have corrected inconsistent p-values presented in the text and table (Page 8, Paragraph 2).

5- Figure legends are needed to understand that Figure 1 corresponds to p53 SNP rs2078486 and Figure 2 to p53 SNP rs1042522. In addition, significant OR should be highlighted on the 2 figures (use bold letters for example).

Revision: We have revised the figure legends to make it clear that Figure 1 corresponds to p53 SNP rs2078486 and Figure 2 to p53 SNP rs1042522. Significant ORs are marked with asterisks in both figures.

Minor points
1- Sentences can be added in the Background section to emphasize the fact that only few publications studied p53 rs2078486 in relation to lung cancer risk (Schildkraut et al, Plos One 2010: SNP rs2078486 and ovarian cancer; Yang et al, Neurosci Lett 2004: SNP rs2078486 and schizophrenia in Chinese population).

Revision: We have added the following sentence in Background section (page 5, paragraph 1): In addition to lung cancer, TP53 rs2078486 SNP has been also linked with risk of ovarian cancer [13] and schizophrenia [14].

2- Population types and number of cases/controls should be given when references are made to other case/control studies in the Background and Discussion sections to allow comparison between statistical power of this study with others.

Revision: We added population types and numbers of cases/controls for references using case-control design in Background section (page 4, paragraph 3): One case-control study conducted among 611 lung cancer cases and 1040 controls in Los Angeles found elevated lung cancer risk associated with the variant genotype of TP53 rs2078486 SNP (doctoral dissertation from Yi Ren Wang) [11]. We also added the following sentence in Discussion section (page 10, paragraph 2):
The less significant association observed in our study might relate to the relatively smaller sample size.

3- Page 9 line 5: Figure 2 instead of Figure 1?

Revision: We have corrected the error and changed ‘Figure 1’ to ‘Figure 2’ (Page 9, Paragraph 2).

**Reviewer 2**

The manuscript of Li et al., reports the association between two TP53 polymorphisms rs2078486 and rs1042522 and lung cancer risk and interactions with lifestyle factors in a Chinese population. The authors found that the variant allele of rs2078486 was associated with a significantly increased risk of lung cancer among smokers and individuals with high indoor air pollution and that homozygous carriers of rs1042522 had a significantly increased risk of lung cancer. The results presented were not corrected for multiple testing.

Revision: We added the following sentence in Discussion section (Page 12, Paragraph 1):

After correcting for multiple comparisons, no significant interactions between lifestyle factors and TP53 rs2078486 SNP remained; therefore we cannot exclude the possibility of spurious associations due to multiple comparisons.

**Minor Essential Revisions**

There are a number of points that need addressing:

1. The gene should be labeled as TP53.

Revision: We have changed gene ‘P53’ to ‘TP53’ in the text and tables.

2. There are probably as many studies (and meta-analyses) on the cancer risks associated with carriage of the TP53 intron 3 duplication polymorphism (rs17878362) as rs1042522 and this should be acknowledged.
Revision: *TP53* intron 3 duplication polymorphism (rs17878362) is the most well studied intronic variation in *TP53*. *TP53* rs2078486 examined in current study is also located in intron.

We added the following sentence in the Discussion section to acknowledge the previous studies conducted on *TP53* intron 3 duplication polymorphism (rs17878362) and cancer risk.

(Page 10, Paragraph 3):

Some other intronic variations in *TP53* were reported to affect disease risk previously and the most widely studied one is *TP53* intron 3 duplication polymorphism (rs17878362) [5, 24].

3. Where is the SNP rs 2078486 located within the TP53 gene and what would be the expected impact of the T>C base change?

Response: *TP53* rs2078486 is an intronic polymorphism, however it might be in linkage disequilibrium with a functional polymorphism and further affects cancer risk. We added a discussion about the possible mechanisms through which *TP53* rs2078486 polymorphism modulates cancer risk.

Revision: We have added the following sentences in the Discussion section (Page 10, Paragraph 3):

*TP53* rs2078486 is located in intron 1 and thus is not likely to be a direct disease-causing polymorphism. However previous studies suggest that *TP53* rs2078486 is in a large linkage disequilibrium block extending from upstream of exon 1 to the first half of intron 1[17]. Therefore it is possible that *TP53* rs2078486 might be in linkage disequilibrium with some functional polymorphisms, which in turn alter susceptibility to human diseases. Some other intronic variations in *TP53* were reported to affect disease risk previously and the most widely studied one is *TP53* intron 3 duplication polymorphism (rs17878362) [5, 25]. The underlying mechanism by which *TP53* rs2078486 modulates cancer risk is not fully understood and warrants further investigations. However prior studies provided some initial evidence that *TP53* rs2078486 is in perfect linkage disequilibrium with *TP53* rs2287498, which is predicted to affect function at a splice site and *TP53* rs2078486 is also in weak linkage disequilibrium with *TP53* rs12951953, which might affect a transcription factor binding site [13].
4. The minor allele frequencies for both SNPs should be given for the cases and controls and are allele ratios compatible with Hardy–Weinberg equilibrium?

Response: We calculated and presented results to evaluate Hardy–Weinberg equilibrium for both SNPs.

Revision: We added the following sentence in the Method section (Page 6, Paragraph 3):
We found no obvious deviations from Hardy-Weinberg equilibrium for both SNPs (rs2078486: \(\chi^2=0.19, P=0.6629\); rs1042522: \(\chi^2=4.24, P=0.0395\)) among control subjects.

5. Are the two SNPs in linkage disequilibrium?

Response: We evaluated the linkage disequilibrium between the two SNPs.

Revision: We added the following sentence in the Method section (Page 6, Paragraph 3):
We did not find strong linkage disequilibrium between the two SNPs (\(D'<0.5\)) in the current study and this is consistent with previous studies [17, 18].

6. Table 3 – what is cOR and aOR (crude and adjusted)? Please use same abbreviations as Table 2.

Revision: We changed ‘cOR’ to ‘crude OR’ and ‘aOR’ to ‘adjusted OR’ in Table 3.

**Reviewer 3**

In this paper, Li et al. present their findings relative to two polymorphisms in the Tp53 gene and interactions with lifestyle factors and lung cancer risk in a Chinese population. While the hypothesis is sound and fairly well presented, I do have some minor concerns with the paper that need addressing.

**Minor Essential Revisions**
1) There is no presentation of the context of rs2078486 with respect to the structure and function of the p53 gene. As this SNP is in intron 1, the discussion regarding a putative functional difference of the genotypes needs to be further developed. Furthermore, this SNP is in a large linkage disequilibrium block, and may be tagging influence from one or more SNPs around it. This possibility needs to be developed by the authors.

Revision: We have added the following sentences in the Discussion section (Page 10, Paragraph 3):

\( TP53 \) rs2078486 is located in intron 1 and thus is not likely to be a direct disease-causing polymorphism. However previous studies suggest that \( TP53 \) rs2078486 is in a large linkage disequilibrium block extending from upstream of exon 1 to the first half of intron 1[17]. Therefore it is possible that \( TP53 \) rs2078486 might be in linkage disequilibrium with some functional polymorphisms, which in turn alter susceptibility to human diseases. Some other intronic variations in \( TP53 \) were reported to affect disease risk previously and the most widely studied one is \( TP53 \) intron 3 duplication polymorphism (rs17878362) [5, 25]. The underlying mechanism by which \( TP53 \) rs2078486 modulates cancer risk is not fully understood and warrants further investigations. However prior studies provided some initial evidence that \( TP53 \) rs2078486 is in perfect linkage disequilibrium with \( TP53 \) rs2287498, which is predicted to affect function at a splice site and \( TP53 \) rs2078486 is also in weak linkage disequilibrium with \( TP53 \) rs12951953, which might affect a transcription factor binding site [13].

2) The authors correctly state in the last phrase of the discussion that the number of analyses carried out increases the chance for spurious associations. No attempt at correction for multiple comparisons is made or even discussed, even though the term "significant" is used seven times in the results section.

Revision: We added the following sentence in Discussion section (Page 12, Paragraph 1):

After conducting Bonferroni correction for multiple comparisons, no significant interactions between lifestyle factors and \( TP53 \) rs2078486 SNP remained; therefore we cannot exclude the possibility of spurious associations due to multiple comparisons.
3) No mention of recall bias is made, which could alter the distribution of established lung cancer risk factors between cases and controls leading to spurious interaction models as well.

Response: We added a discussion about the possible impact of recall bias.

Revision: We added the following sentences in the Discussion section (Page 12, Paragraph 1):
Lastly, recall bias is likely for established or probable risk factors of lung cancer, such as smoking and air pollution, in a case-control study. However the association between smoking and lung cancer observed in the current study is similar to the previous studies conducted in Asian populations [30]. To minimize the possible recall bias on indoor air pollution exposure, we collected information on several relevant variables, such as cooking, heating and window opening behaviors.

Additional editorial concerns:
We note you cite the doctoral dissertation of Yi Ren Wang "One case-control study conducted in Los Angeles found elevated lung cancer risk associated with the variant genotype of p53 rs2078486 SNP (unpublished doctoral dissertation from Yi Ren Wang) in your manuscript. Could you please clarify the following:

1) Was this PhD thesis assessed by experts, or defended by the PhD candidate Dr Wang?


2) Is the thesis available to be read by reviewers and readers, in a citable form?
Response: We added citation for Dr. Yi Ren Wang’s dissertation (page 4, paragraph 3).