Author's response to reviews

Title: No influence of the MDM2 SNP309 on early onset lung cancer in a Caucasian population

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

Thank you for your letter and for making the reviewers' comments available to us. We appreciate the constructive comments of the referees and the opportunity to address them in a revised version of the manuscript.

We have itemized our responses below in a point by point fashion and have revised the manuscript accordingly. All authors have read the revised paper. We hope to have addressed the comments of the referees appropriately and that the manuscript is now suitable for publication in the *Biomed Central Cancer* journal.

Thank you very much for your efforts.

Sincerely Yours,

Kirstin Mittelstraß

Attached:
- The revised manuscript.
Responses to referee #1 - Leah Mechanic:

We thank Dr. Mechanic for the very detailed, clear and helpful comments. We have itemized our responses below with regard to the paragraphs of the Reviewer’s comments.

General comments:

1. “This report is of interest due to the previous inconsistent reports in the literature and the young age of the cases”. We thank the Referee for this favourable comment.

Minor Essential Revisions:

2. **What percent of duplicates were included in the assay?:** We performed a bunch of quality measurements when genotyping the *MDM2* variant including sex determining SNPs, positive and negative controls and 10 % of duplicates. All of the control measurements yielded no discordant genotype. This is now mentioned in the manuscript on page 6, abstract 1, line 6-9 (Materials and Methods) and on page 7, abstract 1, line 1-2 (Results).

3. **What was the concordance between the duplicates?:** Comparing the frequency of the *MDM2* SNP309 with further published studies they are quite similar. This is now also mentioned on page 8, abstract 1, line 10 (Discussion).

4. **When conditioning on age, how was the data adjusted (was age continuous, or were the 3 year age categories used?):** When conditioning on age there were the three groups: =40 years, 41-45 years, 46-50 years of age used.

5. **On page 6, in the last paragraph of the results, authors wrote “Among women, however, we observed a none significantly lower age of diagnosis of eight month in females compared to males (p=0.059).” The meaning of this sentence is unclear:** We have revised the noted sentence (page 8, line 22) and to clarify we have changed the wording to “Women, however, with the *MDM2* SNP309 were diagnosed at a younger age (8 months) compared to males, but this was not statistically significant (p= 0.059, 95% CI: 0 to 16 months)”.

6. **Correct the spelling of “unknown:”** Thank you for pointing us to this mistake, which we corrected now (table 1).

7. **Table 2, the footnotes do not seem to correspond accurately to the models/associations evaluated in the table. For example “stratified by age and adjusted for by smoking”, corresponds with the analysis by gender. Was this data stratified by age, in addition to gender? If so, the age stratification should be shown:** We agree with the Referee in this point and have changed the footnotes in table 2 for a better understanding.
Response to Referee #2 – Qingyi Wei

We thank Dr. Wei for the short summary of our paper and the constructive comments. We have itemized our responses below with regard to the # of paragraphs in the Reviewer’s comments.

General comments:

8. We thank the Referee for pointing out that our study design appears to be sound, the analysis was adequate and that it is an article of importance in the field.

Major Compulsory Revisions:

9. One recent report available online now was missed in the discussion. This report presented a combined analysis of MDM2 SNP309 and risk of several cancers, including lung cancer (Wilkening S et al, Carcinogenesis. 2007 Sep 7; [Epub ahead of print]). It is likely that the authors did not have the opportunity to read it online yet at the time of submission: The Referee stated correctly: that one report available online now was missed in the discussion. We are grateful for the referee’s attention and of course we have read the report by now and have included these findings in our discussion. (Discussion, page 8-9, last paragraph).

10. The authors did mention one (Li et al. [2006]) of the large studies of Caucasian populations published to date in the Discussion, but this report was not listed in the references. Therefore, the authors need to check their references carefully to make sure that other reports mentioned in the text were not missed in the references.: Thank you for pointing us to this mistake, and we have corrected it now (reference list, number 17) and proofed the other references in the reference list.

11. Table 1: for the frequency of MDM2 SNP309, conventionally the common genotype (TT) should be on the top and rare genotype (GG) listed last: We have changed the order of the genotypes as suggested from the referee in table 1.

12. Table 2: it would be more informative if the numbers of the subjects were also presented for each of the strata: The Referee suggested inserting the numbers of subjects for each strata in table 2. These numbers are already presented in table 1. With combining both tables we think that the table would be too large and not clear anymore.
Response to Referee #3 – Gareth Bond

We thank the Referee for the precise summary of our manuscript and for rating our manuscript as a report of “mostly high quality thoroughly analysed and clearly presented”.

Major compulsory Revisions:

13. The authors must further compare and contrast the patient populations they are combining for their analysis. For example, what were the similarities and differences in their patient accruals?: We agree with the Referee that there is more information needed to better compare both case-populations. Therefore we have reconstructed table 1 and now presenting all variables for both case-studies and the control population separately (page 14).

14. Furthermore, in the Results section, the authors must show that the trends they are reporting are similar in all three groups, independently. If the trends are not the same or if the groups differ, this must be clearly shown and discussed: The Referee stated that it is important to know whether the trends of all three groups are similar, independently. We definitely agree with this statement and therefore we included another table in our manuscript comparing the three groups to each other. With this we can show that the trends show all in the same directions (table 3, page 15).

15. Given the literature of the MDM2 SNP309 locus, the analysis and discussion of the average ages of tumor diagnosis is lacking. The authors state that the G-allele was shown in one previous report to associate with an earlier tumor diagnosis in p53 mutation carriers and sporadic soft tissue sarcomas (1). However, in the past 3 years many other reports have further described this phenotype in many other tumor types (2-10). These reports and their conclusions need to be incorporated into their analysis and discussion: The Referee stated that it would be of great interest to discuss the MDM2 variant in other cancer types as well, because three reports demonstrate in four different sporadic tumor types a gender-dependence of this phenotype. Therefore, the authors must minimally show the mean, median and range of ages for both women and male patients separately: Therefore, we should minimally show the mean, median and range of ages for both women and male patients separately. Not to miss any information we have included these numbers of age of diagnosis for both men and women. Along these same lines, the frequencies of the different genotypes of MDM2 SNP 309 must be presented for each gender separately as well: The genotypes for each gender are now presented separately as well (table 4, page 16).

16. Furthermore we have created an additional table with the numbers and frequencies of all genotypes of the different strata (table 4, page 16).

17. However, the authors never directly discuss that if ethnicity plays such a role in these differences why does Lind et al. see a significant increased risk in Caucasian lung cancer patients as well (11)? This
must be discussed: The Referee correctly pointed out, that we never directly discuss that if ethnicity plays such a role in these differences why does Lind et al. 2006 see a significant increased risk in Caucasian lung cancer patients as well (11)? This is now discussed (discussion, page 9, abstract 1). It might be due to age of cases. In our population the mean age is 45 years, whereas the mean age of the population of Lind et al. 2006 is 63.3 years. As the population by Lind et al. 2006 is of Norwegian origin there might be also different gene-environmental interactions. It also can’t be excluded that Lind et al. 2006 showed a false-positive result. Comparing the numbers of subjects of each study populations we have a much higher power than Lind et al. 2006.
Response to Referee #4: Hongbing B Shen

We would like to thank the Referee for the very thorough reading and the constructive comments which helped greatly to improve our manuscript. However, we only partially agree with the Referee that our manuscript is “of limited interest”. There was a lot of debate on the $MDM2$ SNP309. This variant has been investigated in several lung cancer and other type cancer studies with inconsistent results. Therefore it was for special importance for us to look at this SNP in a well characterised and powered case-control study of young lung cancer cases. By finding non significant associations with this variant and lung cancer we think that we made an important contribution to the cancer and lung cancer community.

Minor Essential Revisions:

1. Because the study included cases from two existing studies, please describe and compare the characteristics of two case groups in the Study population and Result sections: Also another Referee pointed out, that it would be important to describe and compare the characteristics of the two case groups. Therefore we have prepared a table with detailed information of the two case groups.

2. In the Result section, the author reported the characteristics of study population, but the difference of selected variables between cases and controls did not be tested: In the first part of the results we are now dealing with the loss of information and have added the suggestions of the Referee (page 6, line 1).

3. In Table 1, the sum of men ($N=405$) and women ($N=228$) was not equal to the total number of cases ($N=635$): Thank you very much pointing us to this problem. We are sorry for this mistake. We lost 1 man and 1 woman. This is now corrected in table 1 and we also checked that the analyses were done with the right numbers.

4. In Table 2, please add the number of different genotypes in cases and controls: To give more information on the numbers of genotypes we have created an additional table with the numbers and frequencies of all genotypes of the different strata (table 4, page 16).

5. And the notes for the stratification may be wrong: * should mean the results stratified by gender and ** should mean the results stratified by histology: We agree with the referee in this point and have changed the footnotes in table 2 for a better understanding.