Author's response to reviews

Title: Early onset lung cancer, cigarette smoking and the SNP309 of the murine double minute-2 (MDM2) gene

Authors:

Kirstin K Mittelstrass (kirstin.mittelstrass@gsf.de)
Wiebke W Sauter (wiebke.sauter@gsf.de)
Albert A Rosenberger (arosenb@gwdg.de)
Thomas T Illig (illig@gsf.de)
Maria M Timofeeva (m.timofeeva@dkfz-heidelberg.de)
Norman N Klopp (klopp@gsf.de)
Hendrik H Dienemann (hendrik.dienemann@thoraxklinik-heidelberg.de)
Eckart E Meese (hgemee@med-rz.uni-sb.de)
Gerhard G Sybrecht (prof.g.w.sybrecht@uniklinikum-saarland.de)
Gabi G Woelke (woelke@gsf.de)
Mathias M Cebulla (mathias.cebulla@sanktgeorg.de)
Maria M Degen (maria.degen@t-online.de)
Harald H Morr (harald.morr.waldhof@t-online.de)
Peter P Drings (peter.drings@thoraxklinik-heidelberg.de)
Andreas A Groeschel (inagro@med-rz.uni-sb.de)
Karsten K Grosse Kreymborg (karsten.g.kreymborg@medizin.uni-leipzig.de)
Karl K Haeussinger (gauting@asklepios.com)
Gerd G Hoeffken (hoeffken@fachkrankenhaus-coswig.de)
Christine C Schmidt (schmidt@fachkrankenhaus-coswig.de)
Bettina B Jilge (b.jilge@skc.de)
Wilhelm W Schmidt (e.schmidt@skc.de)
You-Dschun Y Ko (y.ko@jk-bonn.de)
Dagmar D Taeuscher (d.taeuscher@t-online.de)
Jenny J Chang-Claude (j.chang-claude@dkfz-heidelberg.de)
Heinz-Erich H E Wichmann (wichmann@gsf.de)
Heike H Bickeboeller (hbickeb@gwdg.de)
Angela A Risch (a.risch@dkfz-heidelberg.de)

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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 re-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 Mittelstraße

Attached:
- The re-revised manuscript.
Response to Referee #4 – Hongbing B Shen

We thank the Referee for re-reviewing our manuscript and not having any further Revisions.

Response to Referee #2 – Gareth Bond

We thank Dr Bond for carefully re-reviewing our manuscript and the constructive comments.

Major Compulsory Revision:

We thank the Referee pointing us to the gender specific differences of our two case populations. Now we have incorporated these concerns in our manuscript.

1. Specifically, I had asked that the data and subsequent analysis for each study group be shown separately, as the populations were accrued differently. I had also asked that the two genders be analyzed separately, given the gender-specific effects of this locus previously noted in lung cancer (1) as well as in other tumor types. The data presented in Table 4 seem to show that, in the HLC study group, there could be gender-specific effects on lung cancer risk for this locus.

We now have included the gender specific results of our subgroup analysis in the results, discussion and also in our conclusion. We also present new tables (table 3, 4) with the gender specific results of each case subgroup. All results are adjusted for smoking exposure.

We cannot confirm the p-values of the Referee which might be due to the point that our results are adjusted for smoking exposure. But we also can show that our investigation points towards sex specific differences.

Response to Referee #5 – statistical referee

We would like to thank the Referee for the very thorough reading and the constructive comments and for rating our manuscript as a well-designed case-control study.

Major Compulsory Revisions:

2. Table 4 which shows subgroup characteristics, should also present odds ratios and 95% confidence intervals (or p-values from chi-squared of Fisherâ€™s exact test) comparing the genotypes between the cases (two cases groups separately and combined) and controls within subgroups. These results would clearly reveal the gender specific associations that Reviewer 2 (Dr. Gareth Bond) very importantly points out.
We now have included further sex specific results in our manuscript (see Table 3, 4)

3. I agree with Dr. Bond that these subgroup analyses are counter to the overall conclusion of the paper that the MDM2 SNP309 locus is not associated with early onset lung cancer in this population. I do not believe that the results of the paper should be ignored based on the results of one post-hoc subgroup analysis. Instead, the authors should emphasize that, overall, after controlling for gender and other characteristics no association was found in the combined analysis. The authors should then go on to explain that in their subgroup analysis of gender, among only those from the HLC study, was a gender specific association found.

We would like to thank the referee to suggest ways to address these problems. Now we have incorporated the concerns of Dr. Bond in our manuscript.

Results (page 8):
Even if no significant difference between both case-populations could be stated statistically, we observed twice as many female GG-carriers beyond HLC-cases (21%) than in the control- or LUCY population (10%) (Table 5). We also observed an enrichment of NSCLC- and NSCLC adenocarcinoma-cases in the HLCS-sample (78%) compared to the LUCY-sample (66%). Lind et al. (2006) [6] recently reported a sex specific risk disposing effect of the T-allele of MDM2 SNP309 for NSCLC cases. We therefore additionally performed a subgroup analysis restricted to NSCLC-cases and testing for sex differences. Compared to the GG genotype, the smoking adjusted ORs for the TG and TT genotypes for female NSCLC-cases were 0.78 (95% CI = 0.4-1.6) and 0.71 (95% CI = 0.4-4.0), respectively. For men the ORs were 1.22 (95% CI = 0.7-2.8) and 1.18 (95% CI = 0.7-2.9), respectively (Table 4). Although a possible NSCLC risk modification of MDM2 SNP309 seems to be reverse between men and women, we failed to achieve significance for this sex difference (p=0.237). We also tested sex specific genetic association within both case-samples separately. Neither within all HLCS-cases (p=0.083) nor within all LUCY-cases (p=0.778) we have found significant sex differences (Table 3).

Discussion (page 10):
Lind et al. (2006) [6] recently reported a sex specific risk disposing effect of the T-allele of MDM2 SNP309 for NSCLC cases. They investigated NSCLC-cases and found female carriers of the GG-genotype associated to LC by an OR of 4.06, while male homozygotes showed a non significant OR of 1.25. Our data slightly tends to confirm this finding, but the corresponding OR for NSCLC -women is just 1.4 (1/0.71 of Table 4) , which is much smaller and not significant (p=0.3226). In our study none of the sex specific ORs nor a test of sex differences yielded significance. But we notice discrepancies of such a sex specific genetic association between both case-sample of our investigation. However, a sex difference was
almost significant (p=0.08) for the much smaller single center case-sample of the HLCS-study. There, the OR for female carriers of the GG-Genotype (corresponding to Lind et al.) was 1.9 (1/0.54 of Table 4) and for male carriers 0.6 (1/1.56 of Table 4). With n=67 female and n=96 male cases these subsamples are quite small and the tests for association are underpowered. Thus our investigation provides no significance for such sex specific associations by itself; the findings need to be considered having the results of Lind et al. (2006) in mind. Comparing our study samples to that of Lind at al. (2006) the major difference is the age of onset of LC. The presented investigation focus on early onset LC cases which are in the mean 20 years younger than those of Lind et al. (2006). As the population by Lind et al. (2006) is of Norwegian origin there might also be different gene-environmental interactions.

Conclusions (last paragraph):
[...]. Our investigation provides no significance for sex specific associations by itself; it points only towards sex specific differences. Therefore further studies have to be conducted to explicitly study these gender-specific effects.

Minor essential Revisions.

4. Were haplotypes comprised of SNPs in proximity of SNP309 investigated? No haplotype analysis of SNPs in proximity of SNP309 were investigated. A brief discussion should be provided with regards to the utility of haplotypes in this study.

We concentrated on the often discussed SNP309. Therefore no haplotype analyses were performed. Moreover there are no studies published up to now showing greater risk of haplotypes compared to the analysis of SNP309 alone.

5. On page 5, when describing the KORA study, the sentence “With respect genotyped polymorphisms to date, a major population stratification between KORA and two other cohorts from Northern Germany could not be detected in a genomic control approach”. This sentence is confusing and needs to be reworded. We agree with the Referee in this point and have reworded the sentence as followers: “No major population stratification between KORA (Southwest Germany) and two other cohorts from Northern Germany could be detected in an intensive study using a genomic control approach [12].”