Author’s response to reviews

Title: Characterization and risk association of polymorphisms in Aurora kinases A, B and C with genetic susceptibility to gastric cancer development

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Version: 2 Date: 23 Apr 2019

Author’s response to reviews:

BMC Cancer
Springer Nature

Editor-in-Chief:
Prof. Linda Gummlich
BioMed Central, UK

April 23rd, 2019

Dear Professor Linda Gummlich,

Thank you for your letter – e-mail and the opportunity to revise our manuscript entitled: "Characterization and risk association of polymorphisms in Aurora kinases A, B and C with genetic susceptibility to gastric cancer development" (Reference number: BCAN-D-18-02595R1).

We would like to sincerely thank the reviewers and editor for critical reading of this study and valuable suggestions. We are grateful for your constructive comments and thorough evaluation of the paper. In the accompanying Response Letter below we have addressed all of the reviewers’ comments and revised the manuscript accordingly. Every change is documented individually in the accompanying letter and presented in blue highlighted text in the revised manuscript.
We sincerely hope that the revisions are satisfactory and that the manuscript meets the requirements for publication in the BMC Cancer.

Yours sincerely,

Petra Hudler

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Subject: Response Letter to evaluation of manuscript "Characterization and risk association of polymorphisms in Aurora kinases A, B and C with genetic susceptibility to gastric cancer development", submitted to BMC Cancer (Reference number: BCAN-D-18-02595R1).

Date: April 23, 2019

Dear Editor and Reviewers,

Below are listed responses to Editor and Reviewers comments for the manuscript entitled "Characterization and risk association of polymorphisms in Aurora kinases A, B and C with genetic susceptibility to gastric cancer development", submitted to BMC Cancer (Reference number: BCAN-D-18-02595R1).

The authors would like to thank the reviewers and editor for constructive comments and helpful suggestions. We are grateful for your advice and suggestions, as they have been helpful in our efforts to revise and improve the quality of the manuscript. We have addressed all comments and we hope that we revised the manuscript accordingly.

On behalf of all authors.

Sincerely,

Petra Hudler

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Editor Comments:
1. Please clarify who waived the need for consent from the patients, and please include this information in the Ethics approval and consent to participate section.

R1: We thank the Editor for pointing this out. We corrected this and included this information in Ethics approval and consent to participate section (Page 21, Line 8).

2. Did the control participants consent to this study? If so, please include this information in the Ethics approval and consent to participate section.

R2: Since the control participants were healthy blood donors randomly selected upon regular check-ups, it is the policy of the clinical institution, not to obtain written consent. The participants were informed that the remains of their samples would/could be used in the research experiments. The samples of patients who declined the participation were marked and were subsequently - after obtaining results for requested regular check-ups - destroyed. Therefore, we did not include this information in Ethics approval and consent to participate section.

3. Did the patients consent to their samples being collected during their surgery to be used for purposes of this study? If so, please include this information in the Ethics approval and consent to participate section.

R3: Yes, the patients consented their samples being collected during their surgery to be used for the purposes of this study. We added this information in Ethics approval and consent to participate section (Page 21, Line 8).

Note: All the changes made in the manuscript are highlighted in blue.

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Reviewer 1:

1. The number of samples from the gastric cancer group is greatly reduced. This aspect should be included in the discussion as a limitation of the study.

R1: We are thankful to the reviewer for pointing this out. We included this information in „Conclusions“ section (Page 19, Line 20).

2. The authors performed an "In silico analysis of SNPs" to evaluate the impact of the SNPs in introns and untranslated regions on transcription factors binding sites. Therefore this information must be in the objectives of the study (Introduction: last paragraph) and the findings should be included in the abstract. Also in this last paragraph was described: " In addition, we assessed the association between studied single nucleotide polymorphisms and the clinicopathological characteristics of gastric cancer patients." The authors should rewrite this sentence and
emphasize that the association of polymorphisms with the histological types of gastric cancer (diffuse and intestinal types) has been investigated.

R2: We appreciate this suggestion. For the in silico analysis, we added this information in the objectives of the study (Background: last paragraph, Pages 4-5). Also, in the (Background: last paragraph), we rephrased the sentence „In addition, we assessed the association between studied single nucleotide polymorphisms and the clinicopathological characteristics of gastric cancer patients“ in order to emphasize that we have investigated the association of polymorphisms with the histological types of gastric cancer (diffuse and intestinal types).


R3: We are grateful to the reviewer for noticing this error. In the (Introduction: first paragraph, Page 4), we replaced previous reference, with the more recent reference on the incidence of gastric cancer globally, which you suggested.

4. Methods: Study design and populations: To describe in more detail the clinical and epidemiological characteristics of the case and control groups. Information on sex, age, alcohol and tobacco consumption, histological type of gastric cancer and H. pylori infection should be included in a table. Also highlight if the patients in the case group were subjected to some type of treatment (radiotherapy or chemotherapy).

What is the origin of the individuals in the control group? How were they selected? Are they healthy blood donors?

R4: Thank you for this suggestion. We agree that presenting these data in a table is more visually appealing and easier-to-understand for the readers. We put all the data we have for gastric cancer patients (gender, age and histological type of gastric cancer) in the Table 1, as was suggested (Page 29).

We added information that the patients in the case group were not subjected to any type of treatment (radiotherapy or chemotherapy). Also, we included information that individuals in the control group were healthy blood donors of Bosnian origin (matched to cases for ethnicity), and were randomly selected and signed up for the present study (Methods, Page 5, Line 7).

5. Results: Considering that different polymorphisms of the same gene have been evaluated, I suggest that a haplotype analysis be performed, as well as a combined analysis of these polymorphisms associated with risk of GC to evaluate the synergistic or additive effect of SNPs (Oliveira et al., 2012- Dig Dis Sci, DOI 10.1007/s10620-012-2460-5).
R5: We are grateful to the reviewer for this suggestion. We performed a haplotype analysis as well as a combined analysis of SNPs associated with the risk of GC in order to evaluate the synergistic (additive) effect of SNPs, as you suggested. We followed the article you sent to us, and we also cited it in the manuscript (Methods, Page 7, Line 17, Line 22, Results, Page 10, Line 8, Page 12, Line 15, Discussion, Page 18, paragraphs 2, 3, and 4, Table 5, Figure 3).

6. Discussion: The authors report that bioinformatics analysis of transcription binding sites revealed that some variant alleles have created extra sites for transcription factors, which could affect the rate transcription and protein translation levels. Therefore, it would be interesting to have evaluated the levels of mRNA and/or protein expression of the AURKA, AURKB, AURKC genes in the gastric cancer tissues (at least 40 samples), to assess the influence of this SNPs on the mRNA and protein expression.

R6: We appreciate that the reviewer pointed this out. However, the samples, which we collected, were formalin-fixed paraffin embedded (FFPE) samples and the sections were small. Therefore, it was not possible to obtain enough RNA for experiments and in addition, it was severely degraded. Unfortunately, we also couldn’t obtain proteins or perform immunohistochemistry on these samples, due to limited amount of biological material. Therefore, despite our efforts, it was not possible to perform experiments on the RNA and/or protein level.

Note: All the changes made in the manuscript are highlighted in blue.

Reviewer 2:

Authors need to design a precise experimental plan to demonstrate that the bioinformatic analysis is followed by genotype analysis and supported by expression data. The statistical analysis is acceptable; however, they should use a significant p value cut off.

R1: We appreciate the reviewer’s comment. The study was designed as a candidate gene case-control genotyping study, therefore, additional experiments were not planned at this stage. The bioinformatic analyses were performed in order to evaluate the possibilities of consequences of genotype changes, however, at this point of research the functional assays are not yet warranted as it is not general practice to perform functional experiments in these studies.

The significance of genetic models and best appropriate model was selected based on the AIC criterion, as we did not have multiple comparisons in analyses, which would be the case if performed the study on the genome and particularly on the RNA level. In this study each model was assessed separately, therefore it was suggested in literature that AIC criterion would be the most appropriate method to evaluate models.
There are minor issues with the data interpretation. I was expecting that authors should have performed micro array data analysis and show scatter plots and co-expressed genes. However, in the present investigation, the authors have made conclusions based on the genotype data only.

R2: We thank the reviewer for this comment. The samples were collected from an archive from the Clinical Pathology and Cytology at the University Clinical Center Sarajevo, Bosnia and Herzegovina as formalin-fixed paraffin embedded (FFPE) samples. This procedure for storing strongly compromises the integrity of RNA and it is not generally recommended to use FFPE samples for microarray analyses. However, we did check the quality of RNA in selected few samples, from which we were able to obtain more sections, and it’s quality was not acceptable (RIN numbers (Agilent) and spectrophotometric analysis showed significant level of degradation). Therefore, in this regard and the fact that the amount of samples was limited we could not perform the experiments on the RNA level. In addition, the study was designed as a genetic variation case-control study, and in such studies RNA experiments are generally not included.

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