Author's response to reviews

Title: Associations between GSTM1*0 and GSTA1*A genotypes with the risk of cardiovascular death among hemodialyses patients

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Author's response to reviews: see over
Dear Professor Hayley Henderson,

Thank you for your interest in publishing our paper “GSTM1*0/0 genotype and combined GSTM1*0/GSTA1*A genotype are associated with risk of cardiovascular death among hemodialysis patients” in BMC Nephrology. We have made all the changes in the manuscript as suggested by both reviewers. Because of that our title is modified: “Associations between GSTM1*0 and GSTA1*A genotypes with the risk of cardiovascular death among hemodialysis patients”. We added one author, who responsible for multiple testing analysis. Thank you once again for all the efforts.

Yours sincerely,

Prof. dr Tatjana Simic

RESPONSE TO REVIEWER #1:
Major revisions:

A. Power: “Is the study sufficiently powered to answer the question? The number of individuals and cases during follow up is very little as the authors also point out. Could they include a formal power calculation?”

It is undoubtedly that the number of subjects in our study (n=199) is not sufficient to obtained alpha=0.05 and 1-beta=0.8. According to the formal power calculation, the sample size for cases during follow-up should be 357. Concerning our sample size, 1-beta=0.68. In order to comply with this suggestion the following text was added in the Discussion section:

(Page 15, line 23): “Formal power calculation revealed a 1-beta=0.68.”

B. Multiple testing: “I feel that they have not adequately addressed multiple testing issues. At least 5 different combinations of SNPs are tested and per combination 4 subanalysis are done. Please include either Bonferroni or if they feel this is too stringent FDR correction. If any result is significant after correction the conclusions drawn are fine, otherwise revision is needed.”

We performed extensive multiple testing by using Bonferroni and false waive analysis. Unfortunately, after correction, none of the results obtained reached statistical significance. We made all necessary corrections according to new results.

In Abstract, we changed the following sentences:

(Page 3, lines 13-16): “GSTM1-null genotype in ESRD patients is not a significant independent predictor of overall and cardiovascular mortality. The borderline effect modification by wild-type GSTA1*A/*A genotype on associations between GSTM1-null and analyzed outcomes was found only for death from stroke after false discovery rate and Bonferroni corrections.”

We deleted the following sentence: “Carriers of wild type GSTA1*A/*A genotype exhibited increased risk of cardiovascular death.”

We added new conclusions in Abstract (page 3, lines 21-23): “Combined GSTM1*0/GSTA1*A genotype might be considered as genetic markers for risk of cardiovascular death in ESRD patients, which may permit targeting of preventive and early intervention.”

Appropriate description added in the subsection Statistical analysis:

(Page 8, lines 19-21): “To control for multiple comparisons we used multtest package in R. We reported corrected p values obtained by methods that control family-wise error rate [18] and false discovery rate [19,20].”
In References we added references number 18, 19, and 20. As suggested, additional supplementary tables are added (Supplementary Tables 3A and 3B). According to reviewers suggestion we introduced these negative findings in sections Result and conclusion. The following text was added in the sections Results:

(Page 9, lines 21-22): “However, after multiple testing (Supplementary Table 3) none of models remained significant.”

(Page 11, lines 6-9): “However after multiple testing (Supplementary Tables 3A and 3B) none of models remained significant. Additionally, in controlling for multiple testing, we excluded tests for GSTM1 and GSTA1 genes as they are marginal to GSTM1/GSTA1 interaction.”

Discussion section:

(Page 15, lines 11-12): “…although after multiple testing our models missed statistical significance”

(Page 16, lines 7-8): “Additionally, after multiple testing none of models remained significant.”

C. ESDR related outcome: “It is permissible that ESDR related parameters also contribute to end points. It is not clear if the ESDR related factors are similar between the carriers of the variants vs. non-carriers. Can they at least show the average urea and creatinine level per sub-group.”

The average urea and creatinine concentrations per sub-groups are presented in Supplementary table 4. There were no any statistically significant differences between subgroups.

RESPONSE TO REVIEWER #2

Minor revisions:

1. “Adjust for multiple testing.”

We addressed this issue in answer to reviewer 1.

2. “Tables 2, 3 and 4, include number of individuals included in analyses for each models.”

Numbers of patients are added in Material and method section according to suggestion (page 8, lines 11-15): “The numbers of patients included in regression models were the same for the all tested GST polymorphisms. For overall mortality, the number of patients included in Model 1, 2 and 3 were 186, 183 and 169, respectively; for cardiovascular mortality 180, 177 and 166 patients, respectively; for myocardial infarction 189, 186 and 169 patients and for cardiovascular insult 190, 187 and 169 patients, respectively.”

3. Include definition of outcomes in methods: how information on cause-specific mortality was obtained?

Information of cause-specific mortality was described in sub-section Study subjects and the following text was added

(Page 6, lines 15-16): “Information regarding death and causes of death were obtained from hospital records and other relevant documents.”

4. Include a discussion on multiple testing.

We addressed this issue in answer to reviewer 1.