Author's response to reviews

Title: Genetic contribution of catechol-O-methyltransferase variants in treatment outcome of low back pain: a prospective genetic association study

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Author's response to reviews: see over
EDITOR:

Dear Editor,

We thank you for conveying us the comments of the reviewers, which will help us improve our manuscript. We have now modified the manuscript in light of their comments and hope that you will find the modifications to satisfactory. The manuscript has also been proof read by three researchers.

Best Regards,

Dr Ahmad Omair

REFEREE-1:

Dear Jules Desmeules,

We are thankful to you for your precious comments and guidelines for the modification of our manuscript. These comments were highly valuable and relevant and have helped us to improve our manuscript. We have now revised the manuscript in light of your guidelines.

Important information that we need to convey to you is that VAS LBP change value from 2 patients was calculated wrong (as -84 and 84 instead of the correct -8.4 and 8.4) by the programme we used. This error was evident from the data given in table 1 and table 5 of the previously submitted manuscript, regarding 7-11 year VAS LBP change. The baseline and 7-11 year VAS LBP values were correct for the two above mentioned samples, but the change was computed with error. We apologize that we did not detect this before initially submitting the manuscript. After we realized that the mean VAS change values in table 1 and mean VAS change values according to genotypes in table 5 were not really the subtracted values of the baseline and follow up VAS LBP scores, we scanned the whole dataset for any other errors and can now confidently say that there were no other mistakes beside those two. The whole analysis was performed again after this correction.

Below we have addressed your detailed comments, and described the changes we made in response to them.

Comment-1: Please, give a brief summary of the results of the two previous randomized trials. The lack of association found in this study would be consistent with an absence of significant difference between the groups.

Response: We agree that it will be informative to give a brief summary of the results of the two RCTs from where we recruited our sample. This has now been added to the patient sample part of the methods section of the manuscript (page 7).
Comment-2: 9 subjects who underwent "late" surgery (done between the previous trials and this one) were added to the surgery group. Please, verify if these patients had an impact on the results of your analysis?

Response: We will like to clarify here that in fact there were 14 patients from the group of 42 who were randomized to cognitive therapy at baseline that by the time of follow up had undergone fusion. Similarly, 5 patients from the group of 51 patients who were randomized to surgery at baseline did not undergo fusion by the time of follow up. Therefore regarding classifying the patients according to the treatment they actually underwent, there were 60 fused patients and 33 who underwent cognitive-exercises. So the difference in number of patients randomized to surgery and who actually underwent it were 9. We apologize that we had not explained this well in the previous version of the manuscript, but this has now been described more clearly in the study intervention chapter of the methods section (page 8).

In order to check the impact of these 19 patients on the results, we performed two sensitivity analyses. One according to randomization N=93 (51 fusion + 42 cognitive), and second by removing these 19 patients N = 74. According to randomization, the results were similar to the results from the analysis according to actual treatment the patients underwent N = 93 (60 fusion + 33 cognitive). But when we took out those 19 patients who did not undergo the treatment they were randomized to, the associations become insignificant except for the effect of analgesics that remained significant. It is very difficult to say if the loss of association is actually due to those 19 patients or whether it is due to further loss of power by a decreased patient number.

Information has been incorporated into the manuscript in results section (page 13).

Comment 3: Please, give some details on power calculations. For example if a power was set at 80% what would be the smallest difference that could be detected by the analysis?

Response: We appreciate your advice to add information on power calculations, as this will make it easy to understand and discuss results. This information has been added to the statistical analysis chapter of the methods section (page 11).

Comment 4: Please, give the threshold (and references) of the "genotype success rate" that was used in this study.

Response: It is certainly important to present the threshold for the genotype success rate (GSR) as to what will be the cut off to include the SNPs in the analysis. The threshold has now been given in the statistical analysis chapter of the methods section (page 10) and the actual GSR of the tested SNPs is given in table 2 of the results section (page 12).

Comment 5: Please, give a more detailed explanation on how the mean effect size was calculated.

Response: We agree that in order to let the reader understand the results fully, it is essential to give this explanation. After discussing this with the statistician, we now replace the term mean effect size from the previous submission with mean difference in pain score (β) in the revision, which is what we actually calculated. Explanation of the mean difference has now been given in statistical analysis chapter of the methods section along with the description of recessive genetic model (page 10).
Comment 6: Recessive analysis shows that heterozygote individuals benefited most from the interventions (surgical or conservative). This observation invalidates the hypothesis of a "dose-effect" of the COMT polymorphism. This is consistent with the lack of association found in the additive model that shows that, at least, the effect is not growing regularly.

Response: We totally agree with your comment regarding the results from recessive analysis, which are contrary to the dose effect. We have now used your guidance and have discussed this issue in the discussion section (page 17).

Comment 7: The reported standard deviations would be consistent with a skewed distribution. Please, repeat the main analysis on the median.

Response: We apologize that in accordance with the statistical programme we used (R-package Haplostats), it is not possible for us to perform the analysis as non parametric, as the programme computes the mean itself instead of the median. I will like to also inform you that the standard deviations look less skewed after the correction we made for the errors that occurred for two samples. We have now also tested the normality.

Comment 8: Please, give a more detailed explanation on how the VAS was used (experimental conditions) and what was measured exactly (current pain, pain over the last week, "overall" pain, ...). Was the VAS used in accordance with validated experimental conditions (please provide references)?

Response: In order to give a better understanding of the calculation of the outcome variable, we appreciate your pointing in this direction. We have now given the detailed explanation of measurement of VAS and other variables and covariates in the predictors and outcome variables chapter of the methods section along with the references (page 9).

Comment 9: "Painful experiences" can be influenced directly and indirectly at any given time by a great number of factors. Some are psychological, sociological or cultural, some others are physiological either non-genetically related like a lack of sleep or caffeine/alcohol/drug blood concentration or concomitant headache at the time of the experiment and some are genetically dependant like the activity of a relevant enzyme. Among all these factors the genetic impact is most likely marginal. In order to evaluate its contribution in a regression model, one would need to control for all the other factors (at least for the most important ones). Please, give more details on how these factors were taken into consideration and if not, please, justify.

Response: We highly appreciate the fact that you have discussed the covariates that influence the pain in such a detailed manner. We agree with the importance of such testing, which you have highlighted and therefore we have included in our regression analysis the covariates of age, gender, fusion, pain medication and psychological factor (anxiety and depression). Because of a small sample size, we could not test more covariates, which should rather be tested along with these in a larger study population. The results of our analysis are given in the results section (page 13 and table 3) and have also been discussed in the discussion section. The impact of SNP alone has also been reported and discussed.
Comment 10: Please, provide a more detailed description on how the haplotypes association analysis was done. How was the SNP dimension combined with the haplotypes?

Response: We have now given a detailed explanation of haplotype association analysis in the statistical analysis chapter of the methods section (page 11).

Comment 11: Please, justify the pooling of the operated on and non-operated on patients for this analysis?

Response: We have pooled patients in our analysis, with conduction of the main analysis with the treatment received as a covariate and sensitivity analysis with randomization as a covariate. We have no reason to believe that there is a selection bias according to the genotype, as genotypically both fusion and cognitive-exercise group were not different (p = 0.6).

Comment 12: And then please, provide an explanation on why the response of the GG individuals who were operated on is so different from the response of the non-operated GG's (while the "surgery effect" seems absent for the AA and the AG's) It seems that there could be an interaction between the groups (surgery vs conservative) and the genotypes...

Response: We agree with your observation of the difference in response of GG individuals in two groups. After correcting for errors in dataset and performing the analysis of covariates, we have not found an interaction between treatment and genotypes, rather the covariates of surgical fusion and pain medication were in interaction but the co-linearity was acceptable for entering pain medication in the analysis with fusion as a covariate. Fusion was found to be no more significant in the absence of this co factor. Twice as many fused patients were using pain medication (51 %) compared to the cognitive-exercise group (24 %) (Table1 page 8). In light of the new analysis, we have preferred to present the results from the whole group of 93 patients only, which has a better power compared to the two sub groups.

Comment 13: Therefore, please, give more details on the analysis of residuals. Was the normality tested?

Response: We appreciate your raising of this point. We are pleased to inform you that the analysis of the residuals was performed and normality was tested by QQ-plots and histograms and was found to be acceptable for regression analysis (statistical analysis chapter of methods section, page 10).

We highly appreciate your comments and advice, which has definitely improved our manuscript, and we hope that you find the revised manuscript to satisfactory and in line with your guidelines.

Best Regards

Dr Ahmad Omair, Prof Benedicte A Lie, Prof Olav Reikeras, Marit Holden, Dr Jens Ivar Brox
Dear Frank Skorpen,

We are thankful to you for your precious comments and guidelines for the revision of our manuscript. These comments were highly valuable and relevant and have helped us to improve our manuscript. We have responded to your comments and revised the manuscript accordingly.

Important information that we need to convey to you is that VAS LBP change values from 2 patients was calculated wrong (as -84 and 84 instead of the correct -8.4 and 8.4) by the programme we used. This error was evident from the data given in table 1 and table 5 of the previously submitted manuscript, regarding 7-11 year VAS LBP change. The baseline and 7-11 year VAS LBP values were correct for the two above mentioned samples, but the change was computed with error. We apologize that we did not detect this before initially submitting the manuscript. After we realized that the mean VAS change values in table 1 and mean VAS change values according to genotypes in table 5 were not really the subtracted values of the baseline and follow up VAS LBP scores, we scanned the whole dataset for any other errors and can now confidently say that there were no other mistakes beside those two. The whole analysis was performed again after this correction.

Below have we addressed your detailed comments, and described the changes we made in response to them.

**Major compulsory revisions**

**Comment 1:** There is no information on the use of analgesics or other medication that may modulate pain experience in these patients. Opioids, NSAIDs and also antidepressants are commonly prescribed drugs to patients with chronic LBP and are likely to impact both the patients’ general functioning as well as their pain report. If used by these patients, the authors should provide this important information and also include pain medication as a covariate in their analysis.

**Response:** We highly appreciate the fact that you have discussed the covariates that influence the pain in such a detailed manner. We agree with the importance of such testing, which you have highlighted and therefore we have now included in our regression analysis the important covariate of pain medication along with anxiety and depression, age, gender and fusion. Because of a small sample size, we could not test more covariates, which should rather be tested along with these in a larger study population. The results of our analysis are given in the results section (page 13 and table 3) and have also been discussed in the discussion section.

**Comment 2:** The authors report a significant association between post-treatment VAS LBP and the AA genotype of rs4680 in a recessive model (AA versus AG + GG), whereas no association was seen with neither ODI nor VAS when applying an additive model. On the other hand, when stratifying on rs4680 genotypes, the greatest improvement in VAS-LBP was seen with the heterozygous genotype (which from Figure 1 seems to be significant). Moreover, when analyzing the total cohort (93 patients) least improvement was seen with GG group, but when including the 60 fusion patients only, least improvement was seen with the AA genotype. These findings are difficult to interpret as the recessive model assumes that an
effect is detectable when two copies of an affected allele are present, which fits poorly with the data obtained after stratification on genotypes and after sub analysis. The authors should discuss these findings in the context of the assumed functional effect of the Val158Met polymorphism on COMT activity.

Response: We totally agree that our results after stratification according to genotypes do not fit the model and with what has previously been reported with regard to the genotypes of rs4680 and the effect on the enzyme function and pain sensitivity. We have now discussed this issue in the discussion section of the manuscript (first paragraph, page 17).

Minor Essential Revisions:

Comment 1: The associations do not stand correction for multiple testing, implying that the study is not sufficiently scaled to detect true associations. Thus, there is a possibility that the findings are merely false positives. It should be stated in the abstract that the p-values presented are not formally corrected for multiple testing and that the study is to be considered as explorative.

Response: We totally agree with your observations stated above. Therefore, in accordance with your guidelines, we have now included the mentioning of correction in abstract, statistical analysis chapter of methods section (page 10) as well as we have discussed this issue in the discussion section.

Comment 2: It is stated in the abstract that five polymorphisms in the COMT gene were genotyped. However, as one of these (rs2097603) were not analyzed further due to genotyping failure it sounds better to say that four SNPs in the COMT gene were successfully genotyped.

Response: Thanks for bringing our attention to this point. We agree with you and have now changed the abstract accordingly.

Typographical errors:

1. COMT should not be in italics when referring to the enzyme. E.g. page 4 line 7 from the bottom, page 8 lines 11, 12, 13.
2. Table 2: GRS, abbreviation for genotyping success rate should probably be GSR.

Response: We apologize for not detecting these typographical errors earlier. We have now corrected them as pointed out by you.

Discretionary Revisions

The mean VAS-LBP change for the rs4680 GG group of the total cohort was -12.9, and - 29.2 for the fusion patients only - which is a huge difference and indicates major differences in VAS change between the two treatment groups. In order to understand the background for
these differences, it would be informative to have the characteristics for the patients receiving cognitive intervention and surgery presented separately.

Response: We have now presented the characteristics of the two groups separately (Table 1, page 8).

Once again we highly appreciate your comments, advice, guidance and review of our manuscript, which has surely improved the quality of our paper.

Best Regards,

Dr Ahmad Omair, Prof Benedicte A Lie, Prof Olav Reikeras, Marit Holden, Dr Jens Ivar Brox