Author's response to reviews

Title: Treatment outcome of chronic low back pain and radiographic lumbar disc degeneration are associated with inflammatory and matrix degrading gene variants: a prospective genetic association study.

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Version: 2 Date: 4 March 2013

Author's response to reviews: see over
COVER LETTER REGARDING MODIFICATION OF MANUSCRIPT

MS-1081240955758080

EDITOR:

Dear Editor,

We thank you for conveying us the comments of the reviewers, which will help us improve our manuscript. The comments are quite valid, and we believe that they have helped us improve our manuscript. We have now modified the manuscript and are responding to the review comments you have conveyed us and hope that you will find the modifications to satisfactory.

Best Regards,

Dr Ahmad Omair

REFEREE-1:

Dear Minna Mannikko,

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.

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

Major Compulsory Revisions

Comment-1: The significance of the findings should be discussed more, since only p-values are provided (OR? CI?) and no corrections have been made for multiple testing. Although the authors do bring this out in the methods (“We considered our study to be an explorative genetic association study, and therefore the p-values were not formally corrected for multiple testing”), in interpreting the results, the possibility of a chance finding should be considered. Correcting p-values has reduced the number of false positive findings and improved the possibilities for replication. Nevertheless, here the authors were able to replicate some earlier findings, but also detected association that they found difficult to explain.

Response:

We agree that despite the fact that we replicated the previously reported associations, our sample size and lack of multiple testing may make our results false positive or by chance. We have now discussed the results in the proposed way and have made changes to the discussion section of the manuscript (Last paragraph, page 16 and 3rd paragraph Page 19).
Similarly we have now presented the confidence intervals for the mean difference for ODI and VAS change for individual SNPs as well as for haplotype association analysis (Table 2 on Page 13 and last paragraph on page 15). We have now also presented the Odds Ratio and Confidence interval for the associations reported with severity of degeneration and number of discs involved. (Last paragraph on page 14).

Comment-2: The authors speculate on that different variants in IL18RAP would have an effect on different aspects in the DD phenotype (severity of the degeneration, improvement in outcome). This may well be, but are there any functional data on these variants to support this or is the current data only suggesting the involvement of this gene/gene product in general?

Response:

Thank you so much for raising this important point regarding functional data on IL18RAP SNP, as SNPs from the same gene have shown association with different LDD phenotypes. Most of the data on IL18RAP gene variants is association data and only functional information that indirectly supports our view point is the report of differential expression of IL18RAP gene by the SNP rs917997 in coeliac disease patients (Association study of the IL18RAP locus in three European populations with coeliac disease. Koskinen LL et al, Hum Mol Genet. 2009 Mar 15;18(6):1148-55), which in our study was associated with treatment outcome. This information has now been incorporated in the discussion section of the manuscript (Last line, Page 17).

Minor issues not for publication

We thank you for pointing out these minor mistakes as well which will make the manuscript even better.

1- In the abstract (results) remove italics from “and” that is between IL18R and IL1A.

Response: Italics removed

2- In Statistical analysis, 2nd paragraph, 4th line from the bottom: carriers.

Response: Corrected

3- In Statistical analysis, 3rd paragraph, 3rd line: Reference for R is missing.

Response: Reference added.

4- In table 3, IL18RAP-column, rs is missing in front of the number (rs917997).

Response: We have now added “rs” to SNP number.

5- In the discussion, 3rd paragraph, first line, add rs to rs1420100.

Response: “rs” added to 1420100.
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 Lisbet Haglund,

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 responded to your comments and revised the manuscript accordingly.

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

**Major essential revisions**

**Comment 1:** *It is unclear how the comparison of DNA purification from blood and saliva is relevant to the study and cannot be presented as a result?*

**Response:**

Thank you for raising this point. We agree that the comparison of blood and saliva DNA purification is not directly related to our hypothesis and therefore we have not presented this along with our main results. But we believe that indirectly this is valuable, as the quality of DNA influences the genotyping results. Our motivation to add this small methodological section in this manuscript was the fact that genetic studies require large number of patients and acquiring DNA from blood is more invasive and difficult with regard to patient compliance, storage of sample etc, compared to saliva. So we have presented that we can increase the patient numbers and make long distance genetic association studies more practical by the use of saliva as a source of DNA. We have reported an equally pure DNA and a comparable genotype success rate.

**Comment 2:** *The authors should simple state in what case the analyses were performed on blood and saliva respectively.*

**Response:**

We agree that we should have elaborated our genotyping analyses performed on blood and saliva. For 48 patients, DNA from blood as well as saliva was genotyped together, and genotype success rate of DNA from both sources was comparable for the tested SNPs. We have now added this information to the DNA extraction part of the Methods section (Page 9).

**Comment 3:** *How can IL1 directly activate enzymes? To my knowledge, IL1 activates the production of cytokines and proteases leading to tissue destruction and pain but doesn’t have the capacity to by itself activate the pro form of the proteases. Please clarify.*

**Response:** We are thankful to you for pointing this out. We totally agree with you that IL-1 can activate the production of cytokines and proteases that can lead to tissue destruction. We had a similar understanding but probably failed to give clear wording to this concept. We have now corrected this information, given in the discussion section of the manuscript (2nd paragraph on Page 17).
Comment 4: It is stated that the novelty of the study is that it highlights the influence of the studied genes in patient treated surgically or by cognitive interventions but not clear what the difference was if any between the two treatments and if there was a link to a specific polymorphism? Please clarify

Response:

Your point is pretty valid that the influence of the different treatment regimen must be clarified. We will like to clarify that our sample of 93 patients were from two groups, i.e. surgically treated and cognitively treated patients. Because of a small patient sample, we did not perform analysis to highlight the influence of a specific treatment on the association. On the other hand the novelty of this study is the testing of inflammatory genes not only with radiographic degeneration, but with treatment outcome as well.

Comment 5: It is also unclear how patients were assigned to treatment option? Please clarify.

Response:

We will like to clarify that our patient cohort was originally randomized to two treatment options 7-11 years ago. 51 were randomized to lumbar instrumented postero-lateral fusion with transpedicular screws at L4-L5 and/or L5-S1 and 42 to cognitive intervention and exercises. Later on there were cross over and 19 patients did not receive the treatment they were randomized to i.e. 5 patients randomized to fusion did not undergo surgery and 14 patients randomized to cognitive treatment had later undergone fusion. This information is provided in the patient sample part of the Methods section (Page 6 and 7)

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