Author's response to reviews

Title: Allelic expression analysis of the osteoarthritis susceptibility gene COL11A1 in human joint tissues

Authors:

Emma V A Raine (e.v.a.raine@ncl.ac.uk)
Andrew W Dodd (a.w.dodd@ncl.ac.uk)
Louise N Reynard (louise.reynard@ncl.ac.uk)
John Loughlin (john.loughlin@ncl.ac.uk)

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Author's response to reviews: see over
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Mr. Reynaldo Aldea Jr.
BMC Musculoskeletal Disorders

Dear Sir,

**MS: 1653055969807898 – Allelic expression analysis of the osteoarthritis susceptibility gene COL11A1 in human joint tissues**

Thank you for providing us with the opportunity to revise our manuscript in response to the reviewer’s comments.

As requested, we provide a point-by-point response below.

We thank the reviewers for their comments and we hope that it is now considered suitable for publication in BMC Musculoskeletal Disorders.

Yours sincerely,

Emma Raine, on behalf of all the authors.

**Reviewer: Nils Bömer**

No further requests for changes

**Reviewer: Antonio Gonzalez**

*Maj or poin t of c oncern:*
As already commented, the technical reliability of the assay for testing AEI with rs9659030 is questionable. Discrepancy between the findings with rs1676486 and with rs9659030 put results obtained with rs9659030 in question. Results with rs1676486 are not in question because they are supported by the Mori et al. paper. Once the authors have shown that alternative coding SNPs are not
available, it still would be possible to repeat analysis of rs9659030 with a different test. That is, rs9659030 could be assessed with different primers and probes or with different technology (as single base extension, in which the authors have experience). Assessment of three or four discrepant samples with the new test will be enough to see if the results are confirmed or not.

Author’s response:
We mentioned in the Materials and Methods section of our original manuscript and in our subsequent revision that we subject the AEI assays that we use to QC checks to confirm that the assays function appropriately. We did not however provide that data as we thought it unnecessary, instead writing “data not shown”. We understand the reviewers concerns but we wish to assure him that we have checked the rs9659030 AEI assay to ensure its providence. The assay is a very good one and to convince the reviewer we have now provided in the 2nd revision the QC data that we generated for the assay prior to its use. The relevant text can be found in the Materials and Methods in a new subheading, “Quality control checks on the AEI assays” and in the final paragraph of the “AEI analysis” section of the results. These new sections of text are highlighted in red. We have also created a new figure, Supplementary Figure 1. As is clear from the figure and the accompanying text, the assay is able to accurately discriminate between and measure differences in the levels of the two alleles of rs9659030. It is a very reliable assay and it is not a deficiency in it that is responsible for the varying levels of AEI along the COL11A1 transcript. Instead, and as we suggest, it appears that this gene is subject to differential levels of AEI relative to the physical position at which the AEI is measured - what we have seen is real.

Minor point of concern:
One of the revised sentences in page 13 seems incomplete: “However, the fact that AEI in mature cartilage as measured with rs1676486 is not an OA risk factor makes it hard to understand why the OA risk marked by rs2615977 would be operating via in this tissue”

Author’s response:
We have re-phrased this sentence, and the one preceding it, to express better the point that we are trying to make.

Minor point of concern:
Two similar sentences in the abstract and discussion seem confusing: “These two diseases therefore share a common functional pathophysiology, namely AEI of COL11A1, but this appears to impact on disease risk only in LDH” and “Overall our study has identified that COL11A1 AEI is a common functional pathophysiology between LDH and OA, but that it is not a risk factor for OA when measured in cartilage from mature adults” Would it be more clear to say that the two tissues, spinal disc and articular cartilage, share AEI of COL11A1, but it is involved in disease risk only in LDH, not in OA?

Author’s response:
We have re-phrased these two sentences in line with the reviewer’s suggestion.