Reviewer's report

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

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Reviewer: Antonio Gonzalez

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Review:

The manuscript by Emma VA Raine, Andrew W Dodd and colleagues seeks to identify functional clues explaining the association of genetic variation in COL11A1 with OA susceptibility. Two SNPs were selected for analysis: rs2615977, the top hit in the study identifying this association with OA, and rs1676486, the SNP that was reported as causal for risk to Lumbar Disk Herniation in a previous Japanese study. A decreased level of COL11A1 mRNA with the T allele of the latter is confirmed in articular cartilage, but this SNP is not convincingly associated with OA. On the contrary, the alleles of rs2615977 did not show correlation with the levels of COL11A1 RNA. The authors conclude that OA seems to be indifferent to changes in COL11A1 RNA levels unless they take place at tissues or times not yet explored. Overall, this is an original study addressing an interesting issue with powerful tools and analysis. The work is well described and the conclusions are congruent with the results observed. However, there is a point that limits the value of the conclusion in its current form. It is described in the next paragraph followed for other minor comments:

Major point of concern:

1) Analysis of association of rs2615977 with allele expression imbalance (AEI) is done with rs9659030. The test is not reliable because it does not show the AEI observed with rs1676486. Therefore, the lack of association of rs2615977 with changes in COL11A1 RNA needs to be confirmed with a different test for this SNP or probably better with other coding SNP. At first sight there are three possibly informative SNPs in dbSNP: rs1763347, rs2229783 and rs3753841.

Minor points of concern:

1) There is reference to the work of Mio et al. as describing variation in COL11A1 RNA stability along the length of the gene. I did not find any mention to this variation in the referenced paper.

2) I do not understand the logic behind the analysis reported in figure 5. It seems as if the author’s intent is to show that genotypes of rs9659030 are correlated with expression of COL11A1 RNA. However, this is irrelevant. It will be relevant to check if genotypes of rs2615977 were correlated, but I fear there were too few informative subjects to do the analysis.

3) Also in figure 5, allele expression ratios are represented but the level
corresponding to equal expression is given value = 0. For a ratio, equal expression has value = 1 as in figures 3 and 4.

Suggestions:

4) The use of three reference genes for qPCR is to be commended, but the method of analysis with the mean Ct of the three is not the best possible one. I suggest the authors using other methods like the implemented in software like http://www.gene-quantification.de/download.html#qbase and http://www.gene-quantification.de/rest-2009.html

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

Disclaimer: I collaborate frequently with Prof. John Loughlin in studies of OA genetics.