Author's response to reviews

Title: Allelic expression analysis of the osteoarthritis susceptibility locus that maps to chromosome 3p21 reveals cis-acting eQTLs at GNL3 and SPCS1

Authors:

Fiona Gee (fiona.gee@ncl.ac.uk)
Clare F Clubbs (clare.clubbs@gmail.com)
Emma V A Raine (e.v.a.raine@newcastle.ac.uk)
Louise N Reynard (louise.reynard@newcastle.ac.uk)
John Loughlin (john.loughlin@newcastle.ac.uk)

Version: 2
Date: 16 April 2014

Author's response to reviews: see over
Tim Sands  
Executive Editor  
BMC Medical Genetics

Dear Sir,

We are pleased to submit our revised manuscript, MS: 1506466972119239; “Allelic expression analysis of the osteoarthritis susceptibility locus that maps to chromosome 3p21 reveals cis-acting eQTLs at GNL3 and SPCS1” for your consideration.

We thank the reviewers for their highly constructive comments and suggestions and we have revised our manuscript accordingly. This has entailed editing and adding new text. We believe our manuscript is much improved and we hope that it is now considered suitable for publication in BMC Medical Genetics.

Below we have listed each of the reviewer’s comments and provided our detailed response.

**Francisco J Blanco’s comments:**

*Reviewer’s comment: 1 – Despite the association signal was previously detected in both knee and hip OA, did the authors perform the analysis separating knee and hip samples?*
*Author’s response: For those genes that demonstrate AEI, both the hip and knee samples analysed show a similar range of AEI values.*
*Author’s action: Text has been added to clarify that in cartilage samples, similar levels of AEI were observed in both joint types, and that all analysis in other tissues was performed using knee tissue.*

*Reviewer’s comment: 2 – Would the authors expect to obtain the same results if they had analysed normal healthy samples?*
*Author’s response: Yes - the allelic expression imbalance observed for GNL3 and SPCS1 would be expected to act in all individuals, with the presence of the minor allele of rs6976 leading to lower expression of each gene. However, those individuals in possession of two copies of the minor allele of rs6976 would experience an increased risk of developing OA due to lower levels of each gene*
transcript. A healthy individual may therefore carry copies of the risk allele but will not develop the
disease due to the absence of risk alleles at other loci; the standard polygenic model.

Author's action: An explanation of this point has been added to the discussion.

Reviewer's comment: 3 – The selection of “outside but physically close to 3p21 locus” gene POC1A
should preferably be described in the methods section.

Author's response: We tried to reconfigure the text in the manner that the reviewer advised but we
felt that the flow of the manuscript was negatively impacted by moving the information from the
results section to the methods section. We would prefer therefore to leave the text as it was
originally configured.

Author's action: We have left the text unaltered.

Reviewer's comment: 4 – The SNP rs11177 appears as (G/A) in the text and as (C/T) in additional
file 2.

Author's response: This was our typographical error and we thank the reviewer for pointing this out
to us.

Author's action: This has been amended so that the text now matches the additional file and figures 1 and 2.

Reviewer's comment: 5a – Why did the authors select the transcript SNPs in NT5DC2 and POC1A to
test their association with OA?

Author's response: NT5DC2 and POC1A displayed significant AEI acting in the same direction for the
majority of patients. However, this AEI did not correlate with genotype at rs6976, which is what
would be expected if rs6976 (or a SNP in very high LD with it) was the eQTL causing the AEI. This
implies that NT5DC2 and POC1A are subject to eQTLs acting independently of the association SNP
rs6976. As the transcript SNPs within NT5DC2 and POC1A appear to be subject to AEI that does not
correlate with the genotype at rs6976, we assessed whether the eQTLs responsible for this AEI
could also confer an altered risk of OA. We did this by assessing whether either transcript SNP
showed association with OA and we did this using data from the powerful arcOGEN GWAS.

Author's action: The manuscript has been amended to clarify this point.

Reviewer's comment: 5b – I suggest to write both D’ and r^2 values between rs7639267 and
rs10105543 as well as between rs747343 and rs4687805 in the methods section.

Author's response: We apologise for this omission.

Author's action: This information has now been added to the methods section as suggested.

Reviewer's comment: 6 – Why did the authors analyse only 17-23 OA patients?

Author's response: In the study we analysed a total of 64 patients. For each transcript SNP we
identified from among these 64 individuals those who were heterozygous for that SNP and we then
used these samples for allelic expression analysis. The number of samples analysed was dictated
therefore by the number of heterozygotes and then by the amount of material (tissue, RNA and
DNA) available.

Author's action: We have rewritten the text to clarify this point to the reader.

Reviewer's comment: 7 – A brief explanation of AEI must be described.

Author's response: We agree with the reviewer.

Author's action: An explanation of AEI has been added to the methods section.

Ingrid Meulenbelt's comments:
Reviewer’s comment: Could the authors indicate the level of expression of the 2 genes for example was expression of the genes within the highest quartile?
Author’s response: We agree that this would be of value to the reader.
Author’s action: We have now added relevant text to the third paragraph of the results.

Reviewer’s comment: Could the authors indicate whether the genes were responsive to the OA disease process in previous generated expression datasets of healthy versus osteoarthritic cartilage?
Author’s response: We agree that this would be of value to the reader.
Author’s action: We have now added relevant text to the third paragraph of the discussion.

Reviewer’s comment: It would be informative to elaborate a little on the possible hypothesis how these genes could confer risk to OA due to reduced expression?
Author’s response: We agree with the reviewer.
Author’s action: The conclusions have been expanded to include this point.

Reviewer’s comment: What would the authors suggest as next steps with respect to the indicated “detailed follow up investigations”?
Author’s response: We agree with the reviewer that the next steps should be discussed.
Author’s action: A brief discussion has been added to the conclusions.

We believe that the revisions detailed above have addressed all the suggestions of the reviewers and hope that you consider the revised manuscript suitable for publication in *BMC Medical Genetics*.

Yours sincerely,

Dr Fiona Gee, PhD