Author's response to reviews

Title: Allelic variants of IL1R1 gene associate with severe hand osteoarthritis

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Author's response to reviews: see over
Dear Editor,

We would like to thank the editors and the reviewers for the thorough review and valuable comments concerning our manuscript “Allelic variants of IL1R1 gene associate with severe hand osteoarthritis” (MS: 1200836953248454). The modifications have clearly improved the manuscript and we sincerely apologize for the delays in the resubmission.

After the original submission we have participated in a collaborative effort, in which several SNPs in the IL1R1 and IL1RL2 were analyzed in a population based cohort of 295 dentists and 248 teachers with radiographical evaluation of both hands (Solovieva et al, The Journal of Rheumatology, in press). The single SNPs in the IL1R1 gene did not associate with bilateral DIP OA, although some association was observed with haplotype covering IL1R1 and IL1RL2. There were only less than 30 severe bilateral DIP OA cases among the study subjects, thus this more severe phenotype could not be used in the analysis. Also the study set was underpowered to detect an association with the most probably over-estimated effect size observed in the family based hand OA material. Although this is not a true validation of the observed association, we have now added this information to the Discussion of the manuscript.

We further attempted to validate the results for SNPs rs3917225 and rs2287047 in IL1R1 gene in a larger set of Icelandic severe clinician diagnosed hand OA cases (n=1,676) and controls (n=32,470) genotyped using the genome-wide Illumina 317/370 chip. Some evidence towards association was detected between rs3917225 and severe hand osteoarthritis (p=0.043 males and females combined; p=0.033 females only) in the IL1R1 locus. However, the associated allele differed from the Finnish sample. Interestingly, the frequency of the ancestral A allele varies in different populations (Utah residents with Northern and Western European ancestry from the CEPH collection 0.496; Han Chinese in Beijing, China 0.601; Yoruba in Ibadan, Nigeria 0.969; Italian 0.4583, French0.6071, Han 0.4559; according to HapMap and UCSC data bases) with a significant difference also between Finland and other populations of European ancestry, including Icelandic population. A thorough analysis of the allelic haplotype structure in the studied area and an equivalent radiological phenotype would be needed for true replication effort, which cannot be accomplished in a near future, thus we did not include these findings to the current manuscript. We are however continuing this collaboration to obtain a more clear picture of the role of IL1R1 in severe OA in the future.

Please find below the detailed replies to the comments of the reviewers.

Sincerely,
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Reviewer: Ingrid Meulenbelt

Minor Essential Revisions

1. Page 10 line 17, it is very difficult to interpreter the linkage disequilibrium with the R2 measure as it indicates the redundancies of the SNPs. Please provide D’ as measure of LD.

Our reply: In order to give both perspectives of LD structure of the area, both D’ and r2 measures of LD are now provided. We further performed additional haplotype analysis based on SNP selection by D’.

2. Page 11 second paragraph it is stated in the first line of the second paragraph that allelic association is performed. Yet in the final sentence it is stated that a carrier risk is shown? This is confusing. It is also not completely clear to me what reference genotype is used (22 or 12).

Our response: We have now clarified this chapter by referring to the haplotypes as “3” and “5”, and all other haplotypes “X” and explained more clearly why we were only able to calculate the carrier risk because no homozygotes for the associated alleles were observed. The Haploview program was used for initial calculation of the haplotype association (p-values) and the Phase2 program was used to estimate the individual haplotypes to be able to calculate ORs for those haplotypes that showed association in the Haploview analysis.

3. The rationale of the haplotype analyses is not stated. It could either be a) SNP rs1465325 is the most important SNP since this SNP is tagging the haplotype. Then it would be worthwhile/logical to mention the genotypic OR of this SNP or b) The haplotype analyses actually break downs the effect of the SNP rs2287047 towards haplotype 3 and 5 (haplotype 5 is also showing a protective effect). In this case it would be more likely to provide the OR of rs2287047. Given the fact that the association of rs2287047 alone has the lowest P-value, option b may be most likely as recognized by the authors only mentioning the effect of rs2287047 in the abstract?

Our response: This was a good point and discussed now in the text. The odds ratio for SNP rs1465325 tagging the protective haplotype 3 is also now given.

4. There is quite a difference between the P-value of the Chi2 test en the Pseudomarker program. Could it be that there is one particular family contributing to the observed association?

Our response: The predisposing allele was the major allele of the SNP rs2287047 and the studied families were maximum seven genotyped individuals in size, so several families contributed to the observed association. We hypothesise that rather the reduced amount of individuals in chi2 test lowers the power to detect the association since only unrelated individuals were included.
5. *Table 3* Please provide rs numbers and order of the SNPs making the haplotypes.

Our reply: rs numbers are now provided in the heading of Table 3 for proper interpretation.

**Discretionary Revisions**

6. *Please discuss the fact that the minor haplotype (TAAAG) shows (if anything) a protective effect in hand OA cases whereas a predisposing effect in knee.*

Our response: This is a good point, but as suggested also by the reviewer, the association of the predisposing allele in knee OA is only marginal. We have now discussed this in the text.
Reviewer: Danielle Posthuma

1. The sample size is relatively small compared to similar studies with other phenotypes, resulting in low power in some cases (see also p. 10). This small sample size is probably due to the nature of the phenotype. Increasing the sample size in due time may not be an option. However, searching for replication samples might be an option. Replication will certainly add to confirming this gene's importance in OA.

As suggested by the reviewer, increasing the sample size in due time is not feasible. Thus, we made an attempt to identify sample sets for replication of the results, but were not able to identify any with a matching phenotype. However, we participated in a collaborative effort, in which several SNPs in the IL1R1 and IL1RL2 genes were analyzed in a population based cohort of 295 dentists and 248 teachers with radiographical evaluation of both hands (Solovieva et al, The Journal of Rheumatology, in press). The single SNPs in the IL1R1 gene did not associate with bilateral DIP OA, although some association was observed with haplotype covering IL1R1 and IL1RL2. There were only less than 30 severe bilateral DIP OA cases among the study subjects, thus this more severe phenotype could not be used in the analysis. Also the study set was underpowered to detect an association with the most probably over-estimated effect size observed in the family based hand OA material. Although this is not a true validation of the observed association, we have now added this information to the Discussion of the manuscript. We also initiated a collaboration with an Icelandic group with a large cohort of severe clinician diagnosed hand OA cases (n=1,676) and controls (n=32,470), but the currently available phenotype did not match the one utilized here. An initial analysis with the non-matching phenotype provided some evidence for this locus, but the allele frequencies of the SNPs utilized in the analysis differed significantly between populations, even in populations of European origin, thus a more thorough analysis utilizing several tagging SNPs within this area would have been required for reliable estimation of the significance of this locus. That we cannot complete in due time and we think that publishing the results now will give independent laboratories the possibility to replicate the results.

2. As the most significant association is with a SNP in the intron of the IL1R1 gene, this study is not directly informative to the possible functional impact of this gene on OA. I would suggest to add a second step in this study in which functional SNPs (if known) are also included.

Our response: We agree. As mentioned in the Discussion, three of the associated SNPs are located in the promoter region of the IL1R1 gene, and may have an effect in the binding of transcription factors and thus the expression activity of the gene. Further evaluation of the TF binding efficiency is however out of the scope of this manuscript. We are also currently conducting a deep-sequencing of the entire associated area of the IL1R1 gene including the large promoter region. This will allow us to identify also the putative family-specific variants, but cannot be completed in the time frame of this manuscript.

Minor
3. In the methods section it is stated that HWE and Mendelian errors were checked - please provide details (i.e. how many Mendelian errors etc).

Our response: We tried to add more details on quality control within the limits of practical manuscript length. We have not provided full details of HW for every SNP etc. but rather a description of main definitions of policy followed in genotyping and quality control.

4. In some sentences a couple of words seem to be missing or are misplaced, for example in the abstract, last sentence, before 'known' you probably want to add 'a'; in the introduction, last sentence of 1st para: "There is a strong genetic effects on hand and knee OA heritability..." should be "There is a strong genetic effect on hand and knee OA, with heritability..." I have not collected all instances, but please read the paper critically

Our response: We have done additional proofreading of the manuscript in order to improve the grammatical quality.
Reviewer: Dongquan Shi

Discretionary Revisions
Cite more new papers about IL1 and OA research.

We added a citation (Solovieva et al. 2009) that is a new study on DIP OA in Finnish study subjects and IL1 gene family. Additionally, five new publications are mentioned in the Discussion section (page 14).

- Minor Essential Revisions
  1) page 5, line 3, “IL1RI” should be “IL1R1”;
  2) page 14, line 7, “IL1R1L” should be “IL1R1”.

Our response: We thank for pointing these out, they are now corrected in the text.

- Major Compulsory Revisions
  1) “28 patients had physician-diagnosed hand OA and were also included in the hand OA material to increase statistical power. “.Systematic OA is different from local site OA. Is it a good way to increase the statistical power?

Our response: This is a relevant issue and we considered this during the project. However, we decided to keep the patients affected by knee and hand OA in the hand OA material, since the level of hand OA in these patients was severe. Excluding patients because they are suffering from OA of another joint would be controversial. Otherwise we should have excluded all original hand OA family patients suffering from any other additional OA type, which is not a common practise in OA studies to our knowledge and we did not have the data of OA other sites. We want to emphasize that biologically, it does not matter which joint is the one used for the initial selection for this particular this study. We do agree that this part of the hand OA material is not as strong as the main part of our hand OA material, but we considered that it would still be better to have this part included than excluded from the hand OA material.

2) Due to the limited sample size, we did not analyse males and females separately. It will be better to analyse the males and females separately. As, the author said “based on a Finnish study, the genetic effects seem to be more prominent in females. Is it possible to amplify the sample number? It will be more powerful.

This was a good point and we performed the SNP by SNP association analysis using only females. Hand OA results were similar to the original findings. We also saw association between knee OA and SNPs located in the same D’ LD block than the original finding. However, the amount of male cases is too small to draw any conclusions. These results are now discussed in the manuscript.
3) It would be better to collaborate other center to do replication study. Then it must be more powerful.

Our response: We agree that replication would confirm the results. Since this is an important point, we also mentioned these shortcomings in the discussion and analyzed the most important finding in another sample set. Please see reply to Reviewer 2. Publishing the results now will give independent laboratories the possibility to replicate the results.