Author’s response to reviews

Title: Common genetic variation in the Estrogen Receptor Beta (ESR2) gene and osteoarthritis: results of a meta-analysis

Authors:

Hanneke JM Kerkhof (j.m.kerkhof@erasmusmc.nl)
Ingrid Meulenbelt (i.meulenbelt@lumc.nl)
Andrew Carr (Andrew.Carr@ndorms.ox.ac.uk)
Antonio Gonzalez (Antonio.Gonzalez.Martinez.Pedrayo@sergas.es)
Deborah Hart (deborah.hart@kcl.ac.uk)
Albert Hofman (a.hofman@erasmusmc.nl)
Margreet Kloppenburg (g.kloppenburg@lumc.nl)
Nancy E Lane (nancy.lane@ucdmc.ucdavis.edu)
John Loughlin (John.Loughlin@ncl.ac.uk)
Michael C Nevitt (M.Nevitt@psg.ucsf.edu)
Huibert AP Pols (h.pols@erasmusmc.nl)
Fernando Rivadeneira (f.rivadeneira@erasmusmc.nl)
Eline P Slagboom (P.Slagboom@lumc.nl)
Tim D Spector (tim.spector@kcl.ac.uk)
Lisette Stolk (l.stolk@erasmusmc.nl)
Aspasia Tsezou (atsezou@med.uth.gr)
André G Uitterlinden (a.g.uitterlinden@erasmusmc.nl)
Ana M Valdes (ana.valdes@kcl.ac.uk)
Joyce BJ van Meurs (j.vanmeurs@erasmusmc.nl)

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Author's response to reviews: see over
Dear Dr Harold,

We hereby submit the revision of our manuscript “Common genetic variation in the Estrogen Receptor Beta (ESR2) gene and osteoarthritis: results of a meta-analysis”.

We would like to thank the reviewers and editor for their constructive comments on our manuscript. We have responded to all questions/suggestions and have clarified topics which were not clear to the reviewers without lengthening the manuscript, which we believe has strengthened the manuscript. Please find below our response to the reviewers. In addition, we have changed the manuscript according to the BMC medical genetics format (references, additional files).

We look forward to hearing from you in due course.

On behalf of all co-authors,
With kind regards,

Hanneke J.M. Kerkhof, MD
PhD Student
Genetic Laboratory
Department of Internal Medicine
Erasmus MC, Rotterdam
The Netherlands
Editor’s comments:

1) In addition to this, we also request that you make sure that your study adheres to the PRISMA guidelines for the reporting of Meta-Analyses, details of which can be found here: http://www.prisma-statement.org/.

Author’s response: Please note that in this paper only novel data is included in this prospective meta-analysis as there are no studies published yet on this topic (with this specific genetic variation and this OA phenotype). Therefore, most of the PRISMA guidelines (e.g. the flow diagram) do not apply to the current manuscript. In accordance with PRISMA guidelines we have added additional information on the selection of study populations on page 6, lines 5-11.

2) Specifically, we would like you to elaborate upon why you used these particular studies for the replication meta-analysis and whether you carried out a literature search to identify all relevant studies in the field. If so, you should provide details; if not, you should provide some justification.

Author’s response: we have searched the literature for other studies on OA and genetic variation in the ESR2 gene. Only 1 study as found in Caucasians and we have discussed this paper in the discussion section. Because different genetic variants which cannot be combined with the variants studied in this paper were tested in that study, we could not add this data to out meta-analysis. We have added more information on the selection of studies in the methods section (page 6, lines 5-11).

3) We would also like some clarification concerning any ethical approval sought for the study, including the name of the approving committee.

Author’s response: information on ethical approval is already given for each study in the supplementary material. In order to improve clarity these statements were moved to the main text on pages 6-7, lines 15-16, 19-20, 22-2, 5-7, 9-10, 11-13 and 15-17.

4) Finally, we would be grateful if you could alter the format of the manuscript abstract to include a separated background and methods section.

Author’s action: the abstract has been changed to comply with this request.

Reviewer 1: Young Ho Lee.

Reviewer’s report:

1) I recommend that authors make result tables.
   Author’s response: We have replaced the table with results of the discovery study (the Rotterdam Study) to the main text, and have replaced the table with allele and genotype frequencies for hip OA cases and controls in each study of the meta-analysis to the main text. We will leave it up to the editor to decide whether this is desirable.

2) I think it is needed to do subgroup analysis based on HWE.
   Author’s response: We acknowledge that HWE-deviations should be carefully considered in all genetic association studies. However, it is our view that subgroup analysis based on HWE would not add to the content of the manuscript in any substantial way. There is only 1 SNP (rs1256064) in 1 study which deviates slightly from HWE (p=0.04), although one could also argue it does not deviate from HWE depending on the stringency of p-value thresholds. This SNP was not analyzed in any of the replication studies. More importantly, we do not find an association with this SNP, genotyping accuracy was checked and was good and the allele frequencies for
this SNP are similar to the frequencies reported in HapMap for Caucasians. The SNP which was investigated for replication was in HWE in all cohorts studied. We therefore do not see the need to do a subgroup analysis.

3) Authors did allele analysis, but further genotype analysis will be valuable.
   Author’s response: in the Rotterdam Study, dominant and recessive models were explored, but did not yield any significant associations. We have added this to the manuscript (page 10-11, lines 21-2). Because there were no associations in the Rotterdam Study, we also did not explore this further in any of the other (smaller) studies.

Reviewer 2: Inga Peter

Reviewer’s report:

1) The authors do not state clearly what was meta-analyzed. Are these adjusted logistic regression coefficients? Justification for using random versus fixed effect models as well as estimation of heterogeneity should be moved from Supplemental Material to the main text.
   Author’s response: We appreciate the importance of clearly explaining the methods used therefore we mention in the methods section that meta-analysis was performed using odds ratios and 95% confidence intervals of each study for the rs1256031 SNP and hip OA (page 9, lines 14-15). The justification for random versus fixed effects has now been moved to the main text as suggested (page 9, lines 16-18).

2) No discussion has been provided with regard to the consistency of the present findings with GWAS of osteoarthritis or related phenotypes. Have there been any signals in the ESR2 region identified by GWAS.
   Author’s response: Common genetic variation in the ESR2 gene has not been found associated with OA in a recent GWAS meta-analysis of the TREAT-OA consortium (Evangelou et al. in press, Kerkhof et al. 2010) and is also not associated to other bone-related traits like bone mineral density (Koller et al. 2010, Kung et al. 2010, Guo et al. 2010, Cho et al. 2010, Rivadeneira et al. 2009). This has been added to the discussion on pages 12-13, lines 21-4.

3) Even though it is a negative study, given its significant associations identified in the original cohort in women only, it is suggested to assess rs1256031*gender interaction.
   Author’s response: In the meta-analyses we stratified for gender to assess rs1250631*gender interaction. As shown in Figure 1 the direction and the effect sizes in men and women are similar. This aspect indicates that it is unlikely that interaction is present between the SNP and gender. Although, this observation does not rule out a very subtle difference in the association between males and females and thus SNP*gender interaction, but in the current study power is lacking to robustly assess such small effects (Ref: Patsopoulos et al. JAMA 2007). Furthermore, given the fact that we report a negative finding, adding such analyses may be considered as over interpretation of our data. We have now added to the discussion that given the reasoning mentioned above we consider gender*SNP interaction unlikely (page 12, lines 11-14).