Author's response to reviews

Title: Suggestive evidence of associations between liver X receptor beta polymorphisms with type 2 diabetes mellitus and obesity in three cohort studies: HUNT 2 (Norway), MONICA (France) and HELENA (Europe)

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Version: 2 Date: 2 July 2010

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

Thank you very much for your interest and for the thorough revision of our manuscript.

We mostly agree with the reviewers’ comments and have revised our manuscript in light of their comments. Below you will find our point-to-point response to their concerns. Please find enclosed our revised manuscript re-titled “Suggestive evidence for associations between liver X receptor β polymorphisms with type 2 diabetes mellitus and obesity in three cohort studies: HUNT 2 (Norway), MONICA (France) and HELENA (Europe)”. Our changes are highlighted in red throughout the revised manuscript.

With respect to the need of performing correction for multiple testing, we perfectly understand the reviewers’ comments. As we stated in our reply, if we apply a Bonferroni correction, only the association between rs2695121 and HOMA-IR in the HELENA study (adolescents) would stay significant. However, relationships between LXRbeta SNPs and type 2 diabetes/obesity have already been investigated in two other smaller adult samples, with similar conclusion as ours. Therefore, we feel that our paper confirms previous association study data but still requires confirmation in larger samples to definitely conclude about the association between LXRbeta polymorphisms and obesity/T2DM related phenotypes. This has been clearly stated in the revised version of the manuscript.

Please reply to Hilde Nebb (h.i.nebb@medisin.uio.no) and Aline Meirhaeghe (aline.meirhaeghe-hurez@pasteur-lille.fr) in addition to Karianne Solaas (karianne.solaas@medisin.uio.no) during the summer in case one of us is on holidays when an urgent question is asked, thank you.

Thank you again for your interest.

We do hope the revised manuscript will be acceptable for publication in BMC Medical Genetics.

Sincerely yours,

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Reviewer's report
Title: Liver X receptor beta polymorphisms in type 2 diabetes mellitus and obesity in three cohort studies: HUNT 2 (Norway), MONICA (France) and HELENA (Europe)
Version: 1 Date: 14 June 2010
Reviewer: Hélène Choquet

Reviewer's report:
Review of the paper “Liver X receptor beta polymorphisms in type 2 diabetes mellitus and obesity in three cohort studies: HUNT 2 (Norway), MONICA (France) and HELENA (Europe).” In this report, K. Solaas and colleagues estimated the associations between SNPs in the gene LXRb and risk of type 2 diabetes (T2D), obesity and related traits using three independent cohorts. They also investigated the functional effects of one disease-associated SNP. The study appears comprehensive, however the genetic study design is not strong enough to convince the readers about effects of these LXRb polymorphisms on T2D and obesity.

Minor points:
1. In the Background subsection, the authors said that “We then performed association studies in 3 independent cohorts to determine if the LXRb SNPs were associated with T2DM, obesity …” but in fact only one case-control study (HUNT 2) was used to test association between the LXRb SNPs and T2DM and only two case-control studies (MONICA and HELENA) were used to test association between the LXRb SNPs and obesity. The authors should clarify this issue.
   We have re-written this section to clarify the issue at page 3: ” We then performed association studies to determine if the LXRβ SNPs were associated with T2DM (in one case-control study), obesity or related phenotypes (in two - adult and adolescent - population based-studies).”

2. It may be worthwhile to present briefly the clinical characteristics (sex ratio, age, BMI) of the studied subjects in a single table for each population (96 Norwegian individuals and subjects selected from the HUNT 2, MONICA and HELENA studies). We agree. The clinical characteristics are presented in Additional table 1 for the HUNT 2, MONICA and HELENA subjects.

3. The design used in gene sequencing is not clear enough. The authors started by “re-sequencing” the LXRb gene in 96 Norwegian individuals to search variants, apparently already identified. How did the authors select the 96 individuals sequenced ? Furthermore, it is difficult to understand why the term “re-sequenced” was used ? The authors should more explain why they chose this strategy.
   The issues concerned are made clear at page 5, and the term “sequenced/sequencing” is used instead of “re-sequenced/re-sequencing” at page 2, 3, 5 and 8.

4. The authors gave the genotype success rates for the individual SNPs in each study but we do not know if the authors double genotyped a subfraction of the sample for the individual SNPs. The authors should provide a concordance rate (quality control procedure), especially for the rs2303044 in the MONICA study for which the genotype population distribution deviated from the Hardy-Weinberg equilibrium (p=0.017).
   Two percent of the samples were double genotyped and the concordance rate was 100%. This information has now been added at page 5 of the manuscript.

5. It might be interesting to describe briefly the Breslow-Day tests in the Methods subsection.
We now added a sentence about the Breslow-Day test at page 6.

6. In the Results subsection (“Identification of LXRx SNPs and haplotype blocks” part), the authors noted that “the three tag-SNPs and the two SNPs showing little LD with other SNPs were selected to cover the entire common genetic variability of the LXRx gene”. It would be interesting for the authors to assess the percent of genetic variability at the LXRx locus represented by these five SNPs.

The 5 tag-SNPs selected cover 99.3% of the common genetic variability of the LXRx beta gene. This is now added in the abstract and at page 8.

7. In Tables 2, 3, and 4: rs26955121 should be rs2695121.

Sorry. The misprinted rs number is now corrected in Tables 2, 3 and 4.

8. Concerning the “Association between the LXRx SNPs and T2DM-related quantitative phenotypes in HUNT 2”, do the authors have access to HOMA-IR index and HOMA-B cell index for the HUNT 2 cohort?

Unfortunately we don’t have plasma insulin levels for the HUNT2 individuals and therefore we cannot calculate HOMA-IR and HOMA-B.

9. In the Results subsection, in the “Association between the LXRx SNPs and obesity- or T2DM-related quantitative phenotypes in HUNT 2” part, why only control individuals were selected for these analyses?

The HUNT2 sample is a case-control study for T2DM, so we cannot mix the cases and the controls together as their clinical characteristics differ a lot, by design. The distribution of plasma glucose level for example is bimodal in the combined sample.

10. Depending on the study, p-values were adjusted for different confounding variables: age, gender and centre for HELENA study and age, gender, centre, smoking habit, alcohol consumption and physical activity level for MONICA study. These different adjustments make it difficult to compare results between the three studied populations.

The HELENA study is an adolescent sample. Thus, there are very few smokers and alcohol drinkers in the sample. Moreover, the assessments of smoking and alcohol consumption are often underestimated, if any, in adolescents. In this respect, using these variables might bias the results. In the MONICA study, physical activity level was assessed by questionnaires. In the HELENA study, it has been objectively measured with an accelerometer, but only in 750 individuals (among the 1144) so we lose a lot of power if we use it. Nevertheless, the data are similar in terms of SNP effect (but not in terms of p values) if we correct for physical activity level in the HELENA study.

11. An a posteriori statistical power calculation could be informative for association studies. It may be worthwhile to present these informations in the discussion.

We believe that presenting statistical power for each phenotype (quantitative and qualitative) and for each population in the manuscript would unfortunately be too dreary.

12. Concerning the functional studies, the term “disease-associated SNPs” should be change since only one SNP (rs28514894, a perfect LD with rs17373080) has been tested using in vitro functionality studies.

We agree, and we now say that functional studies were performed for one SNP (page 3) or for rs28514894 (page 10).

Major points:
1. Generally in the manuscript, the authors consider that a p-value of 0.05 is sufficient to confirm that an association is correct. I think this should be discussed quite carefully in the context of multiple testing. We agree with the reviewer and we now better discuss this point in the discussion page 12: “We considered that a p-value of 0.05 was significant. The detected associations would become non-significant if corrected for multiple testing with a Bonferroni correction for example (150 statistical tests), except the association between rs2695121 and HOMA-IR in the HELENA study \( (p=0.002) \). However, such a correction may be too conservative [34], since the tagSNPs are not completely independent \((0.87<D'<1.00)\) and the metabolic phenotypes of interest are intimately correlated (for example, BMI is correlated with waist circumference and waist-to-hip ratio, the HOMA-B and HOMA-IR indexes are derived from plasma glucose and insulin level, etc). In as much as our data confirm results from 2 other studies [32,33], we believe that drawing negative conclusions from a purely statistical point of view would be too harsh. Using additional, large population samples and performing meta-analyses is mandatory to accurately define the nature of the association between \( LXR\beta \) genetic variability and risks of obesity and T2DM.”

2. In Additional Tables 2, 3, and 4: the authors should provide ORs and 95% CI. Additional tables 2, 3 and 4 present quantitative variables (BMI, glucose level etc). Thus, we cannot calculate odds ratios.

3. In the Results subsection, the authors should specify why they chose to present results under a dominant model. If the authors did not obtain better results and p-values under additive and recessive models, they should specify that and if not, they should present these informations and complete all the tables. The additive, dominant (and recessive when possible) models were tested, but only the best model is actually presented (always the dominant model except for rs17373080 and waist-to-hip ratio in additional table 3). This information has been added at page 6.

4. In the Discussion subsection, the authors suggest that “no LXRb SNPs have been significantly associated with obesity or T2M in genome-wide association studies (GWAS) … nominal associations with LXRb SNPs may have gone unreported”. But in my view, it might be interesting to collaborate with consortium and to use data from genome-wide association studies to confirm effect of LXRb SNPs on T2D and obesity and why not to perform a meta-analysis. In fact, the current genetic design is not strong enough to convince the readers about effects of these polymorphisms on T2D and obesity in these populations, especially because the sample sizes of the three cohorts are too weak. Case-control studies with T2D and obesity in largest populations are therefore needed. We fully agree with the reviewer that replication in well-powered case-control or population-based studies would be very useful. Also, stringent threshold in subsequent meta-analyses are needed to be conclusive. In the revised version of the manuscript we made this point extremely clear and also acknowledged the limited statistical power of our studies at pages 12 and 13. The ambition of our work is to point out with caution that LXRbeta might be a possible candidate gene for obesity/T2DM-related phenotypes, and we are sure that researchers that have GWAs in hands will certainly check whether our results are confirmed or not and we will surely be interested in participating in meta-analyses of these associations.

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:
I declare that I have no competing interest's below.
Reviewer's report

Title: Liver X receptor beta polymorphisms in type 2 diabetes mellitus and obesity in three cohort studies: HUNT 2 (Norway), MONICA (France) and HELENA (Europe)

Version: 1 Date: 16 June 2010
Reviewer: Hans-Ulrich Häring

Reviewer's report:

Solaas et al. report on associations between genetic variation in the NR1H2 gene coding for liver X receptor beta (LXRb) and risk of type 2 diabetes mellitus and obesity and three different cohorts. In light of the crucial roles of LXRb in glucose homeostasis and lipid metabolism, NR1H2 appears to be an attractive type 2 diabetes candidate gene and, therefore, this study is highly relevant. The manuscript is well written. The study was thoroughly planned and performed. The results of the present study add important information on potential candidates contributing to the risk of type 2 diabetes mellitus. However, following points should be addressed before the manuscript is acceptable for publication:

Major comments:

1. The authors should perform Bonferroni correction for the number of tested single nucleotide polymorphisms (SNPs).

We agree with the reviewer and we now better discuss this point in the discussion page 12: “We considered that a p-value of 0.05 was significant. The detected associations would become non-significant if corrected for multiple testing with a Bonferroni correction for example (150 statistical tests), except the association between rs2695121 and HOMA-IR in the HELENA study (p=0.002). However, such a correction may be too conservative [34], since the tagSNPs are not completely independent (0.87<D’<1.00) and the metabolic phenotypes of interest are intimately correlated (for example, BMI is correlated with waist circumference and waist-to-hip ratio, the HOMA-B and HOMA-IR indexes are derived from plasma glucose and insulin level, etc…). In as much as our data confirm results from 2 other studies [32,33], we believe that drawing negative conclusions from a purely statistical point of view would be too harsh. Using additional, large population samples and performing meta-analyses is mandatory to accurately define the nature of the association between LXRβ genetic variability and risks of obesity and T2DM.”

The ambition of our work is to point out with caution that LXRbeta might be a possible candidate gene for obesity/T2DM-related phenotypes, and we are sure that researchers that have other cohorts in hands will certainly check whether our results are confirmed or not and we will surely be interested in participating in meta-analyses of these associations.

2. The authors should comment on the associations between common genetic variation in NR1H2 and measures of obesity in the HUNT 2 study. Furthermore, a meta-analysis including all subjects of the three cohorts should be performed to study the impact of NR1H2 SNPs on body mass. Are data on the diabetes prevalence available for the cohorts in the MONICA study and the HELENA study? If this is the case, a replication of the association between genetic variation in NR1H2 and diabetes risk found in the HUNT 2 study could be performed.

We would prefer not to perform a meta-analysis of the three cohorts to study the impact of the polymorphisms on fat mass as we think that adolescents are very different from adults when considering fat mass. The impact of age and fat mass distribution may impact too much on the data in a pooled analysis to draw any valid conclusion. To study the adolescents are a very good strategy to use in association analyses to see if the phenotypes of interest are modulated
by the polymorphisms at an early age. Then the associations in adults may be nicely confirmed.
If we perform a meta-analysis between the adult HUNT 2 and MONICA studies, there is unsurprisingly no significant association like in each study separately. However, we agree that to perform a meta-analysis is a logical next step as we throughout the manuscript.

The MONICA study contains only 209 individuals with type 2 diabetes and the HELENA study (adolescents) none. So these population samples are not suitable for replication of the association between SNPs and diabetes risk found in HUNT2. This aspect has now been added at page 11 in the discussion section.

Minor comments:
1. Though published earlier, subject characteristics should be given as supplementary tables.
We agree and we therefore now added the subject characteristics in additional table 1.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.
Reviewer's report
Title: Liver X receptor beta polymorphisms in type 2 diabetes mellitus and obesity in three cohort studies: HUNT 2 (Norway), MONICA (France) and HELENA (Europe)
Version: 1 Date: 17 June 2010
Reviewer: Kirsten Pruefer
Reviewer's report:
The paper by Solaas et al. demonstrates a potential correlation between a single nucleotide polymorphism (SNP) in the human LXRb gene and the risk of type 2 diabetes mellitus (T2DM) and obesity. After identification of SNPs in a small study, the presence of these SNPs was correlated to T2DM and obesity in 3 separate cohort studies. My expertise in the evaluation and correlation with phenotypes of sequencing data obtained in cohort studies is limited. From this position, however, the presented data are convincing to me. The hypothesis that a specific transcription factor binding site created by the presence of a SNP plays a role in the higher prevalence of T2DM and obesity in subjects with this SNP is plausible; and despite being disproved, the data clearly indicate more potent transcription of LXRb in the presence of this SNP. The paper is mostly well written, the methods appropriate and well described, and the data well presented. The discussion and conclusions are supported by the data and limitations of the work are clearly stated.

Discretionary revisions:
1. The background would benefit from better organization. The review of LXRb functions should lead to a clearly defined question, objective, or hypothesis.
We did our best to better organize the background section and we clearly defined our work hypothesis at page 3: “We performed association studies to determine if the LXRβ SNPs were associated with T2DM (in one case-control study), obesity or related phenotypes (in two - adult and adolescent - population based-studies).”

2. The title could better reflect the main finding of the paper.
We modified the title to better reflecting the main findings in the paper: “Suggestive evidence of associations between liver X receptor β polymorphisms with type 2 diabetes mellitus and obesity in three cohort studies: HUNT 2 (Norway), MONICA (France) and HELENA (Europe)”.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests:
I declare that I have no competing interests.