Author's response to reviews

Title: Association of nineteen COX-2 gene variants to preclinical markers of atherosclerosis The Cardiovascular Risk in Young Finns Study

Authors:

Kati Lähteelä (kati.lahteela@uta.fi)
Tarja Kunnas (tarja.kunnas@uta.fi)
Leo-Pekka Lyytikäinen (leo-peka.lyytikainen@uta.fi)
Nina Mononen (nina.monnen@uta.fi)
Leena Taittonen (leena.taittonen@oulu.fi)
Tomi Laitinen (tomi.laitinen@kuh.fi)
Johannes Kettunen (johannes.kettunen@thl.fi)
Markus Juonala (mataju@utu.fi)
Nina Hutri-Kähönen (nina.hutri-kahonen@uta.fi)
Mika Kähönen (mika.kahonen@uta.fi)
Jorma S Viikari (jorvii@utu.fi)
Olli T Raitakari (olli.raitakari@utu.fi)
Terho Lehtimäki (terho.lehtimaki@uta.fi)
Seppo T Nikkari (seppo.nikkari@uta.fi)

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Author's response to reviews: see over
Enclosed please find a revised version of our manuscript entitled "Association of nineteen COX-2 gene variants to preclinical markers of atherosclerosis. The Cardiovascular Risk in Young Finns Study" (MS 1569882026397415) which we submitted to BMC Medical Genetics. The reviewers’ suggestions have been taken into account as closely as possible. The changes in the manuscript are in red.

Editorial Requirement:
Please name the local ethics committees involved and state it under the methods section

We have added:
All subjects gave their written informed consents in 2001 and the local ethics committees in all five centers (Helsinki, Kuopio, Oulu, Tampere and Turku) approved the study.

Our specific comments to the reviewers are as follows:

Reviewer: Jane Maguire
Minor Essential revisions
1. Is the question posed by the authors well defined?
   The authors investigate whether COX-2 gene variants associate independently with the early subclinical markers of atherosclerosis, carotid intima-media thickness and carotid artery distensibility in a population of young healthy Caucasian adults. The authors could improve the clarity of their research focus by stating up front the study design.

   We have in fact stated in the Abstract: In the present study we analyzed whether COX-2 gene variants associate independently with the early subclinical markers of atherosclerosis, carotid intima-media thickness and carotid artery distensibility in a population of young healthy Caucasian adults. We feel that the methods have also been described in the Abstract.

2. Are the methods appropriate and well described?
   The methods are appropriate and appropriate QC checks have been performed prior to analysis.
3. Are the data sound? Yes
4. Does the manuscript adhere to the relevant standards for reporting and data deposition?
   Tables showing QQ plots and a Manhattan plot would better demonstrate the study findings.

**Our statistician** feels that these plots would be redundant for our negative findings.

5. Are the discussion and conclusions well balanced and adequately supported by the data?
   The authors have acknowledged that this study is underpowered due to the allele frequency of the variant in question. The authors have written the paper well however the discussion is centred on the limited research published to date and does not argue in support of the importance of their own findings. Although the findings are non-significant after adjustment for multiple comparisons the authors should not be discouraged as there are very few significant GWAs findings. This work should be considered as pilot data that should that would do well to be followed up with a meta-analysis and pooling of larger samples.

**Thank you very much** for your positive comments.

6. Are limitations of the work clearly stated? Yes.
7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? Yes- they have done this well.
8. Do the title and abstract accurately convey what has been found? No the title implies an association and could be improved.

We have changed the title to:
**No** association of nineteen COX-2 gene variants to preclinical markers of atherosclerosis.
The Cardiovascular Risk in Young Finns Study

9. Is the writing acceptable? Yes the paper is well written
**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 interests.

**Reviewer:** Marcello Arca
**Reviewer’s report:**
The points that must be addressed are the following:
   1. It seems that the studied cohort was genotyped for other purposes (e.g. for a WGS analysis) and Authors used the data on COX-2 in a post-hoc
analysis: If this true this must be more clearly stated and the main must be described.

The reviewer is right; the studied cohort was genotyped for a number of future studies, as we state in the “Genotyping and imputation”-paragraph: “After quality control there were 2442 samples and 546677 genotyped SNPs available for further analysis”.

In addition must be also explained why these 19 SNPs were considered and how many of them were functionally significant.

In the Introduction we stated:
Functional studies have been made for only one of the tested SNPs (rs 20417)[12], but this does not necessarily mean that the others are not functional. Therefore, in the present study we analyzed a wide range of COX-2 single nucleotide polymorphisms and their association with subclinical markers of carotid atherosclerosis, such as carotid intima-media thickness (CIMT) and carotid artery distensibility (Cdist).

We now explain why these 19 SNPs were considered. We have also found one more functionally tested SNP in the literature. The text has been changed to:
Functional studies have been made for only two of the tested SNPs (rs 20417, rs 5275), but this does not necessarily mean that the others are not functional [12,13,14]. There are 302 known SNPs in the COX-2 gene available from the NCBI dbSNP human database. In the present study we analyzed all the 19 COX-2 SNPs available from the HapMap II CEU (release 22) and their association with subclinical markers of carotid atherosclerosis, such as carotid intima-media thickness (CIMT) and carotid artery distensibility (Cdist).

2. It appears also that the selected SNPs have divergent effect on COX-2 activity (some with increasing and some other with lowering effect) and this may cause biases - e.g. a sort of a null effect-. This aspect must be clarified for example by clustering SNPs or creating haplotypes.

Unfortunately, although we tried very hard, we could not lose the null effect. We feel that inclusion of some of the haplotyping we did would not add to the message of the ms.

Finally, it must be also estimated the amount of variability in the COX-2 gene explained by the selected SNPs.

We have added to the introduction:
There are 302 known SNPs in the COX-2 gene available from the NCBI dbSNP human database. In the present study we analyzed all the 19 COX-2 SNPs available from the HapMap II CEU (release 22) and their association with subclinical markers of carotid
atherosclerosis, such as carotid intima-media thickness (CIMT) and carotid artery distensibility (Cdist).

3. To appreciate the potential role of genetic it is also important to identify the major determinant of CIMT or Cdist in the overall population. This could be done by logistic analysis considering the major cardiovascular risk factors. In fact, if these explain the large proportion of variation in CIMT or Cdist, it is obvious that genetics is of only minimal importance and difficult to reveal. I would suggest including in the manuscript the results of the analysis of major determinants of CIMT and Cdist in this cohort.

We have added to the discussion:
Since CIMT and Cdist are significantly associated with high LDL-cholesterol, elevated blood pressure, obesity and smoking [16,19], it is obvious that genetics may be of only minimal importance and difficult to reveal.

4. It is not clear why Authors postulated only an additive model in evaluating the effect of SNPs on the markers of atherosclerosis. It would be appropriate to test also a recessive model.

We have added to the Statistical analysis:
Association analysis was performed using linear regression with additive and recessive models.

We have also added to the Results:
Finally, we also tested a recessive model in evaluating the effect of SNPs on the markers of atherosclerosis. The results were similar in both the additive and recessive model. After adjustment with gender, age, BMI, smoking status, and multiple testing correction in the recessive model, the only associations that remained significant were total cholesterol (q = 0.014) and LDL cholesterol (q = 0.041) for rs 5275.

5. In the Discussion the biological significance of the SNPs must be underlined. Moreover, the potential implications of the association with cholesterol must be described.

As stated earlier, we have now added to the Discussion:
Since CIMT and Cdist are significantly associated with high LDL-cholesterol, elevated blood pressure, obesity and smoking [16,19], it is obvious that genetics may be of only minimal importance and difficult to reveal.

We already have in the Discussion (and feel that it is sufficient):
Subjects with the minor C allele of rs5275 had lower mean values of total cholesterol and LDL cholesterol compared with the major T allele. The difference was small and not clinically relevant. However, in line with our results, there is a previously reported
interaction of rs5275 and alcohol in relation to plasma cholesterol levels, where the minor allele carriers with low alcohol intake had the lowest lipid levels [8].

Level of interest: An article whose findings are important to those with closely related research interests
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 interests

We thank the reviewers for their constructive comments and hope that these changes and additions will make the manuscript suitable for publication.

Sincerely yours,

Seppo T. Nikkari, M.D., Ph.D.
Professor
Department of Medical Biochemistry