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.monnenen@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)

Version: 3 Date: 18 April 2012

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.

**Version: 2**  
**Date:** 26 March 2012  
**Reviewer:** Jane Maguire  
**Reviewer’s report:**  
Minor essential Revisions: The authors have addressed my comments sufficiently and they have conducted further analysis under a recessive model as requested by Reviewer 2.  
**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

Thank you very much for your positive comments.

**Version: 2**  
**Date:** 3 April 2012  
**Reviewer:** Marcello Arca  
**Reviewer’s report:**  
Authors included some requested changes and included some explanation. However, the main concern - the fact the COX-2 may be the not right target in evaluating CIMT- has not been discussed. I would suggest to include a statement on this.

We had in fact already added this to the discussion, as suggested for version 1 by the reviewer. To make this even clearer, we have now added to the sentence in question: Since CIMT and Cdist are significantly associated with high LDL-cholesterol, elevated blood pressure, obesity and smoking [16,19], it is obvious that genetics, e.g. the variation in COX-2 **genotype**, may be of only minimal importance and difficult to reveal.

Moreover They did not prove that haplotype analyssi left the results unchanged.
For the benefit of the reviewer, we already answered to this concern in the review of version 1. We still feel that inclusion of the haplotyping we did would not add to the message of the ms. More specifically, we evaluated all COX-2 SNPs simultaneously with multiple stage false discovery rate (MSFDR) algorithm, and only rs689470 was left in the model when the threshold q-value was set to be ~0.58. This means that there are no COX-2 SNPs which would constitute a meaningful haplotype nor is the rs689470 statistically significantly associated with the outcome due to multiple testing bias (the q-value used should have been 0.05).

Finally some comment about the functionality of the studied SNPs should be included

We refer to our previous answer in version 1 to the reviewer’s same question (below):

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

We now explain in the Introduction 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).

**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 do not have any 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