Author's response to reviews

Title: Interaction of functional NPC1 gene Polymorphism with smoking on coronary heart disease

Authors:

Weiwei Ma (maweiw82@gmail.com)
Jing Xu (xujingnijux@yahoo.com)
Qianqian Wang (wangqiangqian84@gmail.com)
Ying Xin (xinyin20081115@163.com)
Lin Zhang (EPUSKY@sina.com)
Xinxin Zheng (zhengxxfuwai@gmail.com)
Kai Sun (sunkai@sglab.org)
Hu Wang (whcqq@yahoo.com)
Rutai Hui (huirutai@sglab.org)
Xiaohong Huang (huangxhong12@gmail.com)

Version: 3 Date: 9 May 2010

Author's response to reviews: see over
Dear Biomed Central Editorial,

Enclosed you could find the revised version of the manuscript MS: 1931598682359280 “Interaction of functional NPC1 gene Polymorphism with smoking on coronary heart disease” by Weiwei Ma, Jing Xu, Qianqian Wang, Ying Xin, Lin Zhang, Kai Sun, Hu Wang, Rutai Hui and Xiaohong Huang. The editor’s and the reviewers’ comments have been responded point by point in the attachment. We hope that this will be to your satisfaction.

Thank you very much for your consideration and looking forward to hearing from you at your earliest convenience. Please do not hesitate to contact me if you need any further information.

Yours Sincerely,

Xiaohong Huang
We thank the reviewer Fakhredin Sayed Tabatabaei’s time and the comments were very helpful in improving our manuscript.

Response to Reviewer Fakhredin Sayed Tabatabaei

1. The title of the manuscript indicates the most important findings to be the interaction. However, in the result section only one simple paragraph, and in the tables no actual data is presented. This part should be expanded to include detailed information.

Response: Thank you very much for your comments. We have revised our manuscript as you suggested. We have show the data (table 5, 6) of association between rs1805081 and CHD by non-smoking and smoking group and add the detailed information in result and discussion.

See page 6, line 209 to 225: Smoking is known to raise serum lipid levels, and is considered as one of the major risk factors for the development of atherosclerositc disease. In the present study, we showed that within non-smokers, the coronary heart disease risk did not differ between NPC1 genotypes. In smokers, however, both in recessive modle (GG vs AA+AG) and additive modle (GG vs AG vs AA) NPC1 G carriers showed decreased coronary heart disease risk compared with A carriers. Furthermore in multiplicative interaction mode, there was a significant interaction between NPC1 +644A>G and cigaratte smoking on risk for CHD. However, the biological basis of NPC1-smoking interaction on lipid concertration and CHD risk is still unclear. Smoking might lower NPC1 transcription, by somehow disrupting the binding of transcription factors, therefore, leading to intracellular cholesterol accumulation. Normand Podechard[1] recently found that human macrophages exposed to environmental aryl hydrocarbons and Aryl hydrocarbons (AHs) was found to decrease expression of NPC1 at both mRNA and protein levels. Moreover 2,3,7,8-tetrachlorodibenzop-dioxin (TCDD) and polycyclic AHs (PAHs) , which are especially found in cigarette smoking, had been recently demonstrated to promote the transformation of macrophages to foam cells in the atherosclerosis peocess[2].
2. Table 2 in its current format is not informative. Instead of presenting odds ratio's in three different genetic models, the authors should present two by two comparison between the genotypes. Such comparisons should also be presented separately among smokers and non-smokers in a new table (or graph). If adjustment with co-variates make significant differences in the findings, both adjusted and unadjusted results should be presented here as well.

Response: Thank you very much for your comments. We have revised our manuscript as you suggested. We have separate table2 into two new tables (table2 and table3) and show the crudes OR in table 4. Another we present separated data among non-smokers and smokers in table 5.

3. In Table 1, the association for age, LDL, and TC is not in the expected direction. The authors have to comment on that.

Response: Thank you very much for your comments. We have revised our manuscript as you suggested. See page 6, line 181 to 186: Hypercholesterolemia is traditional cardiovascular risk factor of CHD. However, in this study the CHD patients almost took cholesterol-lowering drugs to control cholesterol levels. Therefore compared with control, LDL-C and TC were significant lower and HDL-C was significant higher in CHD patients \( (P<0.01) \). Because of cholesterol-lowering drugs influences, in the logistic regression model the results did not adjusted by the classical risk factor LDL-C, HDL-C, TG and TC.

4. In the last paragraph of discussion, the authors claim that "...[the findings] suggest therapeutic strategies to promote plaque stability." Also in the abstract they claim "These findings may provide evident that in gene status NPC1 contributes to lipid accumulation in human macrophages." The authors may not claim any conclusion that is not directly supported by their own finding.
Response: Thank you very much for your comments. We have revised our manuscript and deleted those sentences that might bring confusion to readers.

See page 2, line 16 to 19: The results of the present study suggest that NPC1 variants seem to be contributors to coronary heart disease occurrence in Chinese population. Moreover, in smokers, NPC1 variants seem to confer protection to coronary heart disease onset.

5. Not enough information regarding the SNP is provided. For example, it is not mentioned in which part of the gene the SNP is located.

Response: We add the information of NPC1 and rs1805081 in SNP selection part.

See page 4, line 112 to 114: SNPs in NPC1 gene were retrieved from HapMap database for CHB (Chinese Han in Beijing) sample (release No. 24/phaseII Nov08, on NCBI B36 assembly). We searched across a 47-kb region spanning NPC1 gene from 1 kb upstream of the 5’-flanking region to 0.5 kb downstream of the 3’-flanking region. The percent coverage of HapMap variants is subjected to a minor allele frequency (MAF) threshold of 0.05 and an r2>0.8 threshold with pair-wise tagging, using Haploview software (3.31 version). After excluded those SNPs in introns, we identified one important SNP rs1805081 (+644A>G) with a MAF of 0.374, which causes a missense mutation from His215 to Arg in NPC1 protein.

6. The polymorphism, and the alleles, are named differently throughout the manuscript, such as "C vs G," "His vs Arg," "+A644G," and "+644A/G." It is advised to use unique terminology for naming the polymorphism and the alleles in all parts of the manuscript.

Response: We have standardized the name of variant with +644A>G or rs1805081 throughout the manuscript.
We thank the reviewer Pawel Niemiec’s time and the comments were very helpful in improving our manuscript.

**Response to Reviewer** Pawel Niemiec

**Major Compulsory Revisions**

1. **WHOLE TEXT**
   
a) Authors analyze only one polymorphism of NCP1 gene (A644G), but they write in abstract, materials and methods and the discussion sections that examining many.

   **Response:** In this present study, we analyze only one variant (+644A>G) of NPC1, but +644A>G was selected from 380 variants in NPC1, which across a 47-kb region spanning NPC1 from 1 kb upstream of the 5’-flanking region to 0.5 kb downstream of the 3’-flanking region. Then we identified one important tag-SNP rs1805081 (+644G/A, H215R, MAF of 0.374), a non-synonymous coding polymorphism and lie in exon 6.

   b) The names of genes should be written in italics, but there is no standardization in this respect in the text. The name of NCP1 peptide is sometimes written with small letters (Ncp1) sometimes with large (NCP1).

   **Response:** we have standardized the name: gene in italic NPC1 and peptide in non-italic NPC1.

   c) The quality of English is unsatisfactory. There are many grammatical and syntax errors. Some sentences are unintelligible, e.g:

   - “…plays critical roles in vascular injure, which is involved in the progressed of coronary heart disease”…
   - “…These findings may provide evident that in gene status NPC1 contributes to lipid accumulation in human macrophages and interacts with smoking environment factor in the pathogenesis of CHD”…
- “Moreover, in smokers, carriers of NPC1 GG had a decrease age- and sex-adjusted coronary heart disease as compared with those carrying AA and AG.”

**Response:** Thank you very much for your comments. We have revised our manuscript and correct grammatical and syntax errors.

2. MATERIAL AND METHODS
The authors should give a references to recommendations and standards used in the classification of traditional risk factors (including hypercholesterolemia and smoking habit), MI and CAD.

**Response:** Thank you very much for your comments. We have revised our manuscript as you suggested. See reference 16 and 17.

3. RESULTS
a) The groups are not sex matched (84.0% vs 70.8%)

**Response:** Thank you very much for your comments. The mean age of our case group is 50. The number of women that are suffered from CAD in this age is much less than that of men. We tried our best to recrute case-control population and the last thing that we want is to waste any sample we got. It is reported that same or identical outcome are obtained whether the sex and age are matched or not in epidemiological studies [3]. And we also found that the odds ratio and the significance remained almost identical after adjusted by conventional risk factors with or without sex included.

b) table 1: - there is no number of individuals in the case of gender, cigarette smoking, hypertension and DM history - There is no Odds Ratios values for classical risk factors of CAD

**Response:** Thank you very much for your comments. We have revised our manuscript as you suggested. We have added the number of individuals in the case of gender, cigarette
smoking, hypertension and DM history. We presented the P values for classical riskfactors of CAD (*P<0.05, **P<0.01 vs. control).

c) table 2.- The table is incomprehensible. Place the groups in the columns and genotypes in the lines. The ORs and p values assign to concrete genotypes and show in additional column. At present, the left and right sides of table do not correspond. - Authors did not present crude ORs (from the univariate logistic regression analysis) but only the adjusted values. - The alleles frequencies (n, %) and the results of comparison between groups (ORs, P) should be inserted

Response: Thank you very much for your comments. We have revised our manuscript as you suggested. We revised table 2 and 3 following the reviewer’s advice.

d) NCP1 polymorphism – cigarette smoking interactions
There is the weakest part of the text (unclear in all the sections, from abstract to discussion). The logistic regression is not appropriate tool for gene-environment interactions analyses. You must use the 4 x 2 tables approach. Please add the amounts of interaction by using: I. SI - synergy indexes, II. AP-attributable proportion due to interaction and III. RERI-relative excess risk due to interaction, together with their 95% confidence intervals.

Response: The interaction effect between +644A>G and cigarette smoking were calculated in two most widely used modes including multiplicative interaction mode and additive interaction mode. In multiplicative interaction mode, there was a significant interaction between NCP1 genotype and cigarette smoking on risk for CHD (P for interaction=0.0001). Using four-by-two table to estimate additive interaction between +644A/G and cigarette smoking, we found no interaction between NCP1 genotype and cigarette smoking on risk for CHD (Table 6).
e) Because of the NPC1 protein role Authors should give the results of gene-hypercholesterolemia interactions

Response: Hypercholesterolemia is traditional cardiovascular risk factor of CHD. However, in this study we did not get the detailed hypercholesterolemia history of those patients. And considering that many of the CHD patients might have taken cholesterol-lowering drugs to control cholesterol levels, it is difficult to estimate the hypercholesterolemia history judging by patients’ plasma cholesterol level.

4. DISCUSSION
The discussion section is too long and should be focused on NPC1 gene, the A644G polymorphism, and explanation of possible gene-smoking, gene-hypercholesterolemia interactions
The conclusion “We investigated the gene-environment interaction and found carriers of NPC1 GG had a decrease risk of coronary heart disease as compared with those carrying AA and AG in smokers. This result brings an indirect evidence for a repression of NPC1 expression in AHs–exposed macrophages.” Is unclear and written in “baaaaad” English. I suggest to wait for the results of the gene-environment analyses.

Response: Thank you very much for your comments. We have revised our manuscript as you suggested.
We thank the reviewer Robert P. Erickson’s time and the comments were very helpful in improving our manuscript.

1) Ref 5 is not a review but a research article. One of the numerous recent reviews, such as ref 13, should be cited at this point.

2) rs1805081 has been found to be associated with obesity (Meyre, et al, 2009, Nature Genetics, 41:157) and Alzheimers (Erickson, et al, 2008, Neurosci. Letters, 447:153) while studies in mice suggest that the G allele which is protective here, is likely to be associated with decreased activity and increased obesity (Jelllinek, et al, 2009, Obesity, doi:10.1038/oby.2009.415).

Response: Thank you very much for your comments. We have revised our manuscript as you suggested.

See page 6 and line197 to 200: Furthermore, rs1805081 has been found to be associated with obesity[4] and Alzheimers[5] while studies in mice suggest that the G allele is protective here and might be associated with NPC1 activity.