Author's response to reviews

Title: Association of the rs738409 polymorphism in PNPLA3 with liver damage and the development of nonalcoholic fatty liver disease

Authors:

Kikuko Hotta (kikukoh@kuhp.kyoto-u.ac.jp)
Masato Yoneda (yoneda@med.yokohama-cu.ac.jp)
Hideyuki Hyogo (hidehyogo@hiroshima-u.ac.jp)
Hidenori Ochi (hochi@hiroshima-u.ac.jp)
Seiho Mizusawa (mizusawa@genome.med.kyoto-u.ac.jp)
Takato Ueno (takato@med.kurume-u.ac.jp)
Kazuaki Chayama (chayama@mba.ocn.ne.jp)
Atsushi Nakajima (nakajima-ty@umin.ac.jp)
Kazuwa Nakao (nakao@kuhp.kyoto-u.ac.jp)
Akhiro Sekine (sekine@genome.med.kyoto-u.ac.jp)

Version: 2 Date: 2 December 2010

Author's response to reviews: see over
2 December 2010

Dr. Meyre
Editor
BMC Medical Genetics

Dear Dr. Meyre;

On behalf of all the authors, I am sending our revised manuscript MS:1989300951439226, entitled “Association of the rs738409 polymorphism in PNPLA3 with liver damage and the development of nonalcoholic fatty liver disease”.

We thank reviewers for careful reading for our manuscript and for giving useful comments. We have revised the manuscript on the basis of the reviewers' comments. We look forward to a publication of our manuscript in the BMC Medical Genetics.

Sincerely,

Kikuko Hotta, M.D., Ph.D.
Assistant Professor
EBM Research Center,
Kyoto University Graduate School of Medicine
Yoshida-Konoecho, Saky-ku, Kyoto
606-8501 Japan
Phone: +81-75-751-2022
Fax: +81-75-751-2082
kikukoh@kuhp.kyoto-u.ac.jp
Our responses to the reviewer's comments are as follows:
Changes are written in red.

Response for the Reviewer, Dr. Romeo:

1. We have added the median and interquartile ranges in Table 1 and Table 2. The median and interquartile ranges in Table 4 have been indicated in Supplementary Table 2, since the table will be large. In Table 5, linear multiple regression analysis has been performed after the variables of triglycerides, ferritin, and hyaluronic acid were logarithmically transformed. This transformation has been mentioned in the Methods (page 8). Logarithmical transformation could not apply to the fibrosis stage, since this variable has 0 values.

2. I appreciate that the reviewer indicated our mistake of the allele frequency on page 13. We have corrected the mistake.

3. We have added the numbers of individuals across the different genotypes in Table 4.

4. We have corrected liter (l to L) and the kg/m² to kg/m².

Response for the Reviewer, Dr. Michel:

1. We have added 2 papers in our manuscript (references 34 and 35) and added some discussion about these papers (page 13)

2. We have added the discussion about the low serum triglycerides levels in the patients with a risk allele (page 13, 2nd paragraph). Low plasma glucose has not been significant after adjusted with age, gender and BMI. This has been described in results section (page 10, 2nd paragraph).

Response for the Reviewer, Dr. Stefan:

1. Abstract
   a. We have added the model of genotype (additive model) used for calculation of OR in the method of abstract.
b. We have agreed with the reviewer’s comments that our major finding was the association between rs738409 and the progression of NAFLD. Therefore, we have changed the conclusion in the abstract (page4), results (page 9), discussion (page 13, last paragraph to page 16) and conclusion (page16).

2. Introduction
a. We have changed the sentence about the spectrum of NAFLD (page 4).

b. We have added the recent data that fatty liver induce the insulin resistance by such as fetuin-A on page 4, and added 3 references (references 5, 6 and 7).

c. We have added the SNPs in the adiponectin receptor 1 and DGAT2 genes related with NAFLD in the background section. We have added 2 references (references 15 and 16).

3. Methods
a. We have added the number of diabetic patients in the NAFLD group. We also added the information of medication (page 6).

b. We have added the discussion about the differences of metabolic disorders between control and NAFLD groups and about the limitation of our case-control association study (page 12, 2nd paragraph).

4. Results and discussion
a. We have performed case-control association study separately in men and women and the results have been described in Supplementary Table 1 (page 9).

b. We have added the discussion about the higher ferritin, hyaluronic acid and type IV collagen 7S. Ferritin, hyaluronic acid and type IV collagen 7S are the makers for progression/fibrosis of NAFLD. We have added the meaning of these parameters (page 14, 2nd paragraph). Accordingly, some references have been added (references 37 to 44). The implications for the mechanism of action of PNPLA3 have been discussed on pages 15 to 16.

c. We have added the ORs for heterozygotes and homozygotes in Table 3.

d. We have some discussion about the lower serum triglycerides levels in the patients with G-allele (page 13, 2nd paragraph). Low plasma glucose has not been significant after adjusted with age, gender and BMI. This has been described in results section (page 10, 2nd paragraph).
e. We have discussed the different associations of the genotype in the control and NAFLD groups (transaminase on page 12, 3rd paragraph, triglycerides on page 13, 2nd paragraph). We have corrected the legend of Table 4 (“obese” to “NAFLD”).

f. We have agreed with the reviewer’s comment that the discussion about the steatosis grade was not conclusive and insufficient. We have changed and added the discussion about the steatosis grade (page 13, 3rd paragraph). We also added the discussion about the risk allele and fibrosis of NAFLD (page 15, 2nd paragraph).

g. According to the reviewer’s comment, we have removed the discussion about the PPARGC1A and a reference (original reference no. 34).

h. We have changed the discussion about the transaminase according to the reviewer’s comment (page 12, 3rd paragraph).

5. We appreciate the reviewer’s pointing out the typographical error in the title. We have corrected the error in title.

6. Our manuscript has been read and corrected by a native English speaker.