Reviewer's report

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

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Reviewer: Norbert Stefan

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In this case-control study, the G allele of the rs738409 SNP in PNPLA3 was found to be associated with an increased risk for the progress of NAFLD, as well as with increased serum levels of transaminases and with an increased fibrosis stage in patients with NAFLD.

Comments
1. Abstract
   a. It should be mentioned whether the OR was calculated using the dominant or the additive model.
   b. According to the present results, the authors can conclude that the SNP in PNPLA3 may be involved in the progress from simple steatosis to NASH and fibrosis, but not in the development of NAFLD (no relationship with the grade of steatosis was found in the present study). Therefore, the conclusions should be modified accordingly, both in the abstract and the discussion/conclusions. The same applies also for the first sentence on page 9 / last sentence of the case-control association study section.

2. Introduction
   a. The spectrum of NAFLD extends also further than NASH to fibrosis/cirrhosis and possibly hepatocellular carcinoma.
   b. It should be mentioned that, according to recent data, fatty liver may not only be a manifestation of, but also induce insulin resistance by producing specific proteins, such as fetuin-A (see and discuss Endocr Rev. 2008 Dec;29(7):939-60; Diabetes Care. 2006 Apr;29(4):853-7; Diabetes. 2008 Oct;57(10):2762-7)
   c. References referring to SNPs involved in other diseases except NAFLD should be omitted. In contrast, the authors should briefly discuss also other SNPs (besides these investigated by their group) involved in NAFLD, such as in the adiponectin receptor 1 gene, and the DGAT2 gene (Diabetologia. 2005 Nov;48(11):2262-91; Clin Sci (Lond). 2009 Mar;116(6):531-7)

3. Methods
   a. Were there any patients with diabetes among the patients with NAFLD? What medication did they take? Did patients with NAFLD take any medication that could affect liver fat content?
   b. The control group is very specific, consisting of (very) lean, insulin sensitive
subjects having not a single component of the metabolic syndrome (page 6 and table 1). The NAFLD and control groups differed in terms of many metabolically relevant parameters, among which adjustment was performed only for gender, age and BMI. It is, therefore, formally possible that the SNP does not affect liver fat accumulation/progression from steatosis to NASH and fibrosis directly, but indirectly, by affecting e.g. visceral adiposity (known to be closely associated with liver fat) or blood lipids. This has at least to be discussed in the conclusions/limitations of the study.

4. Results and discussion

a. Because there are great differences in gender distribution between the NAFLD and control group, adjustment for gender may not be enough. The analyses should additionally be performed separately in men and women and these data should be shown in supplemental tables.

b. The relevance of higher ferritin, hyaluronic acid and type IV collagen 7s in NASH (table 2) should be discussed. Do the authors imply that these are markers of progression of NAFLD? What is the relevance of the significant association of the G allele in the SNP with increased ferritin and hyaluronic acid, but not type IV collagen levels (table 4)? Are there any implications for the mechanism of action of PNPLA3?

c. The results of the case-control study and the ORs should be shown also separately for the heterozygotes and the homozygotes for the risk allele to show whether there is an allele-dose effect.

d. The findings of lower fasting plasma glucose and (particularly) lower serum triglycerides levels in patients with NAFLD with the risk G allele (table 4) are unexpected and need an explanation.

e. Why is the G risk allele associated with the levels of both transaminases in the NAFLD and only with AST levels in the control group? Generally, is there any explanation for the different associations of the genotype with the same characteristics in the NAFLD and control groups (tables 4 and 5)? There is no group with ‘obese’ subjects (table 4, legend)

f. The studied SNP is consistently not associated with liver fat accumulation per se (page 10, tables 3-5, see also comment 1b). This is in contrast, both, with data from previous studies investigating the effect of this SNP on liver fat content (ref. #14, 17,19,20), and with the arguments provided in the discussion, page 12, paragraph 1. Furthermore, the putative explanation given on page 12, paragraph 2 is not conclusive, because NASH patients had higher and not lower steatosis grade than patients with simple steatosis (table 2). On the other hand, according to the results, the SNP is associated with the progression of simple steatosis to NASH and fibrosis. The authors should discuss putative mechanisms of action of the SNP/risk allele.

g. The hypothesis of an interaction of PPARGC1A with PNPLA3 on NAFLD (page 13) is too speculative and was not investigated in this study. Therefore, it should be removed from the text.

h. The authors imply that the absent relationships of the SNP with serum
transaminases in the African American, European American and German population may be due to the sample size. However, this is not correct (N>2500 subjects). They should carefully discuss the paper referenced as 20 which investigated possible mechanisms explaining these findings.

5. The typo in the title (PNPAL3) needs to be corrected
6. The manuscript needs editing in terms of the proper use of the English.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**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