Reviewer's report

Title: Role of the ABCG8 19H risk allele in cholesterol absorption and gallstone disease

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Reviewer: Markku Nissinen

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Renner and co-workers have examined several aspects in the role of cholesterol metabolism and the D19H polymorphism of the ABCG8 gene among gallstone carriers and healthy controls in their manuscript entitled “Role of the ABCG8 19H risk allele in cholesterol absorption and gallstone disease.”

The major findings of their study are that both gallstone disease and p.D19H of ABCG8 are associated with diminished cholesterol absorption. The polymorphism examined in this study did not affect intestinal expression of ABCG8. This may suggest a gain-of-function of the mutated transporter. Furthermore, the authors conclude that since cholesterol absorption was also low in gallstone carriers without this polymorphism, this genetic trait does not fully explain this characteristic of gallstone disease.

This is an interesting work dealing with alterations in the cholesterol metabolism in the pathogenesis of the gallstone disease.

Unfortunately the study has some major problems.

1) Amount of gallstone carriers is low, only 34 patients. Furthermore, the subgroups of normal weight and overweight gallstone carriers consist of only 11 and 23 patients, respectively. The data in the Table 2 reveal that there are actually no statistical significant findings between gallstone carriers and the control subjects within these subgroups. When the whole group of the gallstone carriers and the controls are compared with each other, the surrogate markers of cholesterol absorption indicate lower cholesterol absorption among the gallstone carriers compared to controls. However, the mean BMI of the gallstone carriers is significantly higher than that of the controls. This difference in the BMI value may explain the difference also in the absorption of cholesterol. Unfortunately, patients and controls are not matched with BMI.

2) Absorption of cholesterol occurs in the proximal part of the human intestine, i.e., in the duodenum and the jejunum. For the purposes of the present study, biopsy samples were collected during colonoscopy from the distal ileum. Would it have been more proper to take biopsies during gastroscopy from the distal part of the duodenum, which is a more essential site in the absorption of cholesterol, and which is easy to access during gastroscopy? Furthermore, in the abstract on line
the authors mention “…ILEAL cholesterol transporters…”.

ABCG5/G8 and NPC1L1 are actually INTESTINAL sterol transporters, not only ileal.

Minor problems/questions.

1) What was the amount of biopsy samples taken from the ileum in a single patient for the purposes of the present study? This could be mentioned in the Methods section on page 7. The amount/volume of mucosal tissue samples is an important issue for other scientists planning studies dealing with, e.g., tissue gene expression.

2) It could be mentioned more clearly in the Methods section dealing with RT-PCR, Western blot analysis, etc., from what tissue/cells were the determinations made. From leukocytes or from the ileal mucosa?

3) Were any of the subjects studied here on cholesterol lowering drugs or were they consuming phytosterol preparations at the time participating the study?

4) In the abstract, on line 4, and also in other parts of the manuscript, it is mentioned that ABCG5/G8 and NPC1L1 are CHOLESTEROL transporters. Is this the exact expression, because particularly the former one transports much more effectively plant sterols than cholesterol out of the enterocytes and hepatocytes? These are actually STEROL transporters.

Overall, the major problems and minor problems/questions mentioned above are so called “Major Compulsory Revisions”, which should be addressed in the manuscript, i.e., corrected if possible or mentioned as weaknesses of the study in the Discussion.

**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