Point-to-point reply to the comments from the reviewers for the manuscript "Meta-analysis reveals up-regulation of cholesterol processes in non-alcoholic and down-regulation in alcoholic fatty liver disease"

Dear Editor,

We would like to thank the reviewers for their constructive comments. Thank you very much for granting us the opportunity to submit a revised version of the manuscript entitled "Meta-analysis reveals up-regulation of cholesterol processes in non-alcoholic and down-regulation in alcoholic fatty liver disease".

We now provide a point-by-point response to the comments and also revised our manuscript accordingly. Revised parts of the manuscript are highlighted in yellow.

Responses to the comments from reviewer #00058872

The reviewer wrote:

Authors, instead to point out the bile excretion mediated role, should emphasise the profibrotic mechanisms stressing that bile acid stimulated cytokine release from macrophages can decrease CYP7A1 transcription via activation of the cJun terminal kinase (JNK) pathway and reduction of HNF4α binding in primary rat hepatocytes and HepG2 cells.

Our reply:

We now integrated a description of the negative feedback mechanisms by which bile acids regulate the level of CYP7A1 via JNK and reduction of HNF4α.

Discussion:

Negative feedback loops down-regulating CYP7A1 by bile acids have already been described [40]: bile acids can down-regulate CYP7A1 via (i) FXR and SHP or (ii) by interaction with liver macrophages (Kupffer cells) whose role in fibrosis has been established as they produce cytokines such as transforming growth factor beta (TGFB) leading to the transformation of stellate cells into myofibroblasts [41]. Furthermore, Kupffer cells secrete cytokines e.g., tumor necrosis factor (TNFα) and interleukin (IL-1β) which in turn induce protein kinase (PKC), c-Jun N-terminal kinase (JNK) and thus inhibit hepatocyte nuclear factor (HNF4α) and consequently CYP7A1 [42],[43]. This gives rise to the question if the lower CYP7A1 levels are a cause of steatosis or are a consequence of the profibrotic stage. Here, systems biology modelling of cholesterol fluxes in the liver including bile acids and regulatory mechanisms of CYP7A1 could be useful in determining under which condition efflux rates are too low.

Remarks to the comments of reviewer #03304651

The reviewer wrote:

Dear author, Greeting from me! The manuscript "Meta-analysis reveals up-regulation of cholesterol processes in non-alcoholic and down-regulation in alcoholic fatty liver disease" summarized the differences of cholesterol processes between non-alcoholic and alcoholic fatty liver disease,having some bright spots.It is helpful for clinical. However, In the paper, the individual place existed in double words such as "in in";individual sentences need to be further polished;additionally, figures are too much, if the figures is to borrow from others,whether some figures may be deleted. sincerely, QU Baoge 10/26/2016

Our reply:

We removed the “in in” from the Core Tip and checked the language. Furthermore, we replaced the figures 4 and 5 which were based on KEGG pathway charts by the good readable schematic view in figure 6.

Core Tip:

With a meta-analysis of newly published liver biopsy-derived transcriptome datasets we identified multiple key genes and pathways in common and mutually exclusive in alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD).

Figure 6:

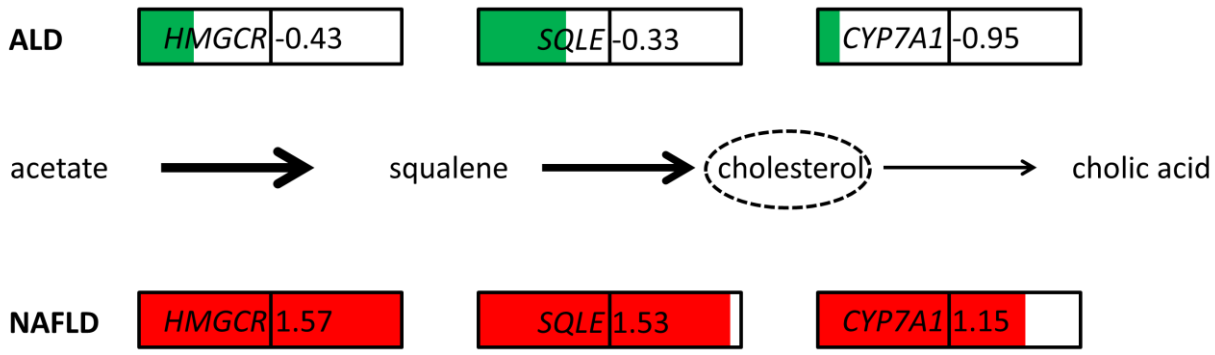

Fig. 6: Rate-limiting genes of cholesterol metabolism are down-regulated in ALD and up-regulated in NAFLD. This schematic figure shows the log2-ratios of HMGCR, SQLE and CYP7A1 indicating down-regulation in ALD (green) and up-regulation in NAFLD (red). There was stronger down-regulation of CYP7A1 (log2-ratio = -0.95) than of the upstream cholesterol genes HMGCR (log2-ratio = -0.429) and SQLE (log2-ratio = -0.33) in ALD while in NAFLD, CYP7A1 (log2-ratio = 1.15) was weaker up-regulated than HMGCR (log2-ratio = 1.57) and SQLE (log2-ratio = 1.53). The size of the arrows points to a disequilibrium between cholesterol production and secretion into the bile via CYP7A1 in both diseases despite the opposite regulation in ALD and NAFLD.

Remarks to the comments of reviewer #00053684

The reviewer wrote:

The manuscript "Meta-analysis reveals up-regulation of cholesterol processes in non-alcoholic and down-regulation in alcoholic fatty liver disease" summarized the differences of cholesterol

processes between non-alcoholic and alcoholic fatty liver disease,having interesting aspects. I suggest minor language polishing and try to reduce the number of figures.

Our reply:

We replaced the figures 4 and 5 by figure 6 as described in the comments for reviewer #03304651 and did some minor text polishing.

Remarks to the comments of reviewer #00069130

The reviewer wrote:

Sir, I read this manuscript titled, 'Meta-analysis reveals down-regulation of cholesterol processes in alcoholic and up-regulation in non-alcoholic fatty liver disease' by Wruck W et al. from Germany, with great interest. This manuscript was informative. The authors found commonalities between both ALD and NAFLD at the level of biological pathways implying some mechanistic similarity between both diseases. It may be noted that I am not a statistician/bioinformatician to judge the methods they have used. Few mistakes in language use I have noticed because of oversight. Refer page 4; do not confuse mutations which reduced risk of becoming alcohol dependent from those which offer a reduced risk of liver damage! SPINK1 is a pancreatic damage marker. Many alcoholics and obese will be having c/c (subclinical) pancreatic inflammation-coincidence/confounder? What was the control control (blue bar)-details? Fig. better resolution would be better. This is rather true with most figures. Will you please include a more detailed description of figures? Many of them are simply copy pasted from the software. Any way, the manuscript is worth publication in WJG. Hope my suggestions would be useful in making the manuscript useful for more readers. Thank you. SMG

Our reply:

We tried to clarify the description of the mutations and their association with a reduced risk of becoming alcohol dependent by adding the sentence:

Introduction:

These severe reactions will impose on most carriers of these variants to abstain from alcohol and thus reduce their risk of becoming alcohol addicts.

With respect to the association of SPINK1 with pancreas we added the following text to the Results:

SPINK1 is secreted in the pancreatic juice to reversibly inhibit activated trypsin thus preventing pancreatic auto-digestion [28] and variants in this gene have been associated with pancreatitis [29] . Obesity and more prominent alcohol abuse are other causative factors for pancreatitis [28] which by its effects on insulin may contribute to liver disease.

We described the details of the controls in the Methods section:

As controls, healthy liver biopsies or liver biopsies with a low grade of fat accumulation were used. For details we refer to the methods sections of the publications associated with the employed datasets [15],[16],[17],[18],[19],[12].

We enhanced the resolution of the figures as far as possible and replaced figures 4 and 5 which might have been problematic with the resolution by figure 6 displaying a schematic view which should be unproblematic concerning the resolution (as described in the comments for reviewer #03304651). However, when there are lists of too many gene names sometimes it will not be possible to read all names in the downscaled format of the publication but in these cases the names will be readable if the figure is zoomed to 100%.