Reviewer’s report

Title: Metabolomic Profiles of Hepatocellular Carcinoma in a European Prospective Cohort

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Reviewer: Andrew Patterson

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The manuscript “Metabolomic Profiles of Hepatocellular Carcinoma in a European Prospective Cohort” profiled the serum metabolome of hepatocellular carcinoma patients and a control cohort from Europe using an NMR approach. The authors report a number of perturbations including changes in fatty acid oxidation, amino acid, lipid and carbohydrate metabolism in the HCC patients, and suggested 16 metabolites to be significantly associated with HCC risk. Uniquely this study examined samples prior to diagnosis and therefore represents an important approach for the much needed identification of biomarkers. This study has some merit, but in its present form is preliminary and requires additional validation in other cohorts and orthogonal detection methods before any claims of biomarkers can be made.

Major Compulsory Reviews

The largest challenge in any human biomarker study is with more validation and considerations related to how these biomarkers perform outside the current cohort. It is important that the authors consider using a separate, distinct cohort in order to more strongly support the conclusions of the study.

The total numbers of HCC patients and control cohort and subgroups are very uneven (Table 1). For example, there are 37 study participants with HBV/HCV in the HCC cases, and 7 in the control cases. Also the uneven distribution of alcohol drinking pattern makes the liver function questionable, since liver is the major organ for alcohol metabolism. There are 17 former drinkers in the HCC group vs 3 in the control group, while 70 lifetime drinkers in the HCC group vs 159 in the control group. The 70 vs 159 difference might lead to a major separation of the two groups. In addition, smoking status 39 former smoker vs 86 former smoker. The potential for these variables to adversely influence the statistical outcomes is an important factor not addressed by the current approaches.

In Figure 1, signals of ethanol and propylene glycol are abnormally big, indicating the significant effects of the exogenous metabolites. These metabolites might lead to two problems: 1) their effects alone would cause significant perturbations on the metabolic profiles, regardless of the HCC case; 2) these signals from the NMR data might be the main factors resulting in the separation of the two groups. The reviewer suggests to remove these two metabolites from the data and rebuild the OPLS model to understand the overall influence of these exogenous
metabolites.

The staging of the patients is not clearly defined and it is unclear if the standard methods were followed including assessments of cirrhosis and those such as Okuda, JIS, BCLC, or CLIP. It is appreciated that HCC is a heterogeneous disease and that not one classification approach is ideal; however, the authors should indicate which they followed as opposed to referencing a previous publication. For example, AFP levels range from 0-18780, which suggest some patients were much more advanced compared to others.

The authors only used CPMG method to collect NMR data, however, this method cannot separate the lipids well. For example, in Figure 1, metabolite “1” comprise of LDL, VLDL and HDL. If these lipids can’t be separate well, the authors cannot claim the change of the lipids in general. According to Figure 3a, signal of LDL and VLDL decreased, but HDL increased, however the authors failed to identify HDL. Therefore, a diffusion NMR is suggested to separate the lipids.

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests.