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Title: The state of play in HCC NASH

Authors List: Bérénice Charrez, Liang Qiao and Lionel Hebbard

Dear Sir/madam, please find attached your corrected manuscript.

We thank the reviewers for their comments and our detailed responses are listed below.

Yours sincerely,

Lionel Hebbard.

Reviewer 1

Charrez et al reviewed recent progress in the understanding of hepatocellular carcinoma as a consequence of non-alcoholic fatty liver disease. These authors did an insightful critical analysis of current literature and often provided innovative ideas regarding the future research directions. It is an outstanding review for physicians and investigators. This reviewer suggest acceptance after minor revision.

Question 1: Minor issues: The reviewer suggests to discuss the fact that the polymorphisms (such as PNPLA3 GG) associated with NASH/HCC are not required for the development of NASH/HCC, and are not sufficient for the development of NASH/HCC. A large portion of healthy population are GG carriers; while the majority of NASH/HCC are CC or GC carriers.

Response 1: To address this we have included two sentences in page 6 (see bold and underlined). We believe that these are sufficient to elude to the reader that clinical studies have suggested links between this SNP and HCC, but the mechanism through which rs738409 promotes HCC remains to be determined. We suggest that other contributing factors are at play.

Of special interest is the work of Liu et al.,^[16] who showed that the carriage of each copy of the rs738409 (G) allele promoted an additive risk of HCC, where GG homozygotes have a 5-fold greater risk than CC. The link with HCC has been further enforced by a recent Japanese

study where the authors showed that SNP rs738409 located in *PNPLA3* was the highest risk factor in their patient cohort. Further stratification of their group showed that the *PNPLA3* G allele was significantly higher amongst HCC patients with type 2 diabetes mellitus. From this group they found a significant association between the *PNPLA3* G allele and the gene for the Juxtaposed with another Zinc Finger Protein 1 (*JAZF1*) rs864745 G allele. *JAZF1* functions as a transcriptional repressor and has been associated with the increased risk of prostate cancer and diabetes ^[18]. Importantly, a recent study has shown that *JAZF1* overexpression in mice suppressed lipid accumulation and decreased droplet size, suggesting that *JAZF1* plays a critical role in the regulation of lipid homeostasis and possibly in the prevention of NAFLD and NASH, and thus progression to HCC ^[19]. **Taken together these data suggest strong links between SNP rs738409 and increased HCC risk, but the mechanism through which this SNP can promote HCC risk remains open and will require the development of genetic murine models.**

Question 2. Page 2, Line 4, “later” should be “latter”. Page 2, Line 12, “the” should be “that”. Page 4, grammatical errors in the last four lines. Page 4, line 2, the first “NASH” should be removed. Page 4, line 12, incomplete

Response 2: These we have corrected.

Reviewer 2

Reviewers' Comments to Author: This review focus on clinical and genomic associations between NAFLD and NASH to liver cancer. The authors discuss new candidate genes as markers for increased HCC risk, and present the latest murine models to clarify some of the mechanisms in promoting liver tumor growth. The idea is new in this field and worth to be published

No questions listed. We thank the reviewer.

Reviewer 3

Charrez and colleagues reviewed the clinical and experimental evidences related to the development of HCC in the evolution of non-alcoholic steatohepatitis (NASH). The authors focused their attention on different aspects strictly related to this serious health burden taking in account polymorphisms, biomarkers, animal models, pathological mechanisms as well as potential therapeutic approaches. The manuscript appears well written and provides a critical and unbiased overview on this specific topic.

Question 1: Minor comments: Page 4 first line from the top: “HCV-related HCC increased from 43.4% to 49.9% NASH, whereas NASH...” should be changed in “HCV-related HCC increased from 43.4% to 49.9%, whereas NASH”.

Response 1: These we have corrected.

Question 2: Page 10 second paragraph the sentence “fed the mice various diets...” should be modified in “fed the mice with various diets...”

Response 2: This we have corrected.

Question 3: Page 14 “rapamycin and its analoges Everolimus” should be changed as “rapamycin and its analogous Everolimus”.

Response 3: This we have corrected to “analog”.

Question 4: Page 15 third line from the top “HCC were divided in to sedentary...” looks better as “HCC were divided into sedentary...”

Response 4: This we have corrected.