Author's response to reviews

Title: Keratin 8 variants are infrequent in patients with alcohol-related liver cirrhosis and do not associate with development of hepatocellular carcinoma

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F. Tacke/T. Shipley, 
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Dear Drs. Tacke and Shipley:

Thank you very much for giving us the opportunity to submit a revision of our manuscript titled “Keratin 8 variants and prognosis of patients with alcoholic cirrhosis” to BMC Gastroenterology and to evaluate our manuscript as an interesting and important contribution to the field. We appreciate the careful review it received and thank the reviewers for their constructive and positive comments. Below is a point-by-point reply to the Reviewer comments [shown in italics followed by our reply in blue font]. In brief, the major revisions include the addition of text throughout the manuscript to address reviewer comments. The overall conclusions of the manuscript remain the same. The edits in the body of the manuscript are highlighted in blue font for easier tracking.

Reviewer 1: We thank the reviewer for his overall positive evaluation of our work and for describing our study as being conducted in an accurate and logical manner.

the title could be a bit more informative providing information about the results. We completely agree with the comment and changed the title to “Keratin 8 variants are infrequent in patients with alcohol-related liver cirrhosis and do not associate with development of hepatocellular carcinoma.”

Reviewer 2: We thank the reviewer for her/his careful evaluation of our work and for describing our study as interesting and relevant as well as being well written.
… the authors do not distinctly state its limits which should be stated in the Discussion section.

We agree with the reviewer that it is important to clearly mention the limitation of our study. To do so, we included following statement in the “Conclusion” section: “The limited numbers of patients available as well as the low frequency of keratin variants within the cohort make it impossible for this study to conclusively clarify the significance of keratin variants in subjects with alcohol-related liver cirrhosis.”

Thus, in genetic association studies a replication cohort is strongly recommended to exclude false negative/positive results. Moreover, since the results reported are negative and the frequency of the mutated variants low, the authors should calculate the power of the analyses with respect to the mains outcomes (HCC and mortality).

Reviewer 2 points to one of the major limitations of such genetic epidemiology studies, namely the low power of analyses conducted in yet insufficient sample-size cohorts. The results of power calculations are provided for both outcomes in the revised version of the manuscript and are indeed rather low (Pages 9). To our point of view, the only way to overcome these limitations would be to conduct genetic explorations in large multicenter cohorts of patients. We emphasized this pivotal point in the conclusions (Page 11):

Furthermore, these findings must be validated in other populations, even though the cohort used herein is well studied and was used in the past to replicate several established genetic associations (references). Up to date, all reports (including ours) focusing on genetic predisposition to life-threatening events in cirrhotic patients are prone to interpretation bias related to low power analyses, a limitation that will not be overcome until coordinated work of international research consortia allows the establishment of large cohorts of patients.

Furthermore, a SUGESTION to overcome this limitation could be to genotype the cohort for a “positive control” that already showed a reproducible association with HCC in various cohorts (e.g. PNPLA3 rs738409 C>G).

We wish to thank Reviewer 2 for this excellent suggestion. Our prospective cohorts of cirrhotic patients are subjected to multiple genotyping according to the progression of knowledge in the field of genetics in chronic liver diseases. In this setting, our group has been able to report in the last ten years several report focused on HFE gene mutations (1), SOD2 (2) or PNPLA3 (3) genotypes that were conducted in the same cohort and were concordant with literature data. In the absence of a validation cohort, we totally agree with Reviewer 2 that previous genetic data obtained in the same patients partially justify the validity of the negative results presented here. As suggested, we added the following comment in the revised version of the manuscript (Page 11):
“Furthermore, these findings must be validated in other populations, even though the cohort used herein is well studied and was used in the past to replicate several established genetic associations (references 1,2,3).”

References:


3- E Guyot et al, personal communication EASL 2012

Please correct the sentence which claims that “14 patients harbored amino-acid-altering K8 variants” when the sum of variants (in brackets) is 13.

We would like to thank the reviewer for carefully reading our manuscript! However, our study indeed found 14 amino-acid-altering variants, i.e. the statement which the reviewer is mentioning is correct. The number 13 in brackets in table 2 highlights the fact, that one of the fourteen amino-acid-altering variants likely represents a polymorphism.

The objectives of the study at the end of the introduction section are confusing. I suggest that the authors clearly outline the primary (presence of HCC) and secondary (mortality) aims.

We thank the reviewer for this important suggestion. We added following statement to the introduction to clearly define the aims of our study: “To further delineate the importance of K8/K18 variants in specific human liver disorders, we studied the impact of these variants on overall and liver-related mortality as well as on development of hepatocellular carcinoma (HCC) in a prospectively monitored cohort of French patients with alcohol-related liver cirrhosis.”

In Table 2, please replace the word mRNA by DNA or cDNA.
We are really sorry about this stupid mistake and thank the reviewer for carefully reading our manuscript. We changed the term “mRNA” to “DNA”.

I suggest the authors suppress from the Abstract, the sentence claiming that K8 R341H variant may predispose to non-HCC-related liver mortality. Indeed, this does not take into account multiple testing corrections and is invalidated by log-rank analysis. We agree with the reviewer that this statement was somewhat speculative and removed it from the abstract.

**Editorial Requirements:**

*Ethics statement: Please document within the methods section of your manuscript the specific name of the organization that granted ethical approval to your study going ahead.*

As requested, we added the following information to the methods section (Page 5):

“The present work was part of a prospective study conducted in cirrhotic patients that was aimed at assessing the performance of HCC screening procedures as well as the rates and risks factors of liver cancer development in the course of various liver diseases (ref). The protocol obtained approval from the Ethics Committee (CPP, Aulnay-sous-Bois, France).”


Research involving human subjects (including human material or human data) that is reported in the manuscript must have been performed with the approval of an appropriate ethics committee. Research carried out on humans must be in compliance with the Helsinki Declaration (http://www.wma.net/en/30publications/10policies/b3/index.html). A statement to this effect must appear in the Methods section of the manuscript, including the name of the body which gave approval, with a reference number where appropriate.

As requested, we added the following information (Page 5):

The protocol obtained approval from the Ethics Committee (CPP, Aulnay-sous-Bois, France). All patients gave written informed consent to participate in the study and all research carried out in participants was in compliance of the Helsinki Declaration.

- **Figure titles:** All figures must have a figure title listed after the references in the manuscript file. The figure file should not include the title or number (e.g. Figure 1... etc.). The figures are numbered automatically in the order in which they are uploaded. For more information, see the instructions for authors: http://www.biomedcentral.com/info/ifora/figures.
We modified figure titles as suggested.

In closing, we wish to thank you again for the careful handling of our manuscript and for considering our submitted work for publication in *BMC Gastroenterology*.

Best wishes,

Pavel Strnad (on behalf of all the authors)