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Decompensated cirrhosis and microbiome interpretation

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The diagnosis of cirrhosis, especially in the advanced/decompensated stages, is made using simple and inexpensive clinico-radiologic-pathological techniques[1]. Qin *et al*.[2], whose paper has replicated prior studies[3–5], reported a relatively novel profile to diagnose cirrhosis using complex stool metagenomics despite having a majority (65% discovery and 76% validation cohorts) decompensated cirrhotic population. We have found that the decompensated cirrhosis cohort, which does not require these complicated diagnostic strategies, was responsible for a significant proportion of these microbiota changes on further analysis of their metagenomics data and using a new cohort of 360 subjects. Therefore, given several confounders and the ease of decompensated cirrhosis diagnosis using current techniques, a careful re-interpretation of newer microbiota-based diagnostic strategies that do not a priori differentiate between early (compensated) and decompensated cirrhosis and treat all people with cirrhosis as one uniform population should be performed. There is a Reply to this Brief Communication Arising by Qin, N. *et al*. Nature **5xx**, http://dx.doi.org/10.1038/nature14852 (2015).

A major confounder in people with cirrhosis are standard of care therapies such as lactulose, rifaximin, antibiotics and acid-suppressants that can affect the gut milieu[1,6]. These alone could explain a large portion of the metagenomics changes and have not been accounted for[5,7–9]. These medications, especially proton pump inhibitors, could also be a major reason why oral origin bacteria are found in the intestine, as has been shown in prospective cirrhotic and non-cirrhotic studies[10,11].

We hypothesized that there was a significant difference in compensated versus decompensated cirrhotic microbiota in Qin *et al*.[2], which needs to be accounted for in the interpretation. Using 66 enriched/depleted metagenomic sequences (MGS) provided by S. D. Ehrlich, we performed linear discriminant analysis (LDA) effect size (LEfSe)[12] after classifying them into healthy, compensated and decompensated subjects. LEFSe uses a factorial Kruskal–Wallis and LDA test to detect features with significant differential

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abundance. We found that even in the selected data set the authors provided, 17 of 66 MGS were different between compensated and decompensated groups (10 MGS overexpressed and 7 MGS underexpressed, Fig. 1a). These included several oral origin species (*Streptococcus* oralis and several *Veillonella* spp.), which were the primary study results. We then enrolled 360 age-matched subjects (45 healthy individuals (age 54 ± 3 years, no chronic diseases), 171 compensated (age 54 ± 4 years, median Child–Pugh score 6) and 141 decompensated cirrhotic patients (age 55 ± 2 years, median Child–Pugh score 9)) for stool multi-tagged pyrosequencing (MTPS)[13]. Using Kruskal–Wallis analysis of relative microbial family abundance >1%, we found that compensated and decompensated patients were significantly different (Fig. 1b). Proteobacteria levels, specifically *Enterobacteriaceae*, were significantly higher in decompensated cirrhotic patients. This pattern is also seen in other recent MTPS studies[4,14]. Although MGS and MTPS are not completely comparable, it is interesting that both resulted in similar conclusions. Therefore, there are significant microbiota differences between compensated and decompensated patients that need to be separated in cirrhosis microbial studies.

In addition, in Qin *et al*.[2] the calculation of the model for end-stage liver disease (MELD) score in Supplementary Table 1 is inaccurate, casting doubt on figure 2. The authors compared diabetes patients with cirrhotic patients to inform their cirrhosis-associated profile. However, diabetes is prevalent and is associated with a poor prognosis in cirrhosis[15]. Therefore these results are not generalizable to patients with cirrhosis and diabetes.

The present need is not for complicated profiles that are unlikely to supplant currently available simple diagnostic strategies, but rather for improving prognostication. This is because gut microbiota are associated with several cirrhosis-related pre-terminal events such as hepatic encephalopathy and infections[1]. A prior study has shown that altered stool microbiota can predict poor outcomes, but further work is required[8].

Therefore, the careful separation of the two groups within cirrhosis, which have different diagnostic criteria and prognoses, and the control of confounders owing to drugs mentioned above are important for the correct interpretation of these results and to avoid epiphenomena.

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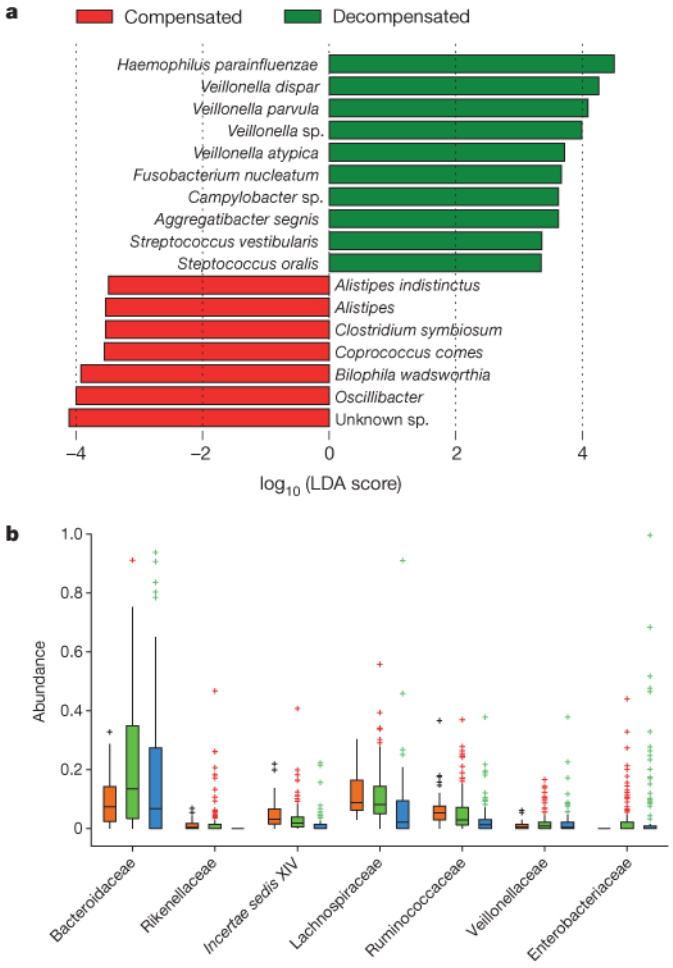

Figure 1. Microbiota distribution between compensated and decompensated cirrhotic subjects

a, LFSe plot showing metagenomic species that are overexpressed (green) and under-expressed (red) in decompensated compared to compensated cirrhosis from Qin *et al.*[2]. **b**, In the new data set using MTPS, boxplots showing interquartile range of median abundance of statistically significant comparisons between controls (orange), compensated cirrhosis (green) and decompensated cirrhosis (blue) using multiple corrections-adjusted Kruskal–Wallis tests at the family level. The line in the centre shows median.