

Reviewer Report

Title: Two distinct metacommunities characterize the gut microbiota in Crohn's disease patients

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Reviewer name: Nadeem Kaakoush

Reviewer Comments to Author:

The manuscript by He et al presents shotgun metagenomics data and analysis on healthy controls and CD patients before and after exclusive enteral nutrition. The results presented are interesting and the authors are to be commended for such deep sequencing (~55 million reads) of their samples. However, I do have several comments that I believe need to be addressed.

1. I am quite surprised at the level of unclassified sequences at the phylum level in Figure 1 and Figure 3. The authors data (in Figure 3 at least) seems to focus on four phyla. Some recent papers have detected up to 21 phyla in fecal samples using shotgun metagenomics (Bonder et al 2016 Nature Genetics). Could this be a classification issue?
2. A marker based tool such as Metaphlan2 would provide taxonomic classification to be compared to the authors classifications. The authors can also perform hierarchal clustering through Metaphlan2 to confirm their metacommunities.
3. There seems to be an absence of microbial eukaryotic and viral annotated reads. Can the authors address this?
4. Based on all the patient metadata available, I believe the authors should apply MaAsLin in addition to their application of LEfSe on the data.
5. The authors refer to ~55 million reads per sample. This level of depth can result in strain level analysis and perhaps even partial genomic assembly of more abundant bacterial strains. Functional inferences can be obtained from much less reads per sample. Can the authors address this?
6. Most studies utilizing EEN as a therapy do this over a long period of time (6-8 weeks). Can the authors justify limiting the EEN to 2 weeks? Could this explain the limited effects the authors observed?
7. Citations of work related to CD and EEN are missing. There are several studies on this topic that have shown microbiome changes, including one study that has utilized shotgun metagenomics. The authors should also correct their statement in the introduction related to this.

Please refer to the following paper (Quince C et al 2015 Am J Gastroenterol).

8. The authors have provided two titles, one during submission and one at the start of the manuscript. I assume the submission title is the relevant one?

Methods

Are the methods appropriate to the aims of the study, are they well described, and are necessary controls included? Yes

Conclusions

Are the conclusions adequately supported by the data shown? Yes

Reporting Standards

Does the manuscript adhere to the journal's guidelines on [minimum standards of reporting?](#) YesChoose an item.

Statistics

Are you able to assess all statistics in the manuscript, including the appropriateness of statistical tests used? Yes, and I have assessed the statistics in my report.

Quality of Written English

Please indicate the quality of language in the manuscript: Acceptable

Declaration of Competing Interests

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