March 15, 2019

Dear Editor,

The revised manuscript has been submitted according to your requirements.

Title: Characterization of mucosa-associated gut microbiota during treatment in Crohn's disease

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Manuscript Type: Basic Study

The responses to the reviewer's comments are as following:

Reviewer #1:

In this work, He et al. compare the microbiome of patients before and after treatment from endoscopic biopsies from different sites. It is important for the field of metagenomics to gather information on as many different populations as possible due to the significant compositional differences those exhibit. The paper is well written and easy to follow. The background section is informative. The discussion is extensive, and the limitations of the paper are described sufficiently.

Although, it is an interesting study there are some points to be addressed in methodology and results sections: The methodology is consistent with previous metagenomic studies but lacks in terms of tools used for the analyses and figure generation. For example, it is evident that Figure S4, generated from the PICRUSt

results, was created via STAMP, but it is not mentioned anywhere.

Author reply: Thank you for your questions and comments. The methods applied in the study have been added in the revised manuscript marked in red.

Furthermore, although they decided to collect samples before and after treatment from the same patients no direct pairwise comparisons were reported. We understand that statistical significance is next to impossible to achieve between two samples, but comparisons could have given a better understanding of the dysbiosis.

Author reply: Thank you for your questions and comments. The paired comparison between two samples before and after treatment has been conducted and the differential taxa were fewer than those identified using Metastats, probably due to the small sample size. Further studies are warranted to enlarge the sample size and validate the specific taxa associated with disease activity by paired comparison.

Regarding the results there are some surprising finds. The authors report that no differences were found between anatomical sites, a finding that contradicts knowledge from previous works (e.g. Tropini C, et al. 2017).

Author reply: Thank you for your questions and comments. A previous study by Tropini C et al. presented that it is necessity to apply not only computational but also imaging tools to investigate the spatial organization of gut microbiota and its relationship with health (Cell Host Microbe 2017 Apr 12;21(4):433-442). They also reviewed the study by Wang et al. who showed that the microbial community in jejunum was different from those in distal ileum, ascending colon and rectum, and that the major phylogenetic groups were similar from distal ileum to rectum using 16S rRNA sequencing, which was consistent with our study (FEMS Microbiol Ecol 2005 Oct 1;54(2):219-231). Similar observations have been recently reported by Nishino K et al., who also found that there were no significant differences in microbial structure among different anatomical sites (the ileum, cecum and sigmoid colon) within individuals. Thus, the overall gut microbial community might be more dependent on inter-individual variation than on anatomical site (J Gastroenterol 2018

Jan;53(1):95-106).

Also, there is no characterization of the CD's behavioral phenotypes in their samples or any reference in this point, which recent works have highlighted to be linked to microbial composition and function (e.g. Dovrolis N, et al 2019).

Author reply: Thank you for your questions and comments. The behavioral phynotype of patients in our study were all nonstricturing and nonpenetrating, which may not affect the analysis of microbial composition and function.

Best regards

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