## COMMENTS TO AUTHORS

Specific comments - I suggest in order to reach a better comprehension of the alpha diversity to choose Shannon or Simpson's index and afterward to give a more complete information associating the index differences with a value of community similarity (i.e. Sorenson's Coefficient). - If you use the Simpson's index you should specify which formula you applied (there are two, both accepted, but they give different results).

**Re**: Thanks for your professional suggestion. We once used the Shannon index, and some other reviewer demanded us to use these two indexes. The two indexes have different performance. The Simpson-based metrics are less affected by sampling effort as the Shannon index. And we even calculated and compared several other indexes, including the Chao index, the nonparametric Shannon index, the Sobs (number of observed OTUs) etc. The significant differences may vary but the overall trend is similar when choosing different indexes. Since it is impossible to cover all the indexes, we choose the most widely used Shannon and inverse Simpson's indexes. In the revised manuscript, we appended the link to the detailed formula.

" The Shannon index and the inverse Simpson index (1/D) were calculated to indicate the diversity in each sample. Both indexes were calculated using Mothur, and the detailed formula can be accessed online (http://www.mothur.org/wiki/Shannon, and http://www.mothur.org/wiki/Simpson)."

Both the Shannon index and the inverse Simpson index indicate the diversity of one given sample, while the community similarity index compare two or more samples. The community similarity has been already demonstrated in the PCA analysis in a more intuitive manner and thoroughly compared using the UniFrac test (Table 2). Thus we deem it unnecessary to calculate the Sorenson's Coefficient.

In the sentence "the 3 groups' distribution in the clusters is significantly different (P = 1.702×10-4, Fisher's test)" I understand that you used the Fisher test on 3 objects while he Fisher's test can be used only on binary nominal value. Did I misunderstand? Could you clarify this point?

**Re**: The Fisher test can indeed be calculated on 3 objects. The Handbook of Biological Statistics (http://udel.edu/~mcdonald/statfishers.html) gives the introduce as following:

" Fisher's exact test is used when you have two nominal variables. A data set like this is often called an "R×C table," where R is the number of rows and C is the number of columns. "…" The most common use of Fisher's exact test is for 2×2 tables…"

So it is OK to use the Fisher test among 3 groups, and it is more precise than the Chi-square test. The SAS software program perfectly do the calculation.

- In the paper you constructed the helix grouping the 75% of the samples. In the figure it looks like not follow this rule (for example the helix Mixed Cluster 1 in figure 4C).

**Re**: Thanks for your careful check. The helix (ellipse) was automatically draw by R and we check the source code:

"ordiellipse(pc12,groups=groups,conf= 0.75,draw="polygon",col="gray",alpha=32)"

The "conf= 0.75" indicate that the ellipse was estimated to cover 75% of the group. You may check the Cluster 3 in Figure 4C (easier to count the dots). 3 of the 16 sample fall outside and 81.25% samples were covered. That does not significantly deviate from the 75% ratio. The Cluster 1 has too few sample (n=3) and was too slim to check the ratio.

- In figure 5, I suggest to indicate the complete taxonomy to the unclassified taxa to give at least the family information to the reader.

**Re**: Thanks for your kind suggestion. We agree that the complete taxonomy of the unclassified taxa should be given. In the revised manuscript, we provid a supplemental file with the bar plot for the biomarkers in figure 5, which contain the full taxonomy.

- Spearman results are in table 3 instead of table 2 as reported on the manuscript.

**Re**: Sorry for the wrong number. We corrected in the revised manuscript.

- it would be better to use the world "genera" as plural of genus instead of "genuses".

**Re**: Thanks for your kind suggestion. We searched and replaced the words.

- the taxonomic meaning of "Clostridium XI" has to be clearly explained.

The Clostridium XI includes the Clostridium difficile, Clostridium litorale, and Clostridium lituseburense. We added the information in the revised discussion section.

- a review paper concerning microbiota analysis and IBS was published quite recently in the World Journal of Gastroenterology (World J Gastroenterol 2014 July 21; 20(27): 8821-8836); in these study, Porphyromonadaceae and Fusobacterium are mentioned as well: could you please comment possible analogies/discrepancies in the scientific informations between that study and yours?

**Re**: Thanks for your kind suggestion. We cited this paper and added relevant discussion:

"A recent review paper [35] summarized 29 relevant original research articles concerning microbiota analysis and IBS. Durbán's pyrosequencing study [36] found that the family Porphyromonadaceae were increased in the fecal samples of IBS subjects. In our study, the Porphyromonadaceae was highest in the control group by week 12. The discrepancy may be explained by the different nature between human patients and rat model."

Fusobacterium was not mentioned in this review.

## COMMENTS TO AUTHORS

In the present paper, entitled "Visceral hypersensitive rats share common dysbiosis features with human irritable bowel syndrome", two different mouse models of IBS have been studied, and correlations with alterations in microbiota have been evaluated. This is an excellent paper. The quality of the study design and experimental investigations are very high. Main comments: A minor linguistic revision is needed.

**Re**: Thanks for your proof reading. This paper has been edited by native English speaker who is a specialist in bioinformatics. We also checked the carefully and made sure that no further linguistic problem exists.

Usually, in human beings, the post-inflammatory IBS develops after an infectious disease, such as gastroenteritis. However, in the present study the equivalent animal model was developed by using a pro-inflammatory molecule (TNBS). Therefore, this model is closer to human IBD rather than IBS. This aspect should be discussed, since even IBD shows visceral hypersensitivity.

**Re**: Thanks for your professional opinion. We do acknowledge that pTNBS resembles the IBD to

some extent. However, we must take the following factors into consideration: 1) Post-inflammatory rodent models are frequently used to study the mechanism of IBS; 2) To date no available model could ideally model the IBS pathogenesis; 3) IBS is heterogeneous and thus unlikely to be modeled in any single model. This is why we used two models (MS and pTNBS) in this study. We believe this design could minimize the bias of each model, and the combined results would be less subject to each model's limitation. We added the following discussion:

"IBS is a human disease with multifactorial pathophysiology[37], and the prevalence of irritable bowel syndrome is associated with social-economic factors.[38] To date no available model could ideally model the IBS pathogenesis. IBS is heterogeneous and thus unlikely to be modeled in any single model. Although common biomarkers were found between human IBS patients and rat models, the limitations of rat models should also be taken into consideration. The pTNBS model was triggered by a pro-inflammatory molecule (TNBS). Therefore, this model resembles the human inflammatory bowel disease to some extent and can only mimic the post-infectious IBS, which is associated only to a percentage of patients."

COMMENTS TO AUTHORS

The manuscript is excellent and addresses adequately the relationship between dysbiosis and visceral hypersensitivity in experimental animals. However, I would add a few points regarding the comparison between the findings in humans with IBS and experimental studies. I think that will be necessary to include them in the discussion of results.

1-IBS is a human disease with multifactorial pathophysiology understood through a biopsychosocial model . This concept needs to be emphasized in the discussion.

**Re**: Thanks for your suggestion. We added relevant discussion:

"IBS is a human disease with multifactorial pathophysiology[37], and the prevalence of irritable bowel syndrome is associated with social-economic factors.[38] To date no available model could ideally model the IBS pathogenesis. IBS is heterogeneous and thus unlikely to be modeled in any single model. Although common biomarkers were found between human IBS patients and rat models, the limitations of rat models should also be taken into consideration."

2-The post-infectious IBS is associated only to a percentage of patients.

**Re**: We added relevant discussion:

"The pTNBS model was triggered by a pro-inflammatory molecule (TNBS). Therefore, this model resembles the human inflammatory bowel disease to some extent and can only mimic the post-infectious IBS, which is associated only to a percentage of patients."

3- I consider necessary discussion about the question "hypersensitivity visceral and dysbiosis are a cause or consequence of the symptoms of IBS "?

**Re**: We added relevant discussion:

" Furthermore, the causal relationship between hypersensitivity visceral, dysbiosis, and the symptoms of IBS is not clear and remains to be untangled in the future."

4-Finally I would like to point out that this study is a consistent contribution regarding IBS dysbiosis and visceral hypersensitivity in experimental models. I suggest reading two articles published by our research group. They provide relevant clinical information about IBS in humans

that can be useful in the discussion. Soares, R. L. (2014). Irritable bowel syndrome: A clinical review. World Journal of Gastroenterolog: WJG, 20(34), 12144–12160. http://doi.org/10.3748/wjg.v20.i34.12144 Soares RL, dos Santos JM, Rocha VR. Prevalence of irritable bowel syndrome in a Brazilian Amazon community. Neurogastroenterol Motil. 2005;17:883.

**Re**: Thanks for your suggestion. We read these two paper and cited them in the revised manuscripts.