

Dear Professor Tang:

Manuscript NO: 51357

Title: Altered profiles of fecal metabolites correlate with visceral hypersensitivity and may contribute to symptom severity of diarrhea-predominant irritable bowel syndrome

Thanks a lot for your precious work in the management of our manuscript. Dr Reviewers' comments help improve the quality of our paper greatly.

All the authors have seriously considered the reviewers' comments and found them extremely helpful. We revised the manuscript in accordance with the reviewers' comments, and carefully proof-read the manuscript to minimize typographical and grammatical errors. The relevant regulations had been made in the manuscript according to the comments of reviewers, and the major revised portions were highlighted in yellow in the revised version of manuscript.

We also responded to the comments point by point as listed below, along with a clear indication of the location of the revision. Hope these will make it more acceptable for publication.

All the best.

Yours Sincerely,

Shu-Kun Yao

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Answering editor

1. the accuracy of general information for our manuscript

Title: Altered profiles of fecal metabolites correlate with visceral hypersensitivity and may contribute to symptom severity of diarrhea-predominant irritable bowel syndrome

Authors: Wen-Xue Zhang, Yu Zhang, Geng Qin, Kai-Min Li, Wei Wei, Su-Yun Li and Shu-Kun Yao

2. The correspondence in author's E-mail address must be issued by his/her institution

Answer: China-Japan Friendship Hospital did not issue E-mail address for their staff. We use the same E-mail address with articles which were published in WJG^[1, 2].

Answering reviewer 03000422

Thank you very much for giving us such valuable suggestions. We have made revisions point by point.

1. It is better to make some Figures to show the significant correlation between IBS-SSS score and metabolites or maximum tolerable threshold and groups in Table 3 or 4.

Answer: Thank you very much for your kind reminder and precious suggestion. We made Figure 2 based on Table 4 and 6 which was Table 3 or 4 previously.

2. Future direction based on this study should be written in discussion if possible.

Answer: Thank you for your kind suggestion. We written future direction in discussion (Page 19, Line 5-7) and in research perspectives of article highlights (Page 20, Line 27-Page 21, Line 6). They were highlighted in yellow in the revised version of manuscript.

Answering reviewer 02531171

Thank you very much for giving us such valuable suggestions. We have made revisions point by point.

Introduction: Justification for another correlative study should be strengthened.

Answer: Thank you for your kind suggestion. These correlative study are credible. The sentence has been corrected in the revised manuscript: There have been only a few articles reporting that some fecal metabolites were correlated with symptoms in IBS (Page 8, Line 9-11).

Methods:

Justify how the group sizes were determined.

Answer: Thank you very much for your kind reminder. A few studies investigated the fecal metabolite compositions were different in subjects with or without IBS^[3, 4]. Based on the sample size of these studies, we recruited thirty patients. We described it in Material and Methods section. (Page 8, Line 20)

Discuss the gender disparity (particularly in the context of female prevalence).

Answer: Thank you very much for your kind reminder and precious suggestion. On the one hand, in contrast to male patients, more female patients refused to participate. On the other hand, women with a history of dysmenorrhea or cesarean section were excluded. We described it in more detail in DISCUSSION section. (Page 16, Line 1-5). They were highlighted in yellow in the revised version of manuscript.

Describe collection of faecal samples.

Answer: With suggestion, we described the collection of fecal samples in Material and Methods section. (Page 9, Line 19 - 21) They were highlighted in yellow in the revised version of manuscript.

Results: Not clear why some of the data is included in supplementary tables – it would be better to present all data in the main manuscript.

Answer: Thank you very much for your kind reminder and precious suggestion. We presented all data in the main manuscript.

Discussion: The statement by the authors that ‘the metabolites in feces are the origin of symptoms in IBS patients’ is very speculative in a multifactorial disorder. Neither does the correlative design of this study denote causality.

Answer: Thank you for your careful review. The sentence has been corrected in the revised manuscript: there has been growing evidence that some metabolites in feces are correlated with symptoms in IBS patients. (Page 15, Line 18 - 20) They were highlighted in yellow in the revised version of manuscript.

Answering reviewer 00008491

Thank you very much for giving us such valuable suggestions. We have made revisions or explanation point by point.

1. My major concern regards the lack of standardization of the diet during the study period. Although few studies showed that a dietary change can improve IBS symptoms, in absence of microbiota change, there were several evidences that dietary habit plays a fundamental role in selecting the microbiota and probably the fecal metabolite composition.

Answer: We appreciate this constructive suggestion. On the one hand, habitual diet determines the repertoire of microbial metabolites. on the other hand, food components also plays a significant role in the pathophysiology of IBS^[5-7]. Although diet was not standardized in this and other studies^[3, 4, 8], it is necessary to standardize diet in the future study. We described it in more detail in DISCUSSION section. (Page 18, Line 21-28). They were highlighted in yellow in the revised version of manuscript.

2. The choice of “individuals who had undergone colorectal cancer screening or polyposis follow-up and had negative results”, as healthy controls is questionable. How was IBS – which is a functional disease - excluded ?

Answer: Thank you very much for your kind reminder and precious suggestion. The sentence has been corrected in the revised manuscript: Healthy controls were recruited from asymptomatic individuals who had undergone colorectal cancer screening or polyposis follow-up and had negative results. (Page 8, Line 24-27). They were highlighted in yellow in the revised version of manuscript.

1 Zhang Y, Qin G, Liu DR, Wang Y, Yao SK. Increased expression of brain-derived neurotrophic factor is correlated with visceral hypersensitivity in patients with diarrhea-predominant irritable bowel syndrome. *World J Gastroenterol* 2019; **25**(2): 269-281 [PMID: 30670915 PMCID: PMC6337018 DOI: 10.3748/wjg.v25.i2.269]

2 Liu DR, Xu XJ, Yao SK. Increased intestinal mucosal leptin levels in patients with diarrhea-predominant irritable bowel syndrome. *World J Gastroenterol* 2018; **24**(1): 46-57 [PMID: 29358881 PMCID: PMC5757124 DOI: 10.3748/wjg.v24.i1.46]

3 Shankar V, Reo NV, Paliy O. Simultaneous fecal microbial and metabolite profiling enables accurate classification of pediatric irritable bowel syndrome. *Microbiome* 2015; **3**: 73 [PMID: 26653757 PMCID: PMC4675077 DOI: 10.1186/s40168-015-0139-9]

4 Tana C, Umesaki Y, Imaoka A, Handa T, Kanazawa M, Fukudo S. Altered profiles of intestinal microbiota and organic acids may be the origin of symptoms in irritable bowel syndrome. *Neurogastroenterol Motil* 2010; **22**(5): 512-519, e114-515 [PMID: 19903265 DOI: 10.1111/j.1365-2982.2009.01427.x]

5 De Giorgio R, Volta U, Gibson PR. Sensitivity to wheat, gluten and FODMAPs in IBS: facts or fiction? *Gut* 2016; **65**(1): 169-178 [PMID: 26078292 DOI: 10.1136/gutjnl-2015-309757]

- 6 Gibson PR, Varney J, Malakar S, Muir JG. Food components and irritable bowel syndrome. *Gastroenterology* 2015; **148**(6): 1158-1174 e1154 [PMID: 25680668 DOI: 10.1053/j.gastro.2015.02.005]
- 7 Staudacher HM, Lomer MCE, Farquharson FM, Louis P, Fava F, Franciosi E, Scholz M, Tuohy KM, Lindsay JO, Irving PM, Whelan K. A Diet Low in FODMAPs Reduces Symptoms in Patients With Irritable Bowel Syndrome and A Probiotic Restores Bifidobacterium Species: A Randomized Controlled Trial. *Gastroenterology* 2017; **153**(4): 936-947 [PMID: 28625832 DOI: 10.1053/j.gastro.2017.06.010]
- 8 Shankar V, Homer D, Rigsbee L, Khamis HJ, Michail S, Raymer M, Reo NV, Paliy O. The networks of human gut microbe-metabolite associations are different between health and irritable bowel syndrome. *ISME J* 2015; **9**(8): 1899-1903 [PMID: 25635640 PMCID: PMC4511929 DOI: 10.1038/ismej.2014.258]