

Dear Professor:

Manuscript NO: 53507

Title: SERT and CCK in diarrhea-predominant irritable bowel syndrome: associations with abdominal pain, visceral hypersensitivity and psychological performances

Thanks a lot for all the work done by staff in revising and publishing our manuscript.

All the authors have seriously considered the opinions of the reviewers, and think that comments are very constructive and greatly improve the quality of our paper.

We have revised the manuscript according to the reviewer's comments. The main revised parts are highlighted in yellow. We have responded to each of the comments listed below, with a clear indication of the location of the revision. Hopefully these will make the manuscript easier to publish.

All the best.

Yours Sincerely,

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Answering editor

Verify the accuracy of general information for your manuscript

Answer: The general information you listed about my manuscript is correct.

Answering reviewer 03723709

the author performed an observational study titled "SERT and CCK in diarrhea-predominant irritable bowel syndrome: associations with abdominal pain, visceral hypersensitivity and psychological performances". The methodology is sound but the manuscript need critical English editing by native English speaker.

Answer: Thank you very much for your advice of language quality. We have had the manuscript edited by American Journal Experts. Grammatical errors were checked thoroughly and corrected. Hope the language of the manuscript reach A level.

Answering reviewer 00504545

This is a very interesting study exploring the pathogenesis of patients with IBS with predominant diarrhea. The authors have found that both CCK and SERT mediators may be involved in the origin os this disease open a new way of conducting their symptoms and suggestin also new strategies for control and treatmente of this disease in the future.

Answer: Thank you for your affirmation of our research direction and significance. We hope it will bring new ideas to other researchers.

Answering reviewer 02546836

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

1. The distribution of male /female patients is an issue since IBS is more common in females and especially in Asia. So generalized conclusions would be hard to make.

Answer: The male/female ratio reported in our manuscript is the real result, which we can guarantee

internal authenticity. We have elaborated the details in the limitations (Page 12, Line 2~5). These limitations might contribute to this result. In addition, it may be related to the following reasons. First, in contrast to male patients, more female patients refused to participate. Second, women with a history of dysmenorrhea or cesarean section were excluded.

2. Measuring a transport protein (SERT) and not receptor site activity specially (S3 or S4) doesn't tell on a lot of the role of serotonin effects on the gut mucosa or the gut-brain axis. It is already well known that serotonin is involved in IBS. CCK is a prokinetic agent but its effect in the colon is mainly by motility.

Answer:

Part 1: Previous studies have shown that SERT in different cells has the same molecular characteristics, so we inferred that the changes of SERT transport function in colonic mucosa also occur in the brain (Jin *et al.*, 2016). We supposed that the expression of mucosal SERT is related to the psychological symptoms of IBS patients. Patients with low SERT protein expression were more likely to have negative emotions (somatization, anxiety, depression, hostility). SERT is a transport protein. 5-HT can not be transported in time owing to low expression of SERT, which makes 5-HT accumulation in synaptic space (Yu *et al.*, 2016). At present, our results do not involve the performance of 5-HT acting on different receptors (S3/S4) (Rebholz *et al.*, 2018), which will be the focus of our next research direction.

Part 2: Previous studies have shown that intravenous CCK can enhance colonic motility and induce abdominal pain. There was a positive correlation between CCK concentration in plasma and sigmoid transit contractile movement (including the amplitude and duration of migrating contractile wave). There was a positive correlation between CCK concentration in plasma and non-transit contractile movement of sigmoid colon (van der Schaar *et al.*, 2013).

3. What was your hypothesis to do this study?

Answer: SERT and CCK might be associated with anxiety, depression and abdominal pain, which were involved in the pathogenesis of IBS-D by regulating the brain-gut axis and affecting visceral sensitivity.

4.Introduction gave a good review of IBS but would expand more on the functions of CCK and 5-HT and why one would theorize there would be a correlation with IBS (You could discuss what each does normally in the gut as well as how they influence depression and anxiety).

Answer: Thanks for your kind reminder. We have supplemented relevant background which was highlighted in yellow in the revised version of manuscript (Page 5, Line 25~30; Page 6, Line 1~9).

5.You mention Rome-IV, would describe the definition for at least IBS-D.

Answer: We have supplemented relevant definitions(Bai *et al.*, 2017) which was highlighted in yellow in the revised version of manuscript (Page 5, Line 8~12,14~16).

6.The sentence, "a meta-analysis found that patients with IBS...depressive symptoms than healthy control, which MEANS (would change means to suggests).

Answer: We have changed wording which was highlighted in yellow in the revised version of manuscript (Page 5, Line 23).

7.Also would define initial, defecating and maximum tolerable thresholds.

Answer: The definition of initial, defecating and maximum tolerable thresholds(Mertz *et al.*, 1995) have been highlighted in yellow in the revised version of manuscript (Page 7, Line 23~26).

8.Please clarify the meaning of "partial patient spouses."

Answer: "partial patient spouses" refers to some IBS patients' spouses who had no symptoms, satisfied our inclusion and exclusion criteria, and had no organic lesions confirmed by colonoscopy, etc.

9.The control group just needs to be age and sex match with no Rome IV criteria.

Answer: As is shown in *Study population*, the patients must be recruited by Rome IV, while the control group just needs to be age and sex match. Related content has been highlighted in yellow in the revised version of manuscript (Page 6, Line 23~25).

10.For the Visceral Hypersensitivity Test, there seems to be a part missing as it is not clear what the

"obstruction," is.

Answer: Before visceral sensitivity test, digital rectal examination is needed to exclude rectal cancer, because some rectal cancer presenting as a mass can found during digital rectal examination. The word "obstruction" is not appropriate here, so we change it to "mass" (Page 7, Line 18).

11.Results 3.2 (Error) States CCK and SERT are higher in IBS-D but only CCK is higher in your study.

Answer: We have made the textual description consist with the statistical results, which highlighted in yellow in the revised version of manuscript (Page 10, Line 19~20).

12.Discussion is a good succinct and accurate deduction of the data. Would address the fact that 75% of patients in study are male and how this may skew data as IBS and depression/anxiety are more common in females.

Answer: See Answer 1 for details.

Reference

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