POINT-BY-POINT RESPONSE

Name of Journal: *World Journal of Gastroenterology*
Manuscript Title: Melanocortin 3,5 Receptors Immunohistochemical Expression in Colonic Mucosa of Inflammatory Bowel Disease Patients: A Matter of Disease Activity?
Manuscript ID: 91102

[REVIEWER 1]

SPECIFIC REVIEWER COMMENTS TO AUTHORS

1) Minor language polishing needed (...Patients with conditions that, in the investigators' judgment, might have led to bias in the study (e.g., patients with decompensated comorbidities or ongoing severe acute conditions such as decompensated diabetes or severe cardiovascular disease)...this sentence doesn't make sense;
2) Methods …in the case of suspected UC (continuous inflammatory involvement without skip lesions and with rectal involvement)…So CD patients L2 (Montreal classification) were considered as UC? - were samples taken from the same site in colon (ascendens/descedens…) in Crohn's and UC (were any samples in CD taken from the left colon (in UC they were all from the left colon/rectum))?
3) in multisegmental involvement and in pancolitis, were samples taken from the most affected area?
4) Results …Like the CD, MC3R labelling in UC samples was significantly higher in inflamed mucosa (fold: +12, $p < 0.01$ vs normal mucosa)…there is a difference in results in the text and in the abstract (+12 and +5,6);
5) Has a correlation been made between the endoscopic scoring system and the number of receptors?
6) Discussion -… in the segments of the intestine most affected by histological damage…– samples were always taken from the most affected site (not clear in methodology)?
7) I understand the therapeutic potential of MC3,5R agonists in UC since the colon is involved, but what the about the patients with CD and ileocolonic or jejuno-ilieal involvement? This could be very briefly discussed in the last paragraph before the strengths and limitations. Limitations -in CD patients only colon was tested for MC3,5R. Is there an evidence in the literature of the presence of those receptors also in small bowel?
8) Fig. 4 a 5 – Data are expressed as % ± S.E.M. of MC3R positive cells / total cells counted…Shouldn't be MC5R instead of MC3R?

RESPONSES TO REVIEWER:

We cordially thank the reviewer for his/her comment on the quality of our review. We thank the Reviewer for the time and effort spent in reviewing our manuscript.

1) Certainly, we appreciate the feedback and thank the reviewer for the precious advice. Here's the revised and corrected sentence: "Patients presenting conditions that, according to the investigators' assessment, could potentially introduce bias into the study (for instance, individuals with decompensated comorbidities or those currently experiencing severe acute conditions such as decompensated diabetes or severe cardiovascular disease) were excluded." We hope this addresses the concern raised by the reviewer.
2) We appreciate the reviewer for seeking clarification. Patients with Crohn's disease (CD) and colonic involvement (L2) underwent biopsy sampling in apparently normal mucosal segments (CDEIS < 3) as well as in segments affected by the disease (CDEIS > 2). Patients with CD L2 were not categorized as ulcerative colitis (UC) since, based solely on our

pancolonoscopies, a patient with UC could be suspected if there was involvement of E1 or E2 (indicating rectal or rectal + sigmoid + entirely affected left colon), which was incompatible with L2 (indicating segmental colitis skipping with normal areas interspersed between macroscopically inflamed areas). For UC, normal mucosa samples in the case of E1 could come from the sigmoid, descending, transverse, and ascending colon. In the case of E2, they could come from the transverse and ascending colon. In the case of Crohn's L2, samples had to be obtained from areas with CDEIS < 3 (including the left side). Similarly, in the case of L3. In the case of samples from diseased mucosa, following the Montreal classification, they could naturally originate from the incriminated region of inflammation (CDEIS > 2 or Mayo endoscopic > 0).

3) We thank the reviewer for this additional request for clarification. Patients with pancolitis (E3) were excluded from the study from the outset. Patients with multisegmental Crohn's disease (CD) received biopsies only in segments with CDEIS > 2. In other words, assuming the endoscopist identified three segments with CDEIS scores of 3, 4, and 6, the biopsies were not exclusively directed towards the segment with a score of 6.
4) Thank you very much to the reviewer for identifying this typographical error. We have promptly corrected it. It should be noted that the error was in the abstract and not in the main body of the text.
5) We appreciate the reviewer for this request. The sample size in our study does not allow us to achieve the statistical power required for the correlation analyses suggested by the reviewer. It's important to note that this aspect was not originally among the study outcomes, but we agree with the reviewer on the importance of conducting this assessment. Consequently, we have expanded the discussion by adding the following paragraph "*Moreover, it could be intriguing from a translational standpoint to investigate whether there exists a correlation between MC_3R-MC_5R expression and endoscopic disease activity, assessed using validated tools and scores [22], aiming to identify a potential direct association. However, it is important to note that the limited sample size in our dataset hinders the execution of correlation analyses necessary to unveil statistically significant findings*".
6) We thank the reviewer for this clarification. The sentence highlighted by the reviewer was indeed confusing and seemed to imply a methodological measure that we did not apply. Therefore, the sentence has been completely restructured as follows "*The present study suggests a distinctive expression pattern of MC_3R and MC_5R in the colorectum of patients with IBD. It proposes that their expression could be hypothetically linked to disease activity, indicating a heightened presence in segments of the large bowel affected by histological damage. To the best of our knowledge, such a profile of immunohistochemical expression has not been previously reported [7]."*
7) We thank the reviewer for this request. We added the text as requested. Please see "*In CD, the data in this study are, by its design, restricted to patients with disease extension L2 or L3 according to the Montreal classification and, in any case, exclusively within the colonic microenvironment. Consequently, it is important to consider this observation and the non-applicability of the data to ileal or ileojejunal locations or other non-colonic disease localizations. Therefore, a greater understanding of the expression of these receptors in the small intestine is absolutely necessary as an additional piece in this already limited research context. A preliminary study by Gantz et al. [39] suggested MC_3R intestinal expression (specifically in the duodenum) through northern blot hybridization and polymerase chain reaction. However, subsequently, there haven't been robust studies that thoroughly detail the expression profiles of these two receptors in different gastrointestinal segments.* ";
8) Sorry for this typo. We corrected the text in figure legends as suggested and as appropriate. Thank you!

[REVIEWER 2]

SPECIFIC REVIEWER COMMENTS TO AUTHORS

1) In this study the authors have analyzed the expression pattern of Melanocortin 3 receptors and melanocortin 5 receptors in colonic \ rectal mucosa. Inclusion criteria is "newly diagnosed (by colonoscopic biopsy) treatment naïve IBD patients". In which case the number of patients screened (46) need not come into picture at all. Of the 46 patients screened for the study, 20 did not meet the criteria. "The remaining 15! patients were included and later excluded. At the end 26 patients were wholly enrolled. The confusion in these statements may be clarified. The authors have shown, in Table 1, in smoking status, 15 patients with CD and 14 patients with UC . This also needs clarification;
2) The description detailed about the distribution of MC2R to MC5R, (*On the contrary, MC2R (mainly expressed in the adrenal cortex and adipocytes) is involved in steroid synthesis [10] and has no putative role in inflammation. MC3R has a widespread expression pattern in the central nervous system and immune cells (mainly B lymphocytes and macrophages) but is also expressed in rat's gut, heart, and placenta [11]. It has a role related primarily to metabolic control and inflammatory response. MC4R is present within the vagal nerve afferents to the stomach and small intestine and is involved in postprandial functions in mice [12]. MC5R has a predominantly ubiquitous distribution and has been related to the immunomodulation of lymphocytic B-T responses and the control of exocrine glandular secretions [13–19], with no information on its localisation and role at the intestinal level. It is not well understood how much MC3R and MC5R are expressed in the colon and their role in the intestinal microenvironment of IBD*) almost appear akin to details seen in article "7" quoted by the authors. But the reference quoted for this detail by the authors is 10 to 19. (7. Gravina AG, Pellegrino R, Durante T, Palladino G, Imperio G, D'Amico G, Trotta MC, Dallio M, Romeo M, D'Amico M, Federico A. The Melanocortin System in Inflammatory Bowel Diseases: Insights into Its Mechanisms and Therapeutic Potentials. Cells. 2023 Jul 19;12(14):1889);
3) Authors study to find out expression of MC3R and MC5R receptors in the colon of patients with IBD. But most patients with CD may have involvement in ileum than colon. So it is presumed that the authors have chosen only patients with colonic CD;
4) Author's statement that no data on MC2R and MC5R expression in colonic mucosa is reported in the literature, may be untrue;
5) "MC3R and MC5R receptor expression is different according to the disease activity" is the conclusion drawn. Quantifying the receptor expression in diseased mucosa and normal mucosa is the basis for arriving at this conclusion. But the disease activity of IBD, assessed by "disease activity index of UC – Mayo score" or "CDAI" are not based endoscopic findings alone but include clinical variables. Even the severity indices based on endoscopic findings alone viz. CDEIS (Crohn's Disease Endoscopic Index of Severity) or the SES-CD (Simple Endoscopic Score for Crohn's Disease) are not measured by examining normal and diseased distal colonic/rectal mucosa. This study only explores the expression of MC2R and MC5R receptors in colonic mucosa. This does not study the molecules that may exert their action through these receptors., like αMSH, or its agonists etc. or the effect of these receptor stimulation. Hence, as rightly stated, this is only a basic study of assessing the expression of receptors in colonic mucosa and the conclusions drawn are based not on this study.

RESPONSES TO REVIEWER:

We thank the reviewer for the efforts in analyzing our work with brilliant attention and the words spent in favor of our work;

1) We thank the reviewer for pointing out these typographical errors. The errors have been promptly corrected as appropriate. We included the number of patients screened for the sake of completeness, but we agree on its non-essential nature. We sincerely appreciate the reviewer for promptly identifying these typographical errors, significantly enhancing the presentation quality of our work. To this end, we have removed the figure on enrollment as we deemed it unnecessary at this point, summarizing the text more clearly in the results;

2) We fully agree with the reviewer; article number 7 was entirely written by us. It is a narrative review that we recently published, which is perhaps why the writing style appears similar to the reviewer. Nevertheless, we acknowledge and have rephrased the sentences, retaining the previous citations and also incorporating the work authored by us, as indicated by the reviewer. We appreciate this valuable advice;
3) Thank to the reviewer for this clarification request: All patients with Crohn's disease (CD) were required to have definite involvement of the colon; otherwise, they were not recruited into the study and were excluded. So it was done in accordance with what was rightly stated by the reviewer;
4) Thank you for your comment. To the best of our knowledge, we are not aware of any previously reported colonic expression of MC2R and MC5R receptors in the literature. For instance, we have added to the manuscript that MC3R is expressed in the gastrointestinal tract (but specifically in the duodenum), but we could not find additional data on this topic. Thank you for your understanding and assistance. In any case, as a precaution, we have added the following phrase to the manuscript: "*The extent of expression of MC3R and MC5R in the colon and their roles in the intestinal microenvironment of IBD are not well understood.*"
5) Thank you for your insightful observations and constructive feedback on our manuscript. We appreciate the thoroughness with which you examined our work, and your comments have been invaluable in refining the focus and interpretation of our study. Upon careful consideration of your remarks, we fully acknowledge and concur with your observation regarding the conclusion drawn about the differential expression of MC3R and MC5R receptors according to disease activity. To address this concern, we have made significant enhancements throughout the manuscript, particularly in the discussion and conclusion sections. We have emphasized the role of colonic immunohistochemical expression rather than attributing the observed expression differences to disease activity. Additionally, we have expanded the discussion on the study's limitations, underlining the need for further mechanistic exploration of this novel finding. Throughout the manuscript, we have explicitly underscored the limited scope of our study, focusing on the assessment of colonic immunohistochemical expression. We recognize the importance of additional preclinical and clinical studies to validate and thoroughly understand this novel data. Your feedback has been instrumental in guiding these revisions, and we are committed to addressing the highlighted aspects comprehensively.

[EDITORIAL COMMENTS]

SPECIFIC EDITORIAL COMMENTS TO AUTHORS

1) Language polishing requirements for revised manuscripts submitted by authors who are non-native speakers of English;
2) Title: Abbreviations are not permitted. Please spell out any abbreviation in the title.
3) Abbreviations must be defined upon first appearance in the Key Words.
4) Please provide the filled conflict-of-interest disclosure form.
5) Please provide the PubMed numbers (https://pubmed.ncbi.nlm.nih.gov/) to the reference list and list all authors of the references. If a reference has no PMID and DOI, please provide the source website address of this reference.
6) The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text (and directly before the References);
7) Editor-in-Chief comments: I have reviewed the Peer-Review Report, full text of the manuscript, all of which have met the basic publishing requirements of the World Journal of

Gastroenterology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. When revising the manuscript, it is recommended that the author supplement and improve the highlights of the latest cutting-edge research results, thereby further improving the content of the manuscript. To this end, authors are advised to apply PubMed, or a new tool, the Reference Citation Analysis (RCA), of which data source is PubMed. RCA is a unique artificial intelligence system for citation index evaluation of medical science and life science literature. In it, upon obtaining search results from the keywords entered by the author, "Impact Index Per Article" under "Ranked by" should be selected to find the latest highlight articles, which can then be used to further improve an article under preparation/peer-review/revision. Please visit our RCA database for more information at: https://www.referencecitationanalysis.com/, or visit PubMed at: https://pubmed.ncbi.nlm.nih.gov/.

RESPONSE TO EDITORIAL COMMENTS:

1) The manuscript has been thoroughly reviewed by an English expert. We have meticulously proofread the entire manuscript, addressing any typographical errors or syntactic expressions. Along with this submission, we have included a completely updated certificate;
2) The title has been modified by eliminating acronyms and keeping it within 18 words in length;
3) The abbreviations have been removed;
4) Provided, thank you;
5) References updated and PMIDs added;
6) Article Highlights added at the end of the main text and directly before the References as requested;
7) Thank you to the Editor-in-Chief for deeming our work publishable in WJG. We have addressed the requested revisions.