Cover Letter January 31st, 2017

Dear Dr. Lian-Sheng Ma,
Editor-in-Chief of *World Journal of Gastroenterology*,

On behalf of my group, I have the pleasure to present this revised version of Manuscript #32199: "Diet and microbiota in IBD: the gut in disharmony" for your consideration.

We thank the three reviewers for finding the results of our study interesting and for reading in detail our review of diet and microbiota in IBD. We feel that the changes and amendments made to the manuscript continue to support and preserve the general idea of the article. We believe that our text is even more consistent after the changes and new references, which have greatly improved the manuscript.

In addition, we hereby certify that all authors concur with the submission of this work and that none of the data presented here have been previously reported or are under consideration for publication elsewhere.

Thank you for your invitation and consideration,

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Manuscript #32199: Diet and microbiota in IBD: the gut in disharmony

Reply to the reviewers

Reviewers' Comments:

Reviewer #1 (2462498)

This is a nice review on diet, microbiota and IBD. There are two comments on article:

1. If the journal cannot accept so long manuscript, authors need shorten it.

Response: We have attempted, as much as possible, to shorten the manuscript while preserving its main content and general ideas. However, the other two reviewers have requested additional references and explanations on several issues.

2. As for the treatment on IBD using microbiota/bacteria, FMT should be the most important therapy. However, the authors only use very limited literature evidence to support it and even said there was no controlled study. The only supporting evidence is the review published in 2012 (Anderson JL, et al. Alimentary pharmacology & therapeutics 2012). Acutally the recent published clinical studies should be strong supportive to highlight the therapeutic role of remodeling microbiota in IBD. Research articles on RCT or the largest samples, instead of review, listed as following: Moayyedi P, Surette MG, Kim PT, Libertucci J, Wolfe M, Onischi C, Armstrong D, Marshall JK, Kassam Z, Reinisch W, Lee CH. Fecal Microbiota Transplantation Induces Remission in Patients With Active Ulcerative Colitis in a Randomized Controlled Trial. Gastroenterology. 2015 Findings From a Randomized Controlled Trial of Fecal Transplantation for Patients With Ulcerative Colitis. [Gastroenterology. 2015] Cui B, Li P, Xu L, Zhao Y, Wang H, Peng Z, Xu H, Xiang J, He Z, Zhang T, Nie Y, Wu K, Fan D, Ji G, Zhang F. Step-up fecal microbiota transplantation strategy: a pilot study for steroid-dependent ulcerative colitis. J Transl Med. 2015 Cui B, Feng Q, Wang H, Wang M, Peng Z, Li P, Huang G, Liu Z, Wu P, Fan Z, Ji G, Wang X, Wu K, Fan D, Zhang F. Fecal

microbiota transplantation through mid-gut for refractory Crohn's disease: safety, feasibility, and efficacy trial results. J Gastroenterol Hepatol. 2015

Response: Though treatment and faecal transplantation were not the major targets of our review, we agree with this reviewer and understand his/her point of view. Therefore, we have included additional text and the suggested references (new references #167-170) in the section regarding treatment and FMT.

We thank Reviewer #1 for the attentive reading of our manuscript and for his/her support of our work.

Reviewer #2 (3661119)

The is a nice review focuses on the relationship of dietary components, epigenetics, immune system and gut microbiota. It starts with an introduction of the host immunity and IBD, followed by a thorough discussion of gut microbiota, dysbiosis, IBD and some diet components.

While diet will certainly influence gut microbiota, the connection from diet to IBD is still lacking. This can be further discussed. I suggest discussing different types of dysbiosis in page 14, paragraph 2 ("Whether dysbiosis consists of a primary or secondary phenomenon in IBD is a question that remains unanswered", "dysbiosis alone is not sufficient to induce IBD"). Different types of diet have differential effects on epigenome. Do these further correlate with the incidence of IBD?

Response: We understand this reviewer's point of view and concerns, and we have expanded the discussion on different types of dysbiosis. However, we also must comply with the space limitations imposed by the editorial office. Regarding epigenetic changes, the studies are still incipient, and there is currently no consistent specific association with IBD, only speculative associations.

The review is focused more on specific components including retinoic acid, iron, vitamin D, folic acid, choline, selenium as well as antibiotics, prebiotics and probiotics than on different types of diets.
I suggest further expanding on different types of diets such as fat-enriched diet and carbohydrate-enriched diet.
What kinds of dietary intervention can we anticipate in the end?

Response: This reviewer is right, and we agree that we focused more on specific components than on diets in general. We have therefore tried as much as possible to expand the discussion on different types of diets, considering the space limitations imposed by the journal. We have added new references in several parts of the text (new references #42-46; #72-75; #176-179).

Some minor errors:

Page 8, paragraph 1: predominantly localized “at” epithelial and immune cells -> “in” Page 8, paragraph 2: Alimentary fibers are not digested by the human gastrointestinal tract but, instead, they are “modified” in the gut by bacteria -> bacteria ferment fibers, which in turn modify gut microbiota.

Response: We agree with the comments, and the errors were corrected as suggested.

We thank Reviewer #2 for the attentive reading of our manuscript and for his/her support of our work. We believe that his/her suggestions have significantly contributed to the improvement of our manuscript.

Reviewer #3 (1490743)

Comments on Diet, Microbiota and IBD The article is well written and provides an comprehensive overview of the field and what is currently known. The review makes it possible to get a quick insight in the intriguing mechanisms that may contribute to IBD. However, I have some comments that could be worth while to consider.

Major

The choice of dietary factors is obscure to me. How have they been chosen among the multtude of factors that have been discussed over the years. See the bottom of this reveiew for reference suggestions.

Response: We understand the concerns of this reviewer, and we agree with the comments. We have focused more on specific dietary components than on diets in general. Of the most recently published reports on dietary elements, we chose to focus on those with a significant impact in the study of IBD. We have accepted the reviewer's suggestions, and we have tried, as much as possible, to expand the manuscript text (according to space limitations).

In general the taxonomic rank is not mentioned thus making the interpretation of the message somewhat more difficult. It is not necessary but it could be worth contemplating the possibility to further clarify the rank of the different organisms mentioned (whether it is about phylum, class, order, family, genus, species etc).

Response: We understand this reviewer's concerns, and we have attempted to address this issue by including information in a new table (Table 1) and an additional 7 references (#181-187).

Minor

Page 9, last paragraph, first sentence (Network-based studies of microbial communities performed with faecal samples of several mammalian species confirmed that diet does determine bacterial diversity, which increases from carnivore to omnivore to herbivore, whereas microbial communities diversify concomitantly with their hosts) is somewhat

unclear. In this sentence two ways for microbial development is described. Are they correlated? Is the second one over time?

Response: We have added further explanation to the indicated sentence.

Page 10, four lines from bottom (more than 160 genetic risk loci) is now more than 200.

Response: This reviewer is right, and the mistake was corrected.

Page 13, in the last last paragraph (In CD, for example, immune reactivity against microbial-derived antigens has long been reported, characterized by several different circulating serum antibodies [64-66].) reference 64 is about ASCA that comes from yeast, reference 65 is appropriate but the substances referred to in reference 66 seems – as far as I can understand – to come from fruit (?). In this context the antibodies against pseudomonas could also be mentioned if further references about bacterial antigens should be warranted.

Response: We have added a reference regarding different antibodies not previously mentioned in the text (new reference #71). However, the previous reference #66 (Dotan I et al, Gastroenterology 2006) is correct and refers to anti-glycan antibodies. Glycans are ubiquitous surface components present in cells such as erythrocytes, immune cells, and several different microorganisms.

Page 13, last paragrpah, 8 lines from bottom (about Faecalibacterium prausnitzii) mentions a reference **71** referring to the occurrence of this bacteria in the postoperative state and risk for relapse. As I see it there are several other more appropriate references that could be used.

Response: We have added additional, more recent references to support the point in question (new references #82-84).

Page 14, last paragraph, about UPR – this type of response could be explained by one sentence (or two) for those readers not fully familiar with this phenomenon.

Response: We understand this reviewer's concern, and we have included an additional explanation of the UPR.

Page 15, second last paragraph (Furthermore, the interaction between single nucleotide polymorphisms of ATG16L1 and IRGM also has been demonstrated in CD [94], indicating the probable integration of defective autophagy with mitochondrial dysfunction and apoptosis) deals with mitocondrial dysfunction but withou explaining it. Some kind of explanation could be valuable.

Response: We again understand this reviewer's concern, and we have included additional explanations of these basic mechanisms.

Page 16, second last paragraph, ref 101 is about maltodextrin, that is one putatively harmful additive. There are other suspects on the crime scene. For example read the aricles by Lerner about the seven possibly harmful additives that could contribute to the present autoimmune epidemic. Although many of these substances have not been definitvely proven to be hazardous to man they could maybe be mentioned as possible contributors.

Response: We thank the reviewer for this specific suggestion, but we had to select the most important points for the addition of further discussion and new references. Regarding this, we feel that this subject does not constitute a major target within the scope of this manuscript.

Page 16, second last paragraph, about sun light, check out Dopico from 2015 about summer and winter genes providing evidence that it is not only about vitamin D. It is outside IBD but worth mentioing. The part about folic acid is contradictory as both high and low levels increase the risk for cancer. This ought to be explained in a more thorough way.

Response: Again, we thank the reviewer for the suggestion, but, although very interesting, we believe this subject should be further discussed in another article.

About the choice of dietary factors When it comes to dietary micronutrients vitamin A, iron, selenium and folic acid are discussed. How have these specific factors been chosen? The list is not complete. It must be explained why only these factors have been chosen. How about zinc that has impact on the risk for both IBD in mice as well as diabetes? How about fat, espe... ?

Response: We previously responded regarding the choice of factors, which was based on the most relevant recent literature. In addition, there are space limitations concerning the article that make it difficult to cover such a complex theme with a vast literature and with frequent controversial data to discuss. Nevertheless, we have managed to add information on zinc, fat, carbohydrates, and other dietary elements (new references #36-48; #72-76; #81; #83; #141-143; #176-180).

We thank Reviewer #3 for the attentive reading of our manuscript and for his/her support of our work. We believe that his/her suggestions have significantly contributed to the overall improvement of the manuscript.