ANSWERING REVIEWERS

Dear Editor,

Title: Unraveling the ties between irritable bowel syndrome and intestinal microbiota

Author: Sung Noh Hong, Poong-Lyul Rhee

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We greatly appreciate the opportunity that we have been given to further revise the manuscript. The thoughtful comments and kind suggestions provided by you and the reviewers have really helped in positioning of this manuscript. We did our best to respond to the reviewers' comments point-by-point. The manuscript has been revised based on the reviewers' comments and recommendations. In addition, we changed the words, sentences and phrases according to the English Proofreading services. We are really grateful for the expert comments and excellent advice we have received.

The point-by-point responses to the reviewers' comments are as follows:

Answers to the comments from REVIEWER # 1

The review is well-written and provides current views on the subject for general readers of WJG. There are a few comments including the following.

1. Authors may need to explain that microbiota refers to the organism and metagenome, the genes, which would be helpful for beginners.

➔ Thank you for your kind suggestion. We mentioned about Metagenomics as following (page 9): A revolution in DNA sequencing technologies would be to define genetic material recovered directly from environmental samples. Metagenomics refers to culture-independent and sequencing-based studies of the collective set of genomes of mixed microbial communities (metagenomes) with the aim of exploring their compositional and functional characteristics.

2. Besides the three enterotypes, there is a "long tail" of low abundance organisms but having specific but important functions including short-chain fatty acid metabolism, which disturbed, would contribute to symptoms. Besides the West, there are some data from Asia (Japanese, Koreans and Chinese) that suggest some differences in microbiota signature, despite having similar diet (Nam, Young-Do et al, PLoS One 2011). This may be relevant for readers from the Asian region.

➔ Thank you for your kind suggestion. We mentioned as following (page 8): Characterization of intestinal microbiota, however, has been limited to Western people. A recent study investigated the overall intestinal microbiota composition of 20 Koreans using pyrosequencing. Microbial communities were dominated by five previously identified phyla: Actinobacteria, Firmicutes, Bacteroidetes, Fusobacteria, and Proteobacteria. Cluster analysis showed that the species composition of intestinal microbiota was host-specific and stable over the duration of the test period, but the relative abundance of each species varied among individuals. The results were compared with those of individuals from the USA, China, and Japan, and it was found that human intestinal microbiota differed among countries, but tended to vary less among individual Koreans. The gut microbial composition may be related to the internal and external characteristics of each country member, such as host genetics and dietary patterns.

3. Despite stability in composition with time, fluctuations can happen in response to diet, antibiotics and stress. Table 1 is important but it is too busy and the authors need to think of ways to revise it, including removing some unnecessary details.

➔ Thank you for your kind comment. We removed some items of table 1, such as IBS definition and case-control matching.

4. The role of small intestinal bacterial overgrowth and antibiotic usage need to be discussed in the context of dysbiosis in IBS since these are also reasons for discrepancies of results across studies.

➔ Thank you for your kind suggestion. We mentioned about Small intestinal bacterial overgrowth (SIBO) and antibiotics as following (page 15-16):

Since Pimentel et al. reported that 84% of IBS patients had SIBO and that patients with IBS were over 26 times more likely to harbor SIBO than controls[61], the potential role of SIBO in IBS pathogenesis has gained considerable research attention[62]. In addition, bacterial fermentation in IBS has been highlighted in recent studies on SIBO[16]. Bacterial overgrowth in stagnant sections of the small intestine leads to malabsorption, diarrhea, bloating, and pain, and it can be treated with antibiotics. However, a subsequent study on the SIBO–IBS link showed similar results, whereas other studies were unable to establish an association[62].
A SIBO diagnosis test includes jejuna aspirate and culture, ^{14}C-xylose breath test, and hydrogen (H_2) breath tests (HBT) using either glucose (GHBT) or lactulose (LHBT) as the substrate. Jejunal aspirate and culture is considered as the gold standard (>10^5 CFU after 48 h of culture); however, it is invasive and time consuming. In contrast, HBT is noninvasive and cheap, but prone to error. Following the ingestion of glucose or lactulose, serial breath H_2 measurements are performed. SIBO is defined by either a rise in H_2 > 20 ppm in <90 min or a "double peak" demonstrating distinct small intestinal and colonic bacterial populations[63]. Meta-analysis of 12 studies containing 1921 subjects meeting the Rome criteria for IBS revealed that the pooled prevalence of a positive LHBT or GHBT was 54% (95% CI, 32–76%) and 31% (95% CI, 14–50%), respectively, but showed marked statistical heterogeneity between study results[64]. In addition, the prevalence of a positive jejunal aspirate and culture was only 4% (95% CI, 2–9%). These results suggested that it is premature to accept a firm etiologic link between SIBO and IBS. Moreover, despite a decade of investigation on the relationship between SIBO and IBS, it remains unclear whether SIBO causes IBS or is a bystander of something else altogether[62].
However, the idea of treating IBS patients with an antibiotic was developed as a consequence of the SIBO concept[65]. Neomycin therapy eradicated SIBO and reduced symptoms of IBS[61, 66]. Considering the chronic, relapsing nature of IBS and the

undesirability of long-term systemic antibiotic therapy, the efficacy of rifaximin, a nonabsorbable antibiotic, began to be explored in IBS[67]. In a RCT, rifaximin treatment for 10 days resulted in symptom improvement that lasted for up to 10 weeks in some IBS patients who did not document bacterial overgrowth[68]. Subsequently, a double-blind, placebo-controlled trial phase III study reported that rifaximin treatment for 2 weeks provided significant relief from IBS symptoms such as bloating, abdominal pain, and loose or watery stools[69]. A recent meta-analysis of 5 studies found rifaximin to be efficacious for global IBS symptom improvement (OR, 1.57; 95% CI, 1.22–2.01) and more likely to improve bloating (OR, 1.55; 95% CI, 1.23–1.96) compared with a placebo[70].

5. The enthusiasm in probiotics from the authors is understandable but at the moment, the role of probiotics actually remains unclear and therefore the enthusiasm should be toned down in the core-tip. There have been a number of reviews on probiotics in IBS, including a recent paper in WJG (Dai C et al, WJG Sep 2013). Probiotics is not the only method in altering the microbiota environment, and there are some interests or potentials in fecal microbiota transplantation.

➔ Thank you for your helpful comment. We removed the some contents about probiotics and added the comments about SIBO and antibiotics.

6. Table 2 is again busy, and the authors should try to revise it, and cutting out unnecessary details.

➔ Thank you for your kind comment. We removed some items of table 3, such as Search strategy (database and periods).

Answers to the comments from REVIEWER #2

This is a nice and complete review. I have only some minor comments

1. microbiota is abnormal in only a subset of IBS patients and not in all (see Jeffery Gut 2012)

➔ Thank you for your kind comment. We changed "IBS patients" → "some IBS patients". For example "The intestinal microbiota is altered in some IBS patients, and the symptoms of IBS can be alleviated by treatments that target the microbiota."

2. the authors do not discuss the questionable problem of a possible small intestinal bacterial overgrowth in a subgroup of IBS patients

➔ Thank you for your kind suggestion. We mentioned about Small intestinal bacterial overgrowth and antibiotics as following (page 15-16). Please, see 4th answer to the comments from REVIEWER #1

3. For the available methods to assess the intestinal composition, a table with the advantages and limitations of each would be probably easier to read than the text

➔ Thank you for your kind suggestion. We added table mentioned about advantages and limitations of the principal techniques used in the characterization of the intestinal microbiota, as following (page 40)

Table 2. Advantages and limitations of the principal techniques used in the characterization of the intestinal microbiota[16, 39]

	Advantages	Limitations

Culture	cheap, easy to use	limited estimate intestinal microbiota
PCR-T/DGGE	high sensitivity in detecting difference in bacterial populations, semi-quantitative	does not identify bacteria unless bands on the gel are cut out and sequenced
FISH	microbial in situ identification, high sensitivity, quantitative	few species can be simultaneously detected, only known species are detected
T-RFLP	low cost	low biodiversity resolution, no species-level identification, not quantitative
Quantitative PCR	can detect small number of bacteria and quantify them	laborious
Phylogenetic microarray	high biodiversity resolution, quantitative	only known species are detected
NGS phylogenetic analysis (i.g. pyrosequencing)	enormous quantities of data at individual species level	very costly, need bioinformatics analysis

16S rRNA, 16S ribosomal RNA; PCR-T/DGGE, PCR temperature/denaturing gradient gel electrophoresis; FISH, fluorescent *in situ* hybridization; T-RFLP, terminal restriction fragment length polymorphism; qPCR, quantitative PCR; NGS, next-generation sequencing

4. the lack of confirmation studies for most of the strains in IBS is an important limitation for the assessment of the efficacy of probiotics in IBS. Ortiz-Lucas et al have published a meta-analysis in 2013.

➔ Thank you for your kind suggestion. We added new meta-analysis by Ortiz-Lucas et al. in table 3, as following:

Table 3. Systemic reviews for randomized controlled trials of probiotics in IBS

	Selection criteria	N. of identified studies	Results
Ortiz-Lucas et al.[77] Rev Esp Enferm Dig 2013	RCTs comparing probiotics with placebo in treating IBS symptoms	24 RCTs → 10 RCTs providing continuous data performed with continuous data summarized using SMDs and 95% CIs	* Pain scores: improved by probiotics containing *Bifidobacterium breve*, *Bifidobacterium longum*, or *Lactobacillus acidophilus species.* * Distension scores: improved by probiotics containing *B. breve*, *Bifidobacterium infantis*, *Lactobacillus casei*, or *Lactobacillus plantarum species.* * Flatulence: improved by probiotics containing *B. breve*, *B. infantis*, *L. casei*, *L. plantarum*, *B. longum, L. acidophilus, Lactobacillus bulgaricus, and Streptococcus salivarius ssp. thermophilus*.

Answers to the comments from REVIEWER #3

Comments to review article:

Unraveling the ties between Irritable Bowel Syndrome and Intestinal Microbiota

This review aims to cover current knowledge on IBS and intestinal microbiota, regarding the possible role of microbiota in the pathogenesis of IBS and as a target for therapeutic treatment of IBS.

In general the authors include many relevant aspects on the subjects and parts of the article is well written, but some sections need major revision.

They are are not well written, some places there is even incorrect statements. There is some major problems with references not matcing or not covering what they were cited for (see specific comments).

Furthermore I think the authors should consider commenting on prebiotics, antibiotics and symbiotics as a possible way of moduling the microbiota as a treatment strategy. Prebiotics are mentioned in the section"human intestinal microbiota", but could be relevant in the section on probiotics. Diet could also be relevant to mention.

I think the article could also benefit from underlining that research in microbiota is in a very novel state and we can still not say for sure if microbiota play a definate role in the pathogenisis of IBS.

➔ Thank you for your kind suggestion. We mentioned about Small intestinal bacterial overgrowth and antibiotics as following (page 15-16). Please, check 4th answer to the comments from REVIEWER #1

Specific comments:

1. Abstract:

… disorder whose causes are… is incorrect. Should be written e.g.: Is a disorder with multifactorial causes.

➔ Thank you for your kind comment. We changed the above sentence as your comment. We changed the words, sentences and phrases according to the English Proofreading services.

The authors only mention three mechanisms that microbiota could contribute to the pathogenis of IBS. I think this is too restrictive. For example some studies have proposed that it could have an effect on the brain gut axis. (Bercik, P. et al. Microbes and the gut-brain axis. Neurogastroenterology and motility 24, 405–13)

➔ Thank you for your kind comment. We added the communication in the gut-brain axis as a one of pathogenesis of IBS.

To reasons why previous studies have been inconsistent, it could be added that there have been different way of handling the samples in the studies. Some samples are frozen, some are fresh etc.

➔ Thank you for your kind comment. We added as following: "different sample collection and handling methods" as a one of cuases of inconsistent results of previous study.

Despite these… microbiota in IBS patients was completely different from that in healthy controls,…. I think it should be stated that: microbiota in some IBS patients…. Because there is also studies where there is no remarkeble difference in microbiota between IBS patients and healthy controls.

➔ Thank you for your kind comment. We changed as following: "IBS patients" → "some IBS patients".

Core tip:

It is stated that IBS symptoms can be improved by treatments that target the microbiota. I think this is a statement that over interprets data on the subject. At least it should be added " in some IBS patients".

➔ Thank you for your kind comment. We changed as following: "IBS patients" → "some IBS patients".

Introduction:

In the first sentence it is stated that IBS is…. in the absence of any identifiable physical, radiological or laboratory abnormalities indicative of organic gastrointestinal disease. I find that radiological being included is a little misleading as radiological investigation is not a part of guidelines for diagnosing IBS.

➔ Thank you for your kind comment. We changed as "IBS is…. in the absence of any identifiable abnormalities indicative of organic gastrointestinal disease" (page5)

In the sentence: … the human microbiobiota with hundreds of phylotypes…. Should be corrected to: ….the human microbiobiota with thousands of phylotypes…. This is clear from figure 1 in the stated reference (11)

➔ Thank you for your kind comment. We changed as following: "hundreds" → "thousands"

In the sentence: The probiotics are composed of the normal intestinal flora… I think it relevant to add "strains" of the normal flora. Otherwise it sounds like a fecal transplantation. The authors could consider to write "microbiota" instead of "flora" to be consistent through out the manuscript.

➔ Thank you for your kind comment. We added "strains" and changed "microbiota" instead of "flora"

Human intestinal microbiome:

The existing of enterotypes have been questioned. E.g. here: http://www.ploscompbiol.org/article/info%3Adoi%2F10.1371%2Fjournal.pcbi.1002863

➔ Thank you for your kind suggestion. We mentioned about recent meta-analysis as following (page 7): Recent meta-analysis including the 16S rRNA sequences and whole genome shotgun (WGS) sequences from the Human Microbiome Project (HMP), Metagenomics of the Human Intestinal Tract (MetaHIT) consortium, and additional studies yielded only bimodal distributions of *Bacteroides* abundances in gut samples[20]. Enterotype identification depends not only on the structure of the data but also on the methods used for identifying clustering strength.

I think the authors should refer to a direct reference in the sentence: Numerous diseases…from systemic disorders, such as obesity and diabetes to gastrointestinal disorders, Including IBS. The reference 9 is not the proper reference for this statement. It is stated in the reference 9 cited from

another reference.

➔ Thank you for your kind suggestion. We added original reference (Shanahan F. The colonic microbiota in health and disease. Current opinion in gastroenterology 2013; 29(1): 49-54) cited by reference 9.

Intestinal microbiota composition in IBS

Put reference after the 1st sentence (The major….) in the section.

➔ Thank you for your kind suggestion. We added reference (Sommer F, Backhed F. The gut microbiota--masters of host development and physiology. Nature reviews Microbiology 2013; 11(4): 227-238, Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome and the immune system. Nature 2011; 474(7351): 327-336)

Metagenomics should be mentioned among culture independent methods!!

➔ Thank you for your kind suggestion. We mentioned about Metagenomics as following (page 9): A revolution in DNA sequencing technologies would be to define genetic material recovered directly from environmental samples. Metagenomics refers to culture-independent and sequencing-based studies of the collective set of genomes of mixed microbial communities (metagenomes) with the aim of exploring their compositional and functional characteristics.

It is 16S rRNA sequencing- not 16S rDNA sequencing!!! This is written many palces in the section!!!

➔ Thank you for your kind comments. We changed all our mistakes.

I do not agree that studies have demonstrated that intestinal microbiota of IBS patients could be grouped in a cluster. As the authors themselves write later in the text different studies have revealed different results and the microbiota from IBS patients cannot be grouped in one cluster.

➔ Thank you for your kind suggestion. We added as following (page 7): Recent meta-analysis including the 16S rRNA sequences and whole genome shotgun (WGS) sequences from the Human Microbiome Project (HMP), Metagenomics of the Human Intestinal Tract (MetaHIT) consortium, and additional studies yielded only bimodal distributions of *Bacteroides* abundances in gut samples[20]. Enterotype identification depends not only on the structure of the data but also on the methods used for identifying clustering strength[20].

The author is describing the 16S method as a DNA method. This is wrong and should be basic knowledge. It is a RNA method!

➔ Thank you for your kind suggestion. We change as following (page 9): In 1977, Woese et al. identified 16S rRNA, which is a component of the 30S small subunit of prokaryotic ribosomes, having relatively short gene sequences and highly conserved primer binding sites and containing hypervariable regions that can provide species-specific signature sequences useful for bacterial identification[40]. Since then, the molecular profiling of

bacterial communities via 16S rRNA-gene based approaches such as terminal restriction fragment length polymorphism (T-RFLP), PCR temperature/denaturing gradient gel electrophoresis (PCR- T/DGGE), and fluorescent *in-situ* hybridization (FISH), has been performed[41].

I think the argumentation on why there is inconsistency in results on IBS microbiota is to shallow. There are more reasons: E.g. handling of samples, how the IBS population is defined, fecal versus biopsi material etc. This needs to cover more aspects.

➔ Thank you for your kind suggestion. We added the explanation why there is inconsistency in results on IBS microbiota as following (page 10-11): This inconsistency in results may be ascribed to several reasons, including differences among the various molecular techniques employed, sample collection and handling methods, , as well as definitions of IBS and IBS subtypes[16]. Table 2 lists the advantages and disadvantages of the principal techniques used for characterizing intestinal microbiota. In studying human intestinal microbiota, classical approaches suffer from individual advantages and limitations[7, 16]. NSG and phylogenic metagenomics update the bacterial community profiles of patients with IBS. The sample collection method can influence the intestinal microbiota composition. Namely, fecal samples show distal colonic luminal microbiota, whereas biopsy samples show only mucosa-attached microbiota. Although feces or fecal swabs are the most convenient samples, they do not accurately reflect the microbiota composition or activities in the proximal colon. Colon biopsies also do not represent the microbiota in its physiologic state because extensive colon preparation for cleaning intestinal contents removes some of the outer mucus layers and, in turn, the mucosa-attached microbes as well as their normal attachment sites[16]. In addition, different studies used different sample handing methods; some studies used frozen samples, whereas others used fresh samples. The use of single samples cannot be linked to fluctuating symptoms and probably to other factors such as diet and patients' phenotypic characterization[7]. Although most studies used the Rome criteria for IBS, the proportions of the enrolled numbers of IBS subtypes differed among the studies. There is suggestive evidence of an association of intestinal microbiota in certain IBS subtypes. Kassinen et al. pooled fecal samples by an IBS subgroup (diarrhea-predominant IBS [IBS-D], constipation-dominant irritable bowel syndrome [IBS-C], and IBS mixed type [IBS-M]) and controls, extracted the bacterial DNA, and analyzed it using high-throughput 16S rRNA sequencing[43]. Population analysis found significant differences between each IBS subgroup and controls[43]

I could be added that in generel there have been found: enriched firmicutes and reduced abundence of bacteroides.

➔ Thank you for your kind suggestion. We mentioned as following (page 3): Despite these difficulties, previous studies found that the intestinal microbiota in some IBS patients was completely different from that in healthy controls, and there does appear to be a consistent theme of *Firmicutes* enrichment and reduced abundance of *Bacteroides.*

Pathogenic role of intestinal dysbiosis in IBS

From this section one gets the impression that the luminal microbiota cannot interact with the

immunesystem, this is not right. It is possible through e.g. metabilites, toxins etc.

➔ Thank you for your kind suggestion. We mentioned as following (page 11): The metabolites and toxins of luminal microbiota can modulate the host immune system[44].

There is a lack of references in the section on mucosal microbiota.

➔ Thank you for your kind suggestion. We added reference: Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome and the immune system. Nature 2011; 474(7351): 327-336

"Tregs"are mentioned with no reference. It should be described in the text if it is mentioned.

➔ Thank you for your kind suggestion. We added reference: Lopez P, Gonzalez-Rodriguez I, Gueimonde M, Margolles A, Suarez A. Immune response to Bifidobacterium bifidum strains support Treg/Th17 plasticity. PloS one 2011; 6(9): e24776

I would be more correct to write. "Intestinal microbiota may be involved in the pathogenesis of IBS" instead of "Responsible for the pathogenesis of IBS".

➔ Thank you for your kind suggestion. We changed as you mentioned:

And again there could be added more than the three aspects (abnormal GI motiliy etc) it can contribute to (see earlier comment)

➔ Thank you for your kind suggestion. We changed as you earlier mentioned (page 13): Intestinal microbiota may be involved in the pathogenesis of IBS by contributing to abnormal gastrointestinal motility, low-grade inflammation, visceral hypersensitivity, communication in the gut–brain axis, and so on.

Ref 43 do not describe muscle dysfunction but visceral hypersensitivity!

➔ Thank you for your kind suggestion. We changed the reference as following: Verdu EF, Bercik P, Bergonzelli GE, Huang XX, Blennerhasset P, Rochat F, Fiaux M, Mansourian R, Corthesy-Theulaz I, Collins SM. Lactobacillus paracasei normalizes muscle hypercontractility in a murine model of postinfective gut dysfunction. *Gastroenterology* 2004; **127**(3): 826-837 [PMID: 15362038]

There is lacking a reference after the sentence: "There also seems to be an inflammatory component…."

➔ Thank you for your kind suggestion. We added reference as following: Collins SM. Dysregulation of peripheral cytokine production in irritable bowel syndrome. *The American journal of gastroenterology* 2005; **100**(11): 2517-2518 [PMID: 16279908 DOI: 10.1111/j.1572-0241.2005.00246.x]

Evidence for the role of potentiallyprobiotic bacteria in IBS

In the this section there is reffered to reference 44 four times, but the reference do not cover what it is written in this section!

➔ Thank you for your kind suggestion. We used reference only one time (during revision the reference number was changed: 44 → 54)

The sentence referring to reference 53 needs some correction. It is not citing correctly! "…to prevent NF-KB" should be corrected to " do not induce NF-KB". Chemokine ligand 20, *clostridium difficile* and *Mycobacterium paratuberculosis* is NOT mentioned in the reference!?!?!

➔ Thank you for your kind suggestion. We changed correct reference (Sibartie S, O'Hara AM, Ryan J, Fanning A, O'Mahony J, O'Neill S, Sheil B, O'Mahony L, Shanahan F. Modulation of pathogen-induced CCL20 secretion from HT-29 human intestinal epithelial cells by commensal bacteria. BMC immunol 2009; 10: 54 [PMID: 19814810 PMCID: 2763856 DOI: 10.1186/1471-2172-10-54])

Can the dose recommendations be standardized to all probiotics? To my knowledge it can differ from strain to strain?

➔ Thank you for your kind suggestion. We mention as following (page 15): The current evidence suggests that probiotic effects are strain specific.

There is five and not four systematic reviews included RCTs for adults. Ref 58 is the 5th.

➔ Thank you for your kind suggestion. We thought the summary of NICE guidance different from other meta-analysis.

There is lacking a reference after the sentence "Recently, new strains, …."

➔ Thank you for your kind suggestion. We added the reference as following: Choi SC, Kim BJ, Rhee PL, Chang DK, Son HJ, Kim JJ, Rhee JC, Kim SI, Han YS, Sim KH, Park SN. Probiotic Fermented Milk Containing Dietary Fiber Has Additive Effects in IBS with Constipation Compared to Plain Probiotic Fermented Milk. *Gut and liver* 2011; **5**(1): 22-28

The description of ref 8 is too detailed compared to description of other studies in the text. It is not clear why this is necessary.

➔ Thank you for your kind suggestion. We modified the description about ref 8

There is a wrong reference after the sentence: "In addition, the treatment was well tolerated, and no…". Reference 10 do not cover this.

➔ Thank you for your kind suggestion. We thought above sentence unnecessary to explain our meaning and removed it.

The whole section from "In another study with composite probiotics, Hong et. Al…" and 15 sentences forward is written very unclear and "messy". The reference to the Hong et al study should come after the first sentence where they are mentioned. The next sentence begins " in this study"- Im not sure which study the authors means, beacuse they describe some different bacteria strains than in the Hong study?? And not the strains from the reference 55 as stated? Maybe its mixed up with reference 56??

➔ Thank you for your kind suggestion. We thought above sentence unnecessary to explain our meaning and removed it.

There is no remark on the conclusion from reference 56? It is not relevant to mention it if it is not mentioned what the study shows.

➔ Thank you for your kind suggestion. We thought above sentence unnecessary to explain our meaning and removed it.

In the section on dietary fiber it should be mentioned that some experience worsening of symptoms.

➔ Thank you for your kind suggestion. We thought above phrase unnecessary to explain our meaning and removed it.

Conclusion

The main part of the first sentence in the conclusion is irrelevant. This aspect on IBS becomming a model of functional GI disorders were not mentioned in the article and should not be included here.

➔ We thought above sentence unnecessary to explain our meaning and removed it.

I also think it is wrong to conclude there is limited resarch on the possible role of microbiota in IBS. There is quite alot (even though many aspects are not wel understood), and much more are beeing conducted. How can this be concluded form the article?

➔ We thought above sentence unnecessary to explain our meaning and removed it. . In addition, we modified the conclusion.

Table 1

In the title it should be clear that it is studies of intestinal microbiota in IBS

➔ Thank you for your kind suggestion. We added and changed as following: "Summary of molecular studies of intestinal microbiota in IBS"

Thank you for your helpful advice on our manuscript. In our opinion, our paper has been corrected according to your recommendations. We believe the revised manuscript has been greatly improved. Thank you again for your consideration. We would be grateful if our revised manuscript could be considered for publication in your journal.

Sincerely yours,

Sung Noh Hong, M.D.

Poong-Lyul Rhee, MD, PhD