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Medical School
Pediatric Gastroenterology
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Septemember 30, 2016

Name of journal: World Journal of Gastroenterology
Manuscript NO.: 29868
Manuscript Type: Review
Can probiotics benefit children with autism spectrum disorders?
Navarro F, et al. Probiotics for children with autism
Fernando Navarro, Yuying Liu and Jon Marc Rhoads

Dear Dr. Gong (Ze-Mao)

We received two very thorough and very helpful reviews of our MS on probiotics in children with autism. We have worked very hard the past 2 weeks to answer the critiques, and we herein submit our answers to the recommendations given.

1. First Reviewer
   This manuscript gives an overview of the possible role of gut microbiota in children with ASD, but it lacks some depth in different parts. My main concern is table 1. Although I like the idea of a table, I argue that all statements are solid and well explained in the text.
   Reviewer 2 suggested that the table was helpful, so we did keep it. We will defer to the Editor to decide if it should be included. A number of references were also added.

   The statement that: oral probiotics change fecal microbiota is in mine opinion not true. The authors give some examples were this is shown, but I can give more examples were it was not shown. See Kristensen et al. Genome Med, 8, 52, 2016.
   On p.6, we have expanded this discussion. It is indeed not clear if the changes attributable to probiotics in general are due to an overall impact on microbiome or rather to change in the metabolites produced in the gut or to immune system modification. The table has been modified to not include the statement that probiotic may change microbiota (below).

   The statement IBS also is associated with an abnormal fecal microbiota is no evidence that probiotics might play a role in the treatment of GI symptoms in ASD
   We have reworded this section. Autism and IBS both appear to be associated with dysbiosis, and accumulating evidence suggests that probiotic therapy ameliorates symptoms in IBS. But we agree that it is not proven that this will apply to ASD.

   Page 10, no reference to the study of Parracho is given, but I guess it is the study published in 2010 in the international journal of probiotics and prebiotics. The study has severe drawbacks,

and concluding that they found improvements without discussing the limitations of the study is not correct.
This reference is not available via PubMed or Google Scholar. It is a Reading University, UK, publication. We have mentioned these limitations.

In the abstract I miss a result/conclusion. page 5, what did Kushak et al. observe exactly. What can be a reason for the contradictionary results?
Their study was larger than others, in an institution that performs many biopsies, and in the authors' opinion is more definitive. Their observations were that, when compared to controls, children with autism had normal mucosal disaccharidases, gut permeability as measured by dual sugar test, and fecal calprotectin. Now stated in MS.

minor comments: - et al needs to be italic and with a . – *Done. Thanks.*

page 4 laboratory measure of intestinal health? – *Yes, re-worded. Thanks.*

page 5 ..bolster their conclusion that ASD ?..by assessing -page 7 16S DNA
The authors did not identify why these tissues of ASD children had different transcriptional profiles; for example, they did not assess for evidence of inflammation by assessing serum cytokines or fecal calprotectin

page 9 anxiety and stress stress – *Deleted. Thanks.*

table GI tract -references: some names are incorrect, e.g. ref 30 (de ML), 40 (van HS), 56 (de VM)
References corrected.

2. Reviewer 2:
Overall, this is a reasonable attempt to review a very complex field. It is generally well-written and well-organized. However, many references are missing, and references sometimes seem to be incorrect, so a careful proof of every reference, and a search for other relevant references, is needed. So, at present, it is a good but not great review, but with work it could become a great review.
Page 3, top – state approximately how many total patients came to your clinic, and what type of clinic it is (pediatric GI?)
During a two-year period, 3% (121/4013) of children seen by 4 pediatric gastroenterologists for various abdominal complaints in our pediatric clinic had an underlying ASD (as indicated by ICD9 codes 299.0 or 299.8* or ICD10 F84.0 or F84.5) (C. Bearden, U.T. Bioinformatics, personal communication 9-24-2016). We have added this information in the manuscript on page 5.*

P 5, top – 3 other studies did find abnormal digestive enzymes in ASD vs. controls: Kushak RI et al., Intestinal disaccharidase activity in patients with autism: effect of age, gender, and intestinal inflammation. Autism. 2011 May;15(3):285-94. Epub 2011 Mar 17.
Kushak did not include nonautistic controls in this study; but in the same group's 2016 follow-up study, the same group did include a control group and found no difference between children with ASD and controls.

Horvath K et al, Gastrointestinal abnormalities in children with autistic disorder," J. Pediatrics 135 no. 5 (1999) 559-563.

This study showed lactase deficiency in 65% of children > 5 years old but did not have a neurotypical (control) comparison group.

Horvath K and Perman JA "Autistic disorder and gastrointestinal disease," Curr. Opinion in Pediatrics, 14 (2002) 583.
This manuscript is a review article, not a clinical trial or report.

P 5, bottom – the following sentence is unclear: "However, the authors did not bolster their conclusion that ASD by assessing serum or fecal inflammatory markers."
We have clarified this sentence. Thanks.

P 6 top – GIS in ASD also includes alternating constipation/diarrhea. *Point well-taken and included.*

P 6, middle – add ref 26 to list of studies showing correlation of ASD severity with GI symptoms.
This was done. Thanks.

P 7 – top – add reference to 2nd sentence (re. less diverse microbial community) P 7 middle, - need reference for Kang et al.
This important reference was not properly cited. It is now.

P 7, middle – the following sentence seems to have the wrong references: Gondalia et al did not find differences in the gut microbiome between children with autism and their siblings [46,48,49,50].
Yes. These references were misplaced. Corrected.

P 7, bottom – SCFA's have been measured in ASD – for example, see ref 26 and search for other references.
We have now said: Ongoing investigations have begun to investigate the importance of SCFA in ASD.

P 8, middle – define prebiotics.
Done: Dietary prebiotics are "selectively fermented ingredients that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health."

Summarize the findings of the RCT's on probiotics for IBS.
We have updated the originally mentioned meta-analysis; there is also one review pertinent to children.

Mention that current probiotics are aerobic, derived from milk cultures, not normally a significant part of the human gut microbiome which are primarily anaerobic, and short-lived in the human gut. *Done.*

P 10, top – discuss results of UK probiotic trial.
This trial was not published in a journal that we can retrieve. It was in a university journal.

Also mention several other probiotic trials: West et al 2013

I could not find this reference but did include one from West on metabolites in ASD, which was cited; Ka1uzna-Czaplinska et al 2012 *Done.*; Tomova et al 2015 *Done*.
Also mention study by Sandler et al on 8 weeks of vanco, followed (in some cases) by probiotics *Done.*

Several studies have reported increased use of antibiotics in children with ASD (primarily for ear infections) Konstantareas MM, Homatidis S: Ear infections in autistic and normal children. Journal of Autism and Developmental Disorders 1987, 17(4):585-594. Niehus R, Lord C: Early medical history of children with autism spectrum disorders. Journal of Developmental and Behavioral Pediatrics 2006, 27(2):S120-S127. Adams JB et al., Analyses of Toxic Metals and Essential Minerals in the Hair of Arizona Children with Autism and their mothers, Biol Tr El Res 2006, 110:193-209. Adams JB et al., Mercury, Lead, and Zinc in Baby Teeth of Children with Autism vs. Controls J Toxicol Environ Health 2007, 70(12):1046-51. Adams JB et al., Mercury in First-Cut Baby Hair of Children with Autism vs. Typically-Developing Children. 2008, 90(4):739-753.
We have cited the ear infection studies that suggest more exposure of children with ASD to antibiotics. It is difficult to reconcile how to integrate the information about mercury and other heavy metal exposure.

FMT is mentioned in the abstract but not in the paper; it should be discussed *Done, and end of manuscript. Thanks.*
Table 1 is a good summary, but also include data that is negative, such as no difference in calprotectin, mixed data on digestive enzymes, etc.
At the request of the other reviewer, we have edited this table.

We sincerely appreciate the opportunity to publish this MS, which we think focuses attention on an increasingly important and complex group of patients receiving care by pediatric (and now adult) gastroenterologists.

Sincerely,

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