

Peer Review File

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Reviewer A

The study presents an interesting point of view about the importance of microbiota and gut state on ASD. The authors constructively point out the limitation of attempts to define this relationship, using the work of Wang et al. as an example.

It would have been a good idea to clearly emphasize in the conclusion that the relationship between ASD and the microbiota is still unclear.

120-126: Information could be added that there are studies that compare microbiota composition and disorders from the gastrointestinal tract of ASD and neurotypical siblings: Son JS, Zheng LJ, Rowehl LM, Tian X, Zhang Y, Zhu W, Litcher-Kelly L, Gadow KD, Gathungu G, Robertson CE, Ir D, Frank DN, Li E. Comparison of Fecal Microbiota in Children with Autism Spectrum Disorders and Neurotypical Siblings in the Simons Simplex Collection. *PLoS One*. 2015 Oct 1;10(10):e0137725. doi: 10.1371/journal.pone.0137725.

It is worth adding the meta-analysis of Morton et al. which indicated that age remains a major factor disrupting the microbiome in early childhood.

Morton JT, Jin DM, Mills RH, Shao Y, Rahman G, McDonald D, Zhu Q, Balaban M, Jiang Y, Cantrell K, Gonzalez A, Carmel J, Frankiensztajn LM, Martin-Brevet S, Berding K, Needham BD, Zurita MF, David M, Averina OV, Kovtun AS, Noto A, Mussap M, Wang M, Frank DN, Li E, Zhou W, Fanos V, Danilenko VN, Wall DP, Cárdenas P, Baldeón ME, Jacquemont S, Koren O, Elliott E, Xavier RJ, Mazmanian SK, Knight R, Gilbert JA, Donovan SM, Lawley TD, Carpenter B, Bonneau R, Taroncher-Oldenburg G. Multi-level analysis of the gut-brain axis shows autism spectrum disorder-associated molecular and microbial profiles. *Nat Neurosci*. 2023 Jul;26(7):1208-1217. doi: 10.1038/s41593-023-01361-0. Epub 2023 Jun 26.

Reviewer A Critique:

1. "It would have been a good idea to clearly emphasize in the conclusion that the relationship between ASD and the microbiota is still unclear."

AU: We have clarified this point in the conclusion (p. 6)

2. "Information could be added that there are studies that compare microbiota composition and disorders from the gastrointestinal tract of ASD and neurotypical siblings:

Son JS, Zheng LJ, Rowehl LM, Tian X, Zhang Y, Zhu W, Litcher-Kelly L, Gadow KD, Gathungu G, Robertson CE, Ir D, Frank DN, Li E. Comparison of Fecal Microbiota in Children with Autism Spectrum Disorders and Neurotypical Siblings in the Simons

AU: We have added this as a new reference on p. 5

3. "It is worth adding the meta-analysis of Morton et al. which indicated that age remains a major factor disrupting the microbiome in early childhood.

Morton JT, Jin DM, Mills RH, Shao Y, Rahman G, McDonald D, Zhu Q, Balaban M, Jiang Y, Cantrell K, Gonzalez A, Carmel J, Frankiensztajn LM, Martin-Brevet S, Berding K, Needham BD, Zurita MF, David M, Averina OV, Kovtun AS, Noto A, Mussap M, Wang M, Frank DN, Li E, Zhou W, Fanos V, Danilenko VN, Wall DP, Cárdenas P, Baldeón ME, Jacquemont S, Koren O, Elliott E, Xavier RJ, Mazmanian SK, Knight R, Gilbert JA, Donovan SM, Lawley TD, Carpenter B, Bonneau R, Taroncher-Oldenburg G. Multi-level analysis of the gut-brain axis shows autism spectrum disorder-associated molecular and microbial profiles. *Nat Neurosci.* 2023 Jul;26(7):1208-1217. doi: 10.1038/s41593-023-01361-0. Epub 2023 Jun 26.

AU: We have added this as a new reference on p. 6

Reviewer B

This is a well-written editorial commentary highlighting the need for more rigorous study design and standardized methodologies to enable a better understanding of the relationship between gut health, microbiome composition, and ASD. Critiques of some of the relevant shortcomings within select current studies, e.g., Wang et al (ref. 6), are instructive and provide useful information regarding steps to facilitate a more meaningful study design and subsequent data collection.

AU. No edits or changes were requested.