

Spotlight

Are changes in the gut microbiome a contributor or consequence of autism—why not both?

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Alterations in the gut microbiome have been associated with autism spectrum disorder (ASD), but whether they are a cause, effect, or confounder remains unclear. In a recent issue of *Cell*, Yap and colleagues report that ASD-associated microbiota changes are likely a consequence of low diet diversity.¹

Interest in microbes and autism spectrum disorder (ASD) largely stemmed from pathogenesis, wherein maternal infection during pregnancy is associated with increased risk for ASD in the offspring.² This aligns with some reports that antibiotic use correlates with improved ASD symptoms.³ Furthermore, subsets of ASD individuals exhibit gastrointestinal disturbances, and anecdotal evidence that elimination diets improve symptoms in ASD⁴ continue to fuel the idea that diet and gut health could be tied to immune and behavioral health.

At the center of these systems, the gut microbiome plays important roles in regulating dietary metabolism and inflammation. Early investigations of the gut microbiota in ASD revealed increases in *Clostridia*.⁵ These were followed by several studies that profiled alterations in the gut microbiota in ASD. While most reported differences relative to controls, there is little consistency in the findings across the different studies, which vary in experimental design, methods, and the assessment of covariates. Yap and colleagues advance the state of the field by examining both the composition and functional potential of the gut microbiome in ASD children and by evaluating how additional variables may play a role in driving microbial signatures.¹

The researchers profiled fecal metagenomes from 247 children: 99 with ASD, 51 undiagnosed siblings, and 97 undiagnosed controls. When considering microbial taxa that discriminate the ASD samples from controls, *Romboutsia timonensis* was identified as reduced in ASD. While this bacterium is of the class *Clostridia*, the results contrast findings from prior studies

that evaluated ASD cohorts by 16S rRNA gene sequencing. No microbial gene families were identified as associated with ASD, but when considering genes derived from *R. timonensis* alone, those related to the metabolism of amino acids, purines, pyrimidines, and galactose as well as spore germination and DNA digestion were particularly reduced in ASD.

To assess potential relationships between microbiome profiles and other traits, the researchers quantified associations of microbial species or genes with ASD diagnosis, age, BMI, Intelligence Quotient-Development Quotient (IQ-DQ) scores, Children's Sleep Habits Questionnaire scores, Bristol Stool scores, and Australian Eating Survey results. Only weak associations were found for neurological traits, where ASD diagnosis explained 7% of variance in rare microbial genes, and IQ-DQ explained 3% and 7% of variance in common microbial species and genes, respectively. In contrast, stool consistency explained 5% and 41% of variance for common and rare microbial species, and 49% and 64% for common and rare microbial genes. Notably, diet diversity explained 13% and 20% of variance for common and rare microbial species and 26% and 58% for common and rare microbial genes. Consistent with restrictive food preferences linked with ASD, ASD diagnosis was associated with decreased diet diversity compared to undiagnosed controls. Diet diversity correlated with microbial taxonomic diversity with a Pearson's correlation of 0.25 and each was a significant predictor of the other in regression analyses. More modest associations were calculated for microbial

taxonomic diversity and stool consistency. Together, these results indicate that there are stronger associations of microbial taxonomic diversity with measures of diet diversity and stool consistency, as opposed to neurological traits related to ASD diagnosis (Figure 1).

To interrogate links between diet diversity and ASD, the authors quantified associations of diet diversity with (1) ASD polygenic score, which quantifies genetic risk for ASD; (2) Autism Diagnostic Observation Schedule version 2 and Generic (ADOS2/G) scores and Social Responsiveness scores, which quantify behavioral features of ASD; (3) ADOS2/G scores particularly for restricted and repetitive behavior; (4) and Short Sensory Profile scores, which assess levels of sensory sensitivity. Overall, the results are consistent with the understanding that the restricted interests, insistence on sameness, and altered sensory perception characteristic of ASD could lead to the consumption of a limited repertoire of foods.

Overall, Yap and colleagues provide evidence that in individuals diagnosed with ASD, variations in the fecal microbiota can be attributed, at least in part, to the consumption of less diverse diets.¹ This raises the important question of whether even diet-driven changes in the gut microbiota could influence the manifestation of ASD-related symptoms or co-morbidities. Animal studies provide proof-of-concept, as the absence or depletion of the gut microbiome impairs social behavior in mice.⁶ In environmental and genetic mouse models for ASD, selectively manipulating the microbiome postnatally improves social and communicative behavior.^{7,8} There



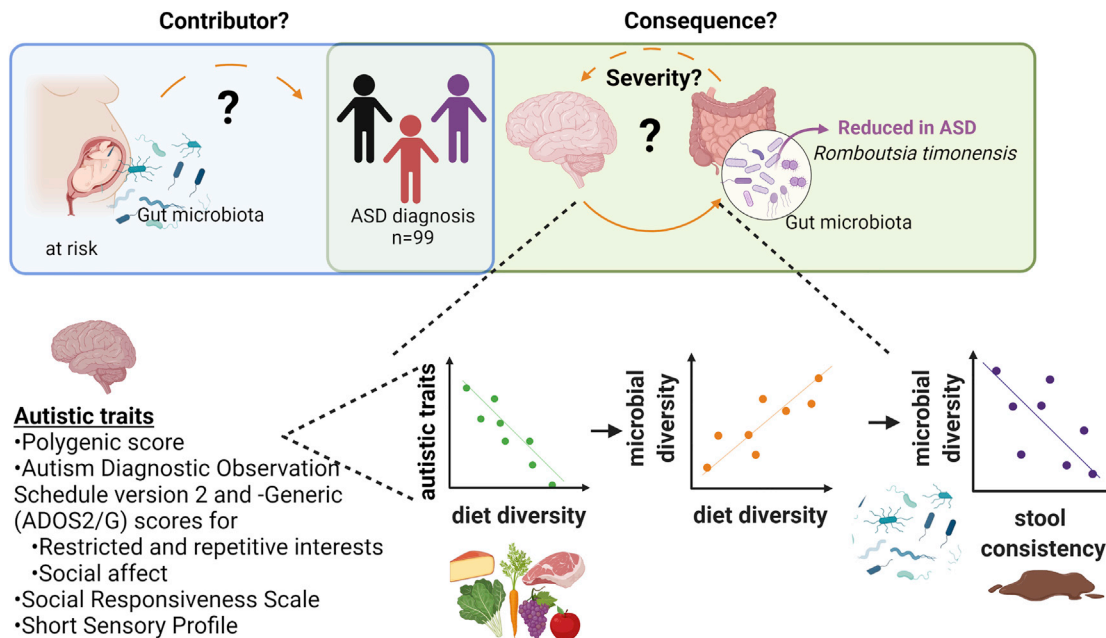


Figure 1. Potential interactions between the gut microbiome and ASD

Yap and colleagues reveal that autistic traits are associated with decreases in diet diversity, which likely drives microbiome alterations and loose stool consistency observed in individuals diagnosed with ASD.¹ Still unclear are whether the gut microbiome could contribute to or modify risk for the early development of ASD, and whether the ASD-driven changes in the gut microbiome in later life could influence the severity of symptoms of disease.

are many biological paths to influence complex behaviors relevant to ASD, and the exact mechanisms by which the microbiome modulates behavior in these animal models remain unclear. In an open-label study of 40 children with ASD, fecal transplantation was reported to correlate with improvements in gastrointestinal symptoms and behavioral measures.⁹ The interventions applied after the diagnosis of ASD suggest that they likely interfere with pathways that inform behavior, rather than reversing the developmental pathogenesis of ASD.

Importantly, in examining the microbiota of individuals already diagnosed with ASD, the question of whether alterations in the microbiota could modify risk for the development of ASD remains unanswered. In a mouse model of environmental risk for ASD, differences in the maternal microbiome tuned the severity of the inflammatory response to environmental challenge, thereby modifying the risk for neurodevelopmental and behavioral abnormalities in the offspring.¹⁰ In humans, evaluating the microbiome as a potential modifier of disease risk for ASD is made especially challenging by the understanding that the etiopathogenesis of

ASD begins *in utero*. Prospective studies of at-risk mother-infant pairs are needed, coupled with longitudinal assessment of the microbiome and clinical outcomes. The study by Yap and colleagues is an excellent demonstration that attention is needed to identify the driving forces, including diet, that can shape microbial diversity.¹ Understanding exactly how factors like diet interact with the microbiome could one day enable the application of such knowledge to create targeted microbial changes that benefit human health.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Yap, C.X., Henders, A.K., Alvares, G.A., Wood, D.L.A., Krause, L., Tyson, G.W., Restuadi, R., Wallace, L., McLaren, T., Hansell, N.K., et al. (2021). Autism-related dietary preferences mediate autism-gut microbiome associations. *Cell* 184, 5916–5931.e17. <https://doi.org/10.1016/j.cell.2021.10.015>.
2. Atladóttir, H.Ó., Thorsen, P., Østergaard, L., Schendel, D.E., Lemcke, S., Abdallah, M., and Parner, E.T. (2010). Maternal infection requiring hospitalization during pregnancy

and autism spectrum disorders. *J. Autism Dev. Disord.* 40, 1423–1430. <https://doi.org/10.1007/S10803-010-1006-Y>.

3. Sandler, R.H., Finegold, S.M., Bolte, E.R., Buchanan, C.P., Maxwell, A.P., Väisänen, M.L., Nelson, M.N., and Wexler, H.M. (2000). Short-term benefit from oral vancomycin treatment of regressive-onset autism. *J. Child Neurol.* 15, 429–435. <https://doi.org/10.1177/088307380001500701>.
4. Lee, R.W.Y., Corley, M.J., Pang, A., Arakaki, G., Abbott, L., Nishimoto, M., Miyamoto, R., Lee, E., Yamamoto, S., Maunakea, A.K., et al. (2018). A modified ketogenic gluten-free diet with MCT improves behavior in children with autism spectrum disorder. *Physiol. Behav.* 188, 205–211. <https://doi.org/10.1016/j.physbeh.2018.02.006>.
5. Finegold, S.M., Molitoris, D., Song, Y., Liu, C., Vaisanen, M.L., Bolte, E., McTeague, M., Sandler, R., Wexler, H., Marlowe, E.M., et al. (2002). Gastrointestinal microflora studies in late-onset autism. *Clin. Infect. Dis.* 35 (Suppl 1), S6–S16. <https://doi.org/10.1086/341914>.
6. Desbonnet, L., Clarke, G., Shanahan, F., Dinan, T.G., and Cryan, J.F. (2014). Microbiota is essential for social development in the mouse. *Mol. Psychiatry* 19, 146–148. <https://doi.org/10.1038/mp.2013.65>.
7. Hsiao, E.Y., McBride, S.W., Hsien, S., Sharon, G., Hyde, E.R., McCue, T., Codelli, J.A., Chow, J., Reisman, S.E., Petrosino, J.F., et al. (2013). Microbiota modulate

- behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell* 155, 1451–1463. <https://doi.org/10.1016/j.cell.2013.11.024>.
8. Sgritta, M., Dooling, S.W., Buffington, S.A., Momin, E.N., Francis, M.B., Britton, R.A., and Costa-Mattioli, M. (2019). Mechanisms Underlying Microbial-Mediated Changes in Social Behavior in Mouse Models of Autism Spectrum Disorder. *Neuron* 101, 246–259.e6. <https://doi.org/10.1016/j.neuron.2018.11.018>.
9. Li, N., Chen, H., Cheng, Y., Xu, F., Ruan, G., Ying, S., et al. (2021). Fecal Microbiota Transplantation Relieves Gastrointestinal and Autism Symptoms by Improving the Gut Microbiota in an Open-Label Study. *Front. Cell. Infect. Microbiol.* 11, 948. <https://doi.org/10.3389/FCIMB.2021.759435/BIBTEX>.
10. Kim, S., Kim, H., Yim, Y.S., Ha, S., Atarashi, K., Tan, T.G., Longman, R.S., Honda, K., Littman, D.R., Choi, G.B., and Huh, J.R. (2017). Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring. *Nature* 549, 528–532. <https://doi.org/10.1038/nature23910>.