Editorial Commentary

Impact of gut health and microbiome on autism spectrum disorder

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Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by challenges in social interaction and communication, as well as restricted interests and repetitive behaviors. The prevalence of this disorder has exhibited a sustained increase over an extended period. Although recent data indicates that it impacts almost 1 in 40 children, the pathophysiological mechanisms underlying the development of ASD remain poorly defined. A prevailing consensus suggests a complex interplay of genetic and epigenetic factors that influence brain development, involving intricate neuronal networks and contributing to the distinctive neurobehavioral phenotype observed in affected individuals. The substantial impact of genetics on ASD development is evident by a strong gender disparity and increased prevalence among siblings, particularly monozygotic twins, with male predominance suggesting a potential association with the X chromosome. However, whole-exome sequencing studies generally have not identified pathogenic sequence variants in most ASD cases, emphasizing the complexity of the genetic basis of ASD and underscoring the significance of potential environmental and perinatal factors in the etiology (1,2).

The gut microbiome constitutes one such potential non-genetic factor, represented by different structured communities of microorganisms that inhabit the intestine. Proper colonization patterns of the gut microbiota play a potential role in infant central nervous system development and function, as well as in preventing inflammatory processes. Described as the microbiota-gut-brain axis, this interrelationship involves mechanisms of gut-brain communication mediated in part by the vagal, sacral and autonomic nervous systems, as well as systemic hormone signaling linking increased stress response with altered gut function and microbiota composition (3). Gut bacteria, as metabolically active organisms, produce neurotransmitters or their precursors, such as gamma-aminobutyric acid (GABA) or short-chain fatty acids (SCFAs), which directly and indirectly impact the nervous systems (4). The microbiota also influences the control of catecholaminergic and serotonergic signaling molecules, in various brain regions including behavioral centers. Imbalances or dysbiosis in the gut microbiota are believed to be associated with the development or exacerbation of neurodegenerative, and neuropsychiatric diseases, including ASD through alteration of microbiota-gut-brain axis signaling.

In clinical observations, it is evident that children diagnosed with ASD often present with digestive disorders and a range of gastrointestinal symptoms, including but not limited to abdominal pain, persistent constipation or diarrhea, and flatulence. Notably, altered diversity in the gut microbiota has been observed across various age groups within the ASD population (1,2). Given the current lack of therapeutic options for ASD, there exists an urgent need to understand whether distinctive principles governing gut microbiota composition in neurotypical children are different from those with ASD. Unfortunately, this requires a central focus on precisely defining a "normal" or "healthy" gut microbiota, and achieving consensus within the

research community remains challenging (5), particularly when considering the dynamic nature of gut microbiota composition during development, which reaches relative stability after 5 years of age. The genetic information of these gut bacteria exhibits considerable individualization and variability. This microbial colonization pattern is also not solely determined by genetic predisposition but is significantly influenced by various environmental factors. These encompass delivery mode, duration of breastfeeding, composition of dietary components such as fiber, exposure to industrially processed foods, geography and lifestyle choices. Thus, understanding the composition of the gut microbiota in ASD reveals a spectrum of diagnostic and therapeutic possibilities that need to be considered when mitigating ASD symptoms through targeted interventions of a dysregulated gut microbiota.

Along these lines, early studies centered on the influence of antibiotics on the core manifestations of ASD, but generally lacked robust control arms to support clinical benefit. While numerous studies have explored the effects of prebiotics and probiotics on ASD symptoms, the outcomes have generally been inconclusive (1). Notably, these interventions can demonstrate beneficial outcomes on gastrointestinal symptoms, which can indirectly impact ASD core behavioral traits in specific instances. Fecal microbiota transplantation (FMT) distinguishes itself by incorporating a full complement of naturally occurring gut bacteria, in contrast to conventional pre- and probiotic approaches. Widely acknowledged and validated, FMT has traditionally been employed in the treatment of recurrent *Clostridioides difficile* infection (5). However, subsequent investigations have expanded its applications to include inflammatory bowel disease and neuropsychiatric disorders. Limited yet promising studies propose FMT as a potential therapeutic approach in ASD treatment, indicating significant reductions in scores on the Autism Behavior Checklist (ABC) and Childhood Autism Rating Scale (CARS), possibly by concurrently addressing gastrointestinal concerns in individuals with ASD (1).

Several of the above issues are considered in a recent publication featured in *BMC Pediatrics*, where Wang *et al.* undertook an investigation aimed at characterizing gut microbiota signatures in children diagnosed with ASD and concomitant chronic gastrointestinal symptoms within the southeastern coastal region of China (6). The study enrolled 42 children presenting with ASD and gut comorbidity, including a control arm of 41 neurotypically developed children. Fecal mid-segment samples, collected shortly after defecation were transferred to a DNA preservation solution and microbiome communities were characterized by 16S rRNA gene amplicon sequencing. The study findings revealed a surprising elevation in microbial richness within the ASD group, although diversity and evenness exhibited no significant changes. A notable variance in beta-diversity was also reported. At the genus rank, a diminished abundance of *Flavonifractor*, UBA1819, and *Acinetobacter* was observed, whereas *Streptococcus*, *Lachnospiraceae_NC2004*, *Ruminococcus*, *Ruminiclostridium*, *Lachnospiraceae_ND3007*, and *Lactococcus* were increased in children with ASD compared to neurotypical controls. Within these genera, noteworthy correlations were identified, with the CARS exhibiting a significant negative correlation with *Ruminococcus* and positive correlations with *Acinetobacter* and *Flavonifractor*. Conversely, none of the genera demonstrated a significant correlation with the ABC. Furthermore, the study revealed differences in the metabolic functionalities of the gut microbiota between the two groups. Specifically, children diagnosed with ASD exhibited a suppressed microbial pathway associated with alpha-linolenic acid and an upregulated pathway involving uncharacterized conserved proteins. While these findings provide a valuable foundational framework for further exploration of the ASD-associated gut microbiota that could potentially be targeted for treatment, they also underscore the challenges inherent in understanding the underlying mechanisms driving microbiome alterations in this context.

Firstly, differences in gut microbiota community structure and composition may be associated with gastrointestinal health, necessitating a precise diagnostic approach. ASD frequently manifests with digestive comorbidities and diverse gastrointestinal symptoms that are often linked to modifications in the gut microbiota composition, regardless of neuropathology. A retrospective prevalence study involving 14,000 individuals with ASD reported a significantly higher occurrence of bowel disorders (excluding inflammatory bowel disease) in individuals with ASD compared to a general hospitalized population (11.74% *vs.* 4.5%) (7). Moreover, another retrospective study revealed that 30.5% of individuals with ASD experienced at least one disorder of brain-gut interaction (DBGI), which was associated with intellectual disability and sleep disorders (8). Numerous investigations have reported alterations in gut microbiota profiles and metabolic functionalities in patients with DBGI (9,10). Considering the close relationship between ASD, gut health and microbiota, it is imperative to assess whether differences in gut microbial profiles in ASD

are dependent on gastrointestinal disorders. However, such an evaluation requires precise and standardized diagnosis of gut health status, such as employing the ROME criteria for diagnosing DBGIs.

In the highlighted study, Wang *et al.* aimed to identify gut microbiota signatures in children with ASD presenting with gastrointestinal symptoms. Nevertheless, the criteria for diagnosing the reported comorbidities were not detailed. Furthermore, despite disclosing the prevalence of symptoms, the inclusion of gastrointestinal disease as an enrollment criterion was not explicitly specified. Furthermore, although the methodology indicated the exclusion of individuals who had received antibiotics or probiotics within 3 months before sample collection, the cohort of 42 individuals with ASD still encompassed those with prolonged probiotic usage or recent antibiotic administration. Additionally, an insufficient analysis was conducted, introducing uncertainty regarding whether the reported gut microbiota signatures were associated with gastrointestinal disease or the use of antibiotics or indeed medications used to treat core ASD symptoms.

Secondly, the comorbidities associated with ASD give rise to concerns regarding the characteristics of the control group. Traditional ASD studies commonly employ individuals without ASD symptoms as the control group, yet the health status of these individuals, particularly pertaining to their gastrointestinal health, is frequently left unspecified. This lack of clarity introduces ambiguity concerning the comparability of the control arm to the ASD group, particularly in relation to factors that may impact gut microbiota composition. Notably, in the study conducted by Wang *et al*., children with ASD exhibited a high prevalence of gastrointestinal symptoms, whereas the health status of the control group was not detailed. Consequently, it remains unclear whether the observed differences in gut microbiota were associated with gastrointestinal symptoms, treatment modalities or the ASD condition itself. In addressing this issue, a more sophisticated study design including siblings of the ASD group as an additional control group should be considered to reveal the significance of genetic similarity and shared environmental factors, encompassing aspects such as diet, lifestyle, and socio-economic status, in elucidating the complex interplay between ASD and gut microbiota (11).

Thirdly, in addition to gastrointestinal symptoms, variations in gut microbiota may be attributable to dietary differences rather than inherent distinctions among disease groups. Diet exerts a profound influence on the composition of the gut microbiota. Considering that children with ASD often contend with eating disorders that significantly impact their dietary intake, it is conceivable that these dietary factors play a role in the observed differences. Notably, a microbiome study involving 247 children reported limited direct associations with autism but revealed strong correlations with dietary traits (12). While children with ASD exhibited lower microbial taxonomic diversity, their diets were also significantly less diverse compared to their siblings or unrelated children without ASD. Moreover, the taxonomic variations were identified as downstream consequences of dietary differences rather than direct associations with the ASD diagnosis. These findings underscore the importance of considering dietary preferences as mediators in microbiota studies related to ASD, which requires a food log or diary to document food intake. In the study by Wang *et al*., 42.86% of the ASD group exhibited selective eating tendencies, 24.81% experienced anorexia, and 7.14% had food allergies. However, the authors did not incorporate these factors or dietary intake into their analyses, leaving uncertainty regarding whether the reported gut microbiota signature is impacted by food intake and eating habits.

Finally, it is noteworthy that bioinformatics and statistical analysis methodologies often lack a standardized approach with clear and comprehensive descriptions, leading to inconsistencies across various studies. For instance, the study conducted by Wang *et al.* lacks clarity in describing the analysis for metabolic pathways. Additionally, the inconsistency in the approach and threshold for multiple testing corrections is prevalent among omics studies; Wang *et al.* did not perform or report multiple testing corrections in their exploratory analysis. Furthermore, the oversight of essential covariates in study design and data analysis is a recurring issue. Extensive and comprehensive systematic meta-analyses of gut microbiota in children with ASD underscores the need to consider these complexities (13,14). These meta-analyses identified three pivotal variables—age, sex, and stool consistency—that require consideration when investigating differences in fecal microbiota composition. Wang *et al.* and certain other studies did not adequately address these factors, potentially contributing to disparities in their research findings. While it is commendable that Wang *et al.* conducted comparisons with age- and sex-matched controls, additional statistical analyses could offer further insight into potential age- or sex-dependent differences in gut microbiota composition.

In conclusion, emerging evidence suggests a potential

role for the gut microbiota in ASD, although the precise relationship between ASD and the microbiota remains unclear. To advance our understanding of microbial taxa that associate with ASD symptoms, it is imperative to meticulously account for covariates and carefully scrutinize the impact of dietary intake and gastrointestinal health within this contextual framework. Adequately addressing these points requires the adoption of precise and standardized methodologies, coupled with a balanced selection of clinical groups to enable rigorous control of environmental, dietary and gastrointestinal co-morbidities.

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References

1. Luna RA, Savidge TC, Williams KC. The Brain-Gut-Microbiome Axis: What Role Does It Play in Autism Spectrum Disorder? Curr Dev Disord Rep 2016;3:75-81.
2. Luna RA, Oezguen N, Balderas M, et al. Distinct Microbiome-Neuroimmune Signatures Correlate With Functional Abdominal Pain in Children With Autism Spectrum Disorder. Cell Mol Gastroenterol Hepatol 2017;3:218-30.
3. Sharkey KA, Savidge TC. Role of enteric neurotransmission in host defense and protection of the gastrointestinal tract. Auton Neurosci 2014;181:94-106.
4. Savidge TC. Epigenetic Regulation of Enteric Neurotransmission by Gut Bacteria. Front Cell Neurosci 2015;9:503.
5. Wu Q, Badu S, So SY, et al. The pan-microbiome profiling system Taxa4Meta identifies clinical dysbiotic features and classifies diarrheal disease. J Clin Invest 2024;134:e170859.
6. Wang H, Liu S, Xie L, et al. Gut microbiota signature in children with autism spectrum disorder who suffered from chronic gastrointestinal symptoms. BMC Pediatr 2023;23:476.
7. Kohane IS, McMurry A, Weber G, et al. The co-morbidity burden of children and young adults with autism spectrum disorders. PLoS One 2012;7:e33224.
8. Penzol MJ, Salazar de Pablo G, Llorente C, et al. Functional Gastrointestinal Disease in Autism Spectrum Disorder: A Retrospective Descriptive Study in a Clinical Sample. Front Psychiatry 2019;10:179.
9. Hollister EB, Oezguen N, Chumpitazi BP, et al. Leveraging Human Microbiome Features to Diagnose and Stratify Children with Irritable Bowel Syndrome. J Mol Diagn 2019;21:449-61.
10. Plantinga AM, Kamp KJ, Wu Q, et al. Exploration of associations among dietary tryptophan, microbiome composition and function, and symptom severity in irritable bowel syndrome. Neurogastroenterol Motil 2023;35:e14545.
11. Son JS, Zheng LJ, Rowehl LM, et al. Comparison of Fecal Microbiota in Children with Autism Spectrum Disorders and Neurotypical Siblings in the Simons Simplex Collection. PLoS One 2015;10:e0137725.
12. Yap CX, Henders AK, Alvares GA, et al. Autism-related dietary preferences mediate autism-gut microbiome associations. Cell 2021;184:5916-5931.e17.

13. West KA, Yin X, Rutherford EM, et al. Multi-angle meta-analysis of the gut microbiome in Autism Spectrum Disorder: a step toward understanding patient subgroups. Sci Rep 2022;12:17034.

14. Morton JT, Jin DM, Mills RH, et al. Multi-level analysis of the gut-brain axis shows autism spectrum disorder-associated molecular and microbial profiles. Nat Neurosci 2023;26:1208-17.

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