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Forum

New insights on gut microbiome and autism

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Autism spectrum disorder (ASD) is a complex neurodevelopmental condition that often coincides with gut dysbiosis. Studies show that alterations in gut microbiota influence brain function and could serve as diagnostic biomarkers and therapeutic targets. This forum article discusses the role of gut microbiota in ASD pathogenesis and its diagnostic and therapeutic potential.

The role of gut microbiota in ASD

ASD is a prevalent neurodevelopmental condition characterized by impairments in reciprocal social interaction and stereotyped repetitive behaviors. Typically diagnosed during preschool years, ASD places a considerable burden on families and society. Despite extensive research into genetics, immunology, and perinatal factors, the exact etiology of ASD is still not fully understood. However, with advancements in sequencing technologies, more and more research has noted the involvement of gut microbiota in pathogenesis and its clinical value in the diagnosis and therapeutics of ASD (Figure 1).

Gut microbiota and ASD development

The gut–brain axis elucidates the bidirectional communication between gut microbiota and the brain, which can also affect host behaviors. This interaction acts through neuronal and chemical signaling pathways (Figure 1), including (i) the vagus nerve that transmits sensory information from gut to brain via neural afferent connections, (ii) microbial derived endocrine and metabolic signals directly affecting brain function, and (iii) microbiota-induced neuroimmune responses in the central nervous system (CNS). Gut microbiota disruption related to an overgrowth of pathogenic microbes and increased gut permeability impairs the integrity of the blood–brain barrier (BBB). This compromise allows peripheral neurotoxic proteins or microbial metabolites to enter the brain, leading to neuronal damage or neuroinflammation. Observational studies have consistently reported that individuals with ASD have a distinct gut bacterial community compared with neurotypical children, characterized by reduced species diversity, altered bacterial composition, impaired synthesis pathways for neuroactive metabolites, and a lagged bacterial developmental trajectory [1,2]. Infants with a higher risk of developing ASD showed lower levels of *Bifidobacterium* spp. and higher levels of *Clostridium* and *Klebsiella* spp. at 5 months of age [3]. The critical role of gut microbiota in ASD development has also been evidenced in animal studies. Transplantation of ASD-associated gut microbiota into germ-free mice resulted in social behavioral deficits, as well as ASD-related gene upregulation in the brain [4]. Mice treated with antibiotics manifested anxiety-like behaviors and reduced social interactions, which is associated with accumulated microbial derived aromatic compounds and disrupted hippocampal dysfunction. Moreover, offspring of mice fed a high-fat diet exhibited social behavior abnormalities, which could be reversed by co-housing with offspring from mothers fed a normal diet [5]. Overall, both preclinical and clinical evidence suggests that alterations in the gut microbiota contribute to the symptoms of ASD. Therefore, ASD-associated microbial signatures hold significant promise for clinical use, both as diagnostic biomarkers and as targets for therapeutic intervention.

Diagnostic potential of gut microbiota

A broad spectrum of studies across diverse geographical regions and populations have characterized a distinct gut microbiota profile associated with ASD (Table 1). Amongst these, an increased abundance of bacteria of the genus *Clostridium*, specifically *Clostridium bolteae* and *Clostridium botulinum*, has been reported in individuals with ASD. By contrast, there appears to be a reduction in the genera *Prevotella* and *Roseburia*. Using these bacterial biomarkers, machine-learning models have achieved an accuracy of 70–80% in distinguishing individuals with ASD from neurotypical peers [1,2]. Beyond compositional differences, dysfunctions in the microbial metabolism of amino acids, carbohydrate, and lipid profiles have also been observed and accompanied analogous brain metabolism differences in individuals with ASD [6]. The combination of microbial metabolic functions and bacterial biomarkers has demonstrated superior diagnostic accuracy compared with the bacterial markers alone [1]. This suggests that a complex neurodevelopmental disorder like ASD requires a more comprehensive characterization of other multi-kingdom microbiota, including viruses, fungi, and archaea.

Gut viruses directly or indirectly affect human health, either by infecting human cells or by interacting with other gut microorganisms. Administration of microbiota with a higher level of *Caudovirales* in mice enhances novel object recognition capacity and memory as well as upregulating memory-involved genes in the brain [7]. Research has identified an association between ASD and higher levels of several bacteriophages, including those that infect *Clostridium* spp., *Bacillus* spp., and enterobacteria. Gut phage dysbiosis disrupted the homeostasis of bacterial and viral communities, potentially impairing functional pathways responsible for

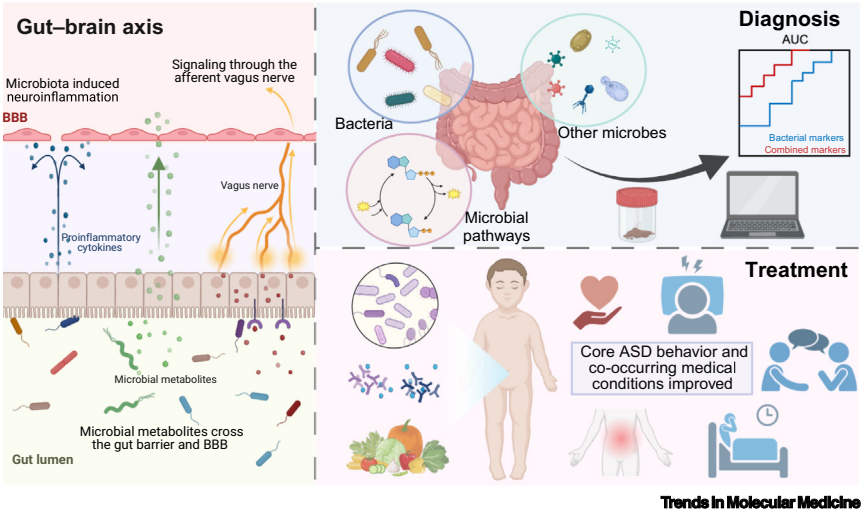

Figure 1. Microbiota gut–brain axis and gut microbiota potential in diagnosis and therapeutics of autism spectrum disorder (ASD). The communication between gut microbes and the brain through the gut–brain axis involves the vagus nerve pathway, microbially derived endocrine and metabolic signaling, and the immune system (left panel). The ASD-associated microbial markers have promising value for aiding diagnosis (right top panel) and as targets for therapeutic intervention (right bottom panel). Abbreviations: AUC, area under the curve; BBB, blood–brain barrier. Figure created with BioRender.

neuroactive metabolite synthesis in individuals with ASD [8]. The extraintestinal effects of gut fungi and archaea have also been revealed in the gut–brain axis. Transfer of gut fungi from stressed to non-stressed mice has been shown to induce irritable bowel syndrome-like symptoms, which can be restored by administration of *Saccharomyces boulardii* [9]. Mice colonized with a specific consortium of mucosa-associated fungi have a significantly increased social preference, which is mediated by the interleukin (IL)-17R-dependent signaling pathway of neuroimmune modulation [10]. Individuals with ASD have higher levels of gut fungi (*Candida* and *Saccharomyces cerevisiae*) but a lower level of *Aspergillus versicolor*. Furthermore, the altered gut methanogenic archaea are associated with worsened gut permeability and inflammation, leading to pathogenic factors crossing the gut barrier, and increasing the risk of neuroinflammation. The involvement of each microbial kingdom – bacteria, viruses, fungi, and archaea – in shaping phenotypes of neurological disorders has become increasingly evident. Hence, integrated analysis of the multi-kingdom gut microbiota warrants consideration as a novel biomarker panel in ASD diagnosis. In addition, different geographic regions involving various populations, diets, and lifestyles may contribute to variations in gut ecology (Table 1), indicating a need to eliminate/assess the confounders' effects on ASD models.

Therapeutic potential of gut microbiota

Until now, no pharmaceuticals have been officially and widely approved to treat ASD. The strong connection between gut microbiota and ASD has highlighted the potential approach of modifying the gut microbiome to improve behavioral symptoms associated with ASD. Fecal microbiota transplantation (FMT) has been evaluated in ASD. There was a marked improvement in both the severity of autism and gastrointestinal (GI) symptoms in individuals with ASD after FMT,

Table 1. Microbial markers associated with ASD

Distinct microbial signatures from healthy controls	Biomarkers	AUC based on biomarkers	Refs
Veillonella, Enterobacteriaceae increase Microbial glutamate degradation and acetate synthesis decrease and 15 other microbial metabolism changes	Two taxa and 17 microbial metabolic functions	Discovery cohort: 0.86 Validation cohort 1: 0.78 Validation cohort 2: 0.82 Validation cohort 3: 0.67	[1]
Faecalibacterium decrease *Clostridium*, *Dialister*, *Coprobacillus*, *Alistipes indistinctus*, *candidate division_TM7_ isolate_TM7c*, *Streptococcus cristatus*, *Eubacterium limosum* and *Streptococcus oligofermentans* increase	Five bacteria species	Discovery cohort: 0.83 Validation cohort: 0.76	[2]
Eubacterium decrease *Paraprevotella*, *Granulicatella*, *Peptoniphilus* increase	Three differentially abundant gut or oral genera and dysbiosis markers	Oral marker: 0.71 Gut marker: 0.72 Dysbiosis marker: 0.69	[13]
Sutterella, *Prevotella*, and *Bacteroides* decrease	24 bacterial genera	Discovery cohort: 0.93	[14]
Bifidobacterium longum, *Prevotella copri* decrease *Veillonella parvula* and *Lactobacillus rhamnosus* increase Microbial detoxification enzymes decrease	Five detoxification pathways	Discovery cohort: 0.88	[15]

and a long-term benefit was shown [11]. The beneficial effects of multiple probiotics (e.g., *Lactobacillus* spp., *Bifidobacterium* spp.) and prebiotics (e.g., oligosaccharides, galacto-oligosaccharide) supplements in alleviating ASD symptoms – as well as improving speech communication, sensory cognitive awareness, and GI symptoms – were revealed in clinical trials. Modulating microbial metabolites provides another viable therapeutic option. Reducing gut bacteria-derived phenols not only targets core ASD symptoms but also addresses commonly associated conditions such as anxiety and irritability [12]. Notably, diets provide substrates and energy for gut inhabitants that could plausibly affect gut ecology and microbiota diversity. Children with ASD have selective eating behavior associated with poorer dietary quality and reduced microbiota diversity. However, multiple nutritional interventions (e.g., ketogenic diet, specific carbohydrate diet) enabled a reduction in opportunistic pathogens and improved behavior, underlining the importance of diet-driving microbiome in ASD therapy. Accumulative evidence has revealed that gut microbiota regulates autism-like behaviors through several mechanisms: (i) upregulating the γ-aminobutyric acid receptor gene expression in the brain, (ii) restoring the excitatory/inhibitory neurotransmitter balance, (iii) increasing oxytocin synthesis in the hypothalamus, and (iv) decreasing the proinflammatory cytokines. These interventions not only improve core autism-like behavior but also alleviate the highly prevalent co-occurring medical conditions: GI symptoms, anxiety, irritability, and sleep disturbance. This suggests a potential shared etiology of genetic and environmental factors between these comorbidities and ASD, as well as shared pathways through which gut microbial interventions can modulate brain function and host phenotypes. There is no universal remedy for ASD; thus, the challenge remains in personalized microbial interventions to suit children with varying clinical traits, which may enhance the efficacy of treatments.

Concluding remarks

The linkage between gut microbes and brain health could be key to helping us understand and treat ASD. Treatments like FMT, probiotics/prebiotics, and special diets that change gut bacteria have shown promise, but they need to be personalized, because the gut microbes of individuals with ASD vary significantly. To help those with ASD, we need to characterize the entire ecosystem of gut microbes, including bacteria, viruses, and other microorganisms. Research considering all these factors will lead to better tests and treatments that meet broader needs, offering more effective management of ASD.

Declaration of interests

S.C.N. has served as an advisory board member for Pfizer, Ferring, Janssen, and Abbvie, and has received honoraria as a speaker for Ferring, Tillotts, Menarini, Janssen, Abbvie, and Takeda. S.C.N. has received research grants through her affiliated institutions from Olympus, Ferring, and Abbvie. S.C.N. is a founder member, non-executive director, non-executive scientific advisor, and shareholder of GenieBiome Ltd. S.C.N. receives patent royalties through her affiliated institutions. Q.S. is a Scientist (Diagnostics) of GenieBiome Ltd. Y.W., Q.S., and S.C.N. are named inventors of patent applications held by the CUHK and MagIC that cover the therapeutic and diagnostic use of microbiomes related to ASD.

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