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Autism Spectrum Disorder as a Brain–Gut–Microbiome Axis Disorder

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Abstract

While there are numerous medical comorbidities associated with ASD, gastrointestinal (GI) issues have a significant impact on quality of life for these individuals. Recent findings continue to support the relationship between the gut microbiome and both GI symptoms and behavior, but the heterogeneity within the autism spectrum requires in-depth clinical characterization of these clinical cohorts. Large, diverse, well-controlled studies in this area of research are still needed. Although there is still much to discover about the brain-gut-microbiome axis in ASD, microbially mediated therapies, specifically probiotics and fecal microbiota transplantation have shown promise in the treatment of GI symptoms in ASD, with potential benefit to the core behavioral symptoms of ASD as well. Future research and clinical trials must increasingly consider complex phenotypes in ASD in stratification of large datasets as well as in design of inclusion criteria for individual therapeutic interventions.

Keywords

Autism spectrum disorders; Gastrointestinal; Microbiome; Brain-gut axis

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by persistent deficits in social communication and repetitive behavioral patterns. Current statistics suggest that ASD affects 1 in 59 children throughout the USA [1]. Although the diagnosis of ASD is exclusively neurobehavioral, ASD is accompanied by many medical comorbidities that often occur in much higher prevalence than in neurotypical children. Among these co-occurring medical conditions, gastrointestinal (GI) problems are among the most common.

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GI disorders are approximately fourfold more prevalent in children with ASD than in the neurotypical population. Although all of the same GI disorders that present in neurotypical individuals can also be found in those with ASD, constipation and diarrhea tend to be the most common [2, 3]. There have been strong correlations found between GI dysfunction and multiple other comorbidities. For example, functional constipation in children with ASD has been associated with worsened behavioral symptoms, as well as an increase in cortisol, stress, and anxiety [4–14]. Other CNS-based comorbidities in the ASD population, such as seizures and sleep disorders, also present more commonly in association with GI dysfunction [3, 15–17].

The high prevalence of GI dysfunction in ASD and its significant correlations with challenging behaviors and psychiatric comorbidities suggest that there is a relationship between gut and brain dysfunction in a significant subset of these individuals. Insight into the mechanisms that cause dysfunction in brain-gut communication may thus lead to a greater understanding of the underlying pathophysiology of the brain-gut axis in patients with ASD and, moreover, lead to the discovery of novel therapeutic targets. There is increasing evidence to suggest that one of the key modulators of gut-brain communication in ASD is the intestinal microbiome.

The brain-gut-microbiome axis has become a compelling area of investigation in ASD, specifically in the pediatric population (Fig. 1). Characterization of the gut microbiome, usually profiled by sequencing the 16S rRNA gene in bacteria isolated from stool, has been increasingly employed in pediatric GI disorders over the past decade. Unique microbial patterns have been identified in a variety of functional GI disorders like irritable bowel syndrome and recurrent abdominal pain as well as in inflammatory disorders such as inflammatory bowel disease [18–23]. Pilot studies with small cohorts have expanded into large multicenter studies that are now beginning to provide the power to identify robust patterns in these GI conditions, and ASD research is just beginning to benefit from these studies. In this review, we will discuss the potential role of the gut in ASD with a focus on the current state of knowledge of the gut microbiome in this population.

Gut Microbiome and ASD

The role of the gut microbiome in ASD was first questioned with the emergence of anecdotal reports of young children who developed regressive ASD after repeated exposure to antibiotics for chronic otitis media [24, 25]. The clinical course described in this subset of children included the development of chronic diarrhea post-antibiotic administration that was quickly followed by a loss of language and social skills [26, 27]. The initial hypothesis, which was generated in part by Ellen Bolte [26], a mother of a child who with regressive ASD, implicated anaerobic bacteria, namely Clostridiales, based on their ability to produce neurotoxins [28]. Bolte was also the first individual to report an overall improvement in ASD symptoms when her son was given additional antibiotics targeted to eradication of Clostridiales, leading to further research on the possibility that antibiotics could effect change in the core symptoms of ASD [29].

The notion that Clostridiales could be an underlying factor in ASD-associated symptoms for regressive ASD set the foundation for the first open-label clinical trial of vancomycin, an antibiotic known to treat Clostridia [30], in kids with regressive ASD. The inclusion criteria for the study created a unique subgroup of ASD, specifically those who developed ASD-like symptoms and diarrhea after antibiotic exposure in early childhood and who continued to have GI issues and significant communication difficulties post-antibiotic exposure. Each subject was given an 8 week course of vancomycin followed by 4 weeks of oral probiotics (*L. acidophilus L. bulgaricus*, and *B. bifidum*) [24]. Interestingly, eight of the 11 individuals who received vancomycin showed substantial behavioral improvements. The majority of subjects, however, experienced substantial behavioral deterioration within 2 weeks after vancomycin cessation [24]. Although there were significant limitations in the study, including small sample size, lack of an untreated control group, and an open-label study design [31, 32], it was a crucial first step in support of the hypothesis that changes in the gut microbiome could elicit changes in the core symptoms of ASD.

A number of studies over the past 20 years have reconfirmed the finding that stool from some individuals with ASD may harbor distinct Clostridial species relative to neurotypical individuals [27, 33–40]. *Clostridium (Lachno-clostridium) bolteae* has specifically been associated with ASD patients with GI problems [34, 36, 41]. Despite these differences in proportions, the ways in which Clostridiales impacts host physiology in ASD are not yet known. Blood samples from some ASD patients demonstrate increased levels of metabolites that are chemically similar to p-cresol (e.g., 4-Ethylphenylsulfate), a neurotoxin produced by Clostridiales [42, 43]. This finding supports the hypothesis that Clostridiales produce neurotoxins. Studies have not been done, however, to confirm a connection between increased levels of 4-Ethylphenylsulfate and deleterious effects on brain or enteric nervous system development or function.

Extensive Diversity of Current Studies: Background and Implications

In addition to Clostridiales, multiple distinct microbiota populations have been demonstrated in individuals with ASD compared to neurotypical controls, and these studies have been done almost exclusively in children [27, 33–36, 39–41, 44]. It is important to note that these studies have generated highly divergent results [45]. Underlying reasons for this discordance in findings include recruitment of small cohorts, utilization of unaffected siblings as the singular control group, and failure to control for key modulators of the microbiome, including diet, antibiotic exposure, probiotic intake, and both over-the-counter and prescription medications (Fig. 1) [46–48]. Individuals with ASD often have highly self-restrictive diets, which have been shown to affect gut microbiota [49]. GI motility issues, including constipation and diarrhea, have also been shown to affect the microbiome (and vice versa) and, as stated above, are also common in individuals with ASD [2, 3, 12, 50, 51]. Finally, it is exceedingly difficult to account for the extensive genetic and medical heterogeneity inherent in the ASD population which may also impact microbial composition [2, 3, 52].

Beyond variation in study design and population, laboratory and analytic techniques employed in the study of the microbiome have differed vastly as technology has rapidly

advanced. As the cost of sequencing has decreased, studies have exponentially increased average sequencing depth. This continuous advancement, paired with more complete microbially focused databases, has increased the level of resolution in the microbiome field yet has also contributed to the diversity of outcomes based on the type of technology utilized. For example, the earlier studies, that detected an increased abundance of *Clostridium* species in the GI tracts of ASD patients [27, 36, 39], utilized stool-based culture and RT-PCR. The results of this research was much more limited in that stool bacteria was examined at higher taxonomic levels rather than at the species or OTU level [33, 34, 38, 41]. The next phase of studies, that incorporated next-generation sequencing, were more specific but continued to exhibit clinical study limitations, as detailed above. As the studies in the USA evolved from PCR-based or culture-enhanced characterization of the microbiome, the findings of previous studies in pediatric ASD failed to be replicated [33, 38] with some studies unable to identify any statistically significant differences in microbiota between the ASD and control groups [37, 40].

Geographic location is known to affect microbiome profiles, and diet is thought to be the major driver behind those differences. While the initial studies that sought to evaluate the microbiota in ASD were mainly conducted in the USA, recent publications are now offering a glimpse into how gut microbiome profiles in ASD differ across several countries including Italy [53, 54], India [55], Spain [56], China [57], and Ecuador [58]. As expected, even though these individual studies highlight differences between the ASD and control groups, there is very little consistency of findings across these studies. For example, two recent studies both conducted in Italy failed to reach consensus at even the phylum level, with one study reporting a decrease in Bacteroidetes [54] while the other reported an increase [53]. It is worth noting that, in contrast to the former study, the latter focused on young children (ages 2–4 years) and age stratification is a crucial point of analysis in the evaluation of the developing gut microbiome in pediatric ASD. Studies with larger cohorts, including multi-site recruitment from diverse geographic locations, that are integrating a critical mass of data related to the clinical phenotype (Fig. 1), with GI and behavioral components, as well as addressing dietary differences, are thus greatly needed in this population.

Stool Versus Tissue Microbiota: Is There a Difference?

Most current studies have evaluated the intestinal microbiome in ASD patients through stool. Though stool collection is a less invasive option, evaluation of microbiota that lay directly on the intestinal mucosa may be more highly indicative of the direct interactions that specific microbiota have with the gut mucosa, as well as their impact on host physiology [59–64]. There have been two studies published thus far that have sought to identify a mucosal microbiome in ASD [44, 65]. The initial study utilized next-generation sequencing to evaluate ileal and cecal mucosal biopsy specimens from children with and without ASD. There were differences in the microbiota composition between the children with ASD and neurotypical children, including increases in Clostridiales, specifically *Lachnospiraceaea* and Rumino-coccaceae [44]. The investigators further found that these microbiota shifts were associated with lower mRNA levels of genes important for carbohydrate digestion (e.g., disac-charidases and hexose transporters). This was an interesting finding because there have been multiple anecdotal reports describing behavioral and GI improvements in

children with ASD placed on gluten-free/low carbohydrate diets [66]. Lack of adequate carbohydrate breakdown and absorption has been shown to lead to fermentation, gas production and increased gut osmotic load which results in bloating, abdominal pain and diarrhea with carbohydrate intake [44]. Although the double-blind, placebo-controlled studies have not shown differences in behavior or GI symptoms after a gluten-free diet, this study was important in that it was the first to provide a potential explanation for why some children with ASD may respond well to gluten free and/or low carbohydrate diets. The current study, however, was small and included patients with a diverse array of GI motility problems (e.g., constipation versus diarrhea). If these findings were to be reconfirmed in a larger population of children whose clinical metadata is more clearly defined, this could be an important insight into how diet can be used as therapy for a subset of individuals with ASD. It is also important that the precise functional roles of the bacteria associated with these changes in hexose transporters and dissacharidases be investigated.

A more recent study that focused on the relationship between the mucosal microbiome in children with and without ASD specifically was advantageous in that included children with ASD who also met the criteria for a functional GI disorder (FGID) [2, 67], the most common type of GI problems affecting this population. The ASD and neurotypical (NT) children evaluated were grouped based on their FGID status as assessed by the Questionnaire on Pediatric Gastrointestinal Symptoms-Rome III (QPGS-RIII) and endoscopic findings (ASD-FGID, NT-FGID, NT). The investigators found novel correlations from not only those patients with ASD compared to neurotypical children, but also those individuals with ASD and abdominal pain compared to those without pain. As seen in prior stool-derived microbiota studies, there was a significant increase in several Clostridiales species, but here it was noted specifically in the population of children with ASD who also had a diagnosis of FGID, indicating a novel association between Clostridiales, ASD, and FGIDs [65]. Several of the *Clostridium sp*. were also found to correlate significantly with proinflammatory cytokines (IL-6, IL-1, IL-17, and INF-gamma) as well as tryptophan and serotonin, indicative of newly identified potential links between the intestinal microbiota, serotonin and/or tryptophan secretion as well as inflammation [65]. These correlations thus yielded the first multi-omic profile specific to ASD-FGID and ASD-FGID with abdominal pain and identified novel human-relevant associations between specific microbiota, gut neurotransmitters and immunity. Finally, the data also may provide some insight into how serotonin affects gut function in these individuals. Almost a third of individuals with ASD have high blood serotonin levels [68]. Children with ASD and hyperserotonemia have also been found to exhibit higher levels of lower GI tract problems (e.g., constipation) [4]. This may be linked to bidirectional communication with the gut microbiota; gut bacteria can synthesize serotonin, increases in host serotonin levels can impact gut microbiota composition and, further, specific bacteria (i.e., *Turicibacter sanguinis*) may actually possess a serotonin “sensor” that takes up serotonin with consequent effects on its colonization and host physiology [61, 62, 69]. These correlates have been demonstrated in several transgenic murine models, including the BTBR T + Itpr3tf/J (Black and Tan BRachyury with inserted *tufted* inositol 1,4,5-triphosphate receptor 3 delta as a marker) and the SERT Ala56 (serotonin reuptake transporter alanine 56 substitution) mice, that exhibit core behavioral

phenotypes of ASD, as well as alterations in intestinal microbiota that correlate with delayed GI transit and impaired serotonin signaling [70, 71] (and pilot data by Luna and Margolis).

Altogether, these data suggest that alteration of the microbiota is associated with changes in the gut-neuro-immune axis. They also provide the basis for much larger studies, both clinical and functional, to identify the roles of specific microbiota in behavioral and gut dysfunction in ASD and also the utility of serotonin-based modulators as potential therapeutic targets.

Genetic Abnormalities and Environmental Exposures Contribute to ASD Risk: Understanding the Underlying Mechanisms in Relation to the Gut Microbiome

Although there have been many studies that demonstrate that the microbiota community in individuals with ASD is different, the specific microbiota involved and their functional effects on ASD development and/or the perpetuation of ASD-associated behaviors remain largely unknown. Although still in its infancy, the examination of this field in murine studies has begun to elucidate potentially important links between environmental risk factors, the microbiota and ASD development.

A number of murine studies support the notion that genetic abnormalities and/or environmental exposures that affect brain development and function also impact gut function [72–77]. The microbiome has been increasingly shown to play a role in these gene-environmental interactions. Gnotobiotic mice and/or rats, for example, that are born and raised in the absence of microbial colonization, exhibit aberrations in several complex behaviors diagnostic of ASD including decreased sociability, repetitive stereotyped behaviors and impaired social behaviors, when introduced to new partners [78]. Some of these deficits, further, can be reversed by the administration of probiotics or co-housing with WT mice [79–84]. Underlying these observations are evidence that intestinal microbiota impact brain development in areas critical for emotion and mood. Gnotobiotic mice, for example, exhibit gene expression changes in the amygdala, a key region for emotional responses [85]. They also have increased hippocampal serotonergic neurons, in male mice, and decreased hippocampal brain-derived neurotrophic factor levels [80, 86]. The dorsal hippocampus plays a role in spatial learning and memory, while the ventral hippocampus preferentially regulates anxiety and the stress response. These abnormalities in CNS development correlate with behavioral disturbances in ASD, including stereotypies and decreased socialization [80].

In addition to its impact on the brain and behavior, the microbiome may also impact the gut itself. Gnotobiotic mice also exhibit gut abnormalities, including increased gut permeability and slowed motility [79, 87–91]. Gut dysfunction may be causative of CNS disturbance; it has been shown in prior mouse studies that increased intestinal permeability allows for the passage of proinflammatory mediators and/or hormones into the circulation, where they may be transported through the bloodstream to the brain, where they may ultimately impact CNS neurodevelopment and/or function [62–64, 69, 75, 92, 93]. Consequently, any genetic

predisposition or maternal exposure that impacts the intestinal microbiome could have implications on both brain and intestinal function.

Two exposures that have been linked to ASD development in clinical epidemiological studies are maternal obesity and maternal inflammation [60]. Mouse models of maternal obesity and maternal infection are also linked with development of ASD in progeny and, further, have implicated the microbiome in their underlying pathology.

In the USA, ~ one-third of women are obese [94, 95] and there has been a coincident rise in both obesity and neuropsychiatric disorders [96]. A mouse model of maternal obesity, induced by a maternal high-fat diet (MHFD), has been shown to result in ASD-like behaviors as well as changes in the CNS in resulting progeny. MHFD-exposed pups exhibit reduced vocalizations and disruptions in exploratory, cognitive, and stereotyped behaviors [72, 73, 82]. They also have fewer oxytocinergic neurons in the hypothalamus and impaired mesolimbic (dopaminergic) system function. Interestingly, these pups exhibit a dysbiosis characterized, in part, by lower levels of *Lactobacillus reuteri*, compared to controls [72, 82]. When *Lactobacillus reuteri* is replenished or oxytocin is administered, however, there is a reversal of the abnormal brain and behavioral findings, implicating a potential role for the microbiome in brain oxytocin production [82]. It was further shown by these investigators that the vagal nerve stimulation that induces oxytocin release, by projecting fibers into the paraventricular nuclei (PVN) which synthesize oxytocin in the brain, is triggered by *L. reuteri*, a hypothesis further confirmed by the lack of oxytocin release after vagotomy. A later study by the same laboratory determined that *L. reuteri* supplementation also increased the number and intensity of fluorescence of oxytocinergic neurons in the PVN in Shank3BKO mice, another mouse model of ASD with a genetic mutation found in Phelan McDermid syndrome. These findings together suggest that the microbiome may alter brain activity and behavior by stimulating the vagus nerve to increase PVN production of oxytocin. These findings have a clinical correlate in that there are anecdotal reports of behavioral improvements after oxytocin administration [97, 98].

L. reuteri is unlikely, however, to be the only microbe affected by high-fat diet exposure. A complementary study was done in which pregnant female mice were subjected to a microbiome depletion/transplantation paradigm. Antibiotics were used for microbial clearance and were followed by transplantation with microbiota isolated from donors on a high-fat diet, throughout pregnancy and breastfeeding. Male and female high-fat diet fed pups both had significantly decreased communication compared to controls while males exhibited significantly decreased amounts of exploratory time, an increase in stereotyped behaviors and impaired learning. Metagenomic R16S analysis suggested that decreased representation of particular species of the Firmicutes phylum was predictive of behavioral declines in male HFD pups [72].

The link between maternal infection and ASD originates from a large epidemiological study that showed strong associations between peripartum infections, specifically first trimester viral infections or second trimester bacterial infections, and ASD risk [99]. A subsequent prospective cohort analysis of over 100,000 women showed that maternal influenza infection

or episodes of fevers lasting more than 7 days during pregnancy are associated with a two- and three-fold increased risk of ASD, respectively [100].

A highly validated mouse model of maternal inflammation is the maternal immune activation (MIA) model, in which mouse dams are injected with the viral mimetic, poly(I:C). Studies of this model have revealed connections between maternal inflammation, enteric microbiome changes and abnormalities in brain-gut development and function [75, 76, 84, 101, 102]. Resulting offspring of maternal dams exposed to poly(I:C) exhibit deficits in core communication and social behaviors as well as stereotypical movements reminiscent of ASD [75]. In addition to the neurobehavioral deficits, these mice exhibit increased gut permeability accompanied by decreased expression of gastrointestinal tight junction proteins, and microbial dysbiosis characterized by changes in the populations of Clostridia, Bacterioides and Lachnospiraceae. These differences were associated with several abnormalities in serum metabolites, including increased levels of indolpyruvate, 5-HT, and 4-ethylphenylsulfate (4-EPS). Interestingly, oral gavage of *B. fragilis* corrected the dysbiosis, corrected the serum abnormalities in indolpyruvate and 4-EPS, and resulted in improvement of the behavioral abnormalities [84]. Because the gut permeability was also normalized after *B. fragilis* exposure, the investigators suggested that this microbial supplement repaired the intestinal barrier, thereby preventing leakage of toxic molecules (e.g., 4-EPS) into the serum which could not then circulate to the brain.

The proinflammatory cytokine, IL-6 has been implicated in the pathogenesis underlying the MIA models [75, 103]. In addition to its proinflammatory effects, Il-6 exhibits a robust inverse relationship with full scale intelligence quotient (FSIQ) scores and socialization scores in ASD patients, indicating a potential relationship between inflammation and behavior [5]. Interestingly, increases in IL-6 have also been linked to changes in intestinal tight junction expression and intestinal barrier integrity suggesting that the cytokine could be a link between inflammation, tight junction integrity and behavioral dysfunction [104]. Given the apparent microbial modulation of gut and brain function in MIA models and the repeated observation that IL-6 and the microbiome impact each others' regulation, it is possible that the underlying connection between IL-6, inflammation, and gut-brain dysfunction lites in its ability to modulate the microbiome, or vice versa [105–107]. Conversely, however, enteric microbes are also known to regulate intestinal tight junction and cytokine levels, suggestive of the idea that the microbiome may be a cause of the inflammation associated with maternal infection [108].

Other environmental exposure or genetic mouse models that exhibit impaired social behaviors and microbial dysbiosis, for which there is less data available, include a valproic acid exposure model and the BTBR T + Itpr3tf/J mice [70, 109]. Interestingly, social deficits observed in the BTBR T + Itpr3tf/J mice can be reversed by co-housing with their WT counterparts, making the microbiota a possible modulator for ASD-associated behavior in these mice as well [110].

In contrast to maternal infection/maternal immune activation and maternal obesity, an environmental exposure that may be protective against ASD is the ketogenic diet (KD). The KD has been used to manage epilepsy for nearly a century [111], including the treatment of

epilepsy syndromes where ASD is a core symptom [112, 113]. Interestingly, however, the KD has also been shown to relieve ASD-associated behaviors in some of these patients. There are several case reports of Rett syndrome patients who exhibited improvements in sociability and contact behavior after being placed on a KD [114]. These findings have been replicated in several mouse models; the KD has been shown to improve sociability in environmental exposure mouse models, including those subjected to MIA or valproic acid during development [77, 111, 115] and also in three genetically modified mouse models of ASD, the BTBR model [116], the EL strain (a strain used in epilepsy research) [117], and the genetic variant *Engrailed 2 gene* [118].

The mechanism linking the KD to seizure amelioration and sociability improvement remains unclear [111] though it has been suggested that these changes may result from microbial differences that emerge from the dietary changes. KD alters the composition of gut microbiota in mice [119, 120], as well as in humans [121, 122] BTBR mice have been shown to have fecal microbiota composition similar to that of some individuals with ASD, including elevated levels of Clostriadiales cluster XI [39], decreased Firmicutes and increased Bacteroidetes sp [33, 34] as well as increased levels of *Akkermansia muciniphila* [33, 34, 120]. The fecal and cecal low Firmicutes: Bacteroidetes ratio in BTBR mice, as well as the elevated levels of *Akkermansia muciniphila*,is both reversed by the KD [120]. The studies evaluating the effects of the KD on the gut microbiota, however, have not been entirely consistent; a KD has also been shown to result in an increase in *Akkermansia* and *Parabacteroides* species in a different study, and, moreover, confers the protective effects of KD against electrically induced seizures in the 6 Hz seizure-induced model [81].

It has been suggested that the link between ASD, the microbiota and seizure activity may all evolve from an underlying mitochondrial disturbance. Mitochondrial instability is found in many neurodevelopmental disorders, including ASD [123]; moreover, abnormalities in adenosine levels in ASD patients have been observed [124]. Improved mitochondrial function is thought to be a mechanism by which KD functions [125]. In accordance with this postulate, KD has been shown to increase adenosine levels in the CNS, purinergic therapies have been described as improving core symptoms of ASD [126, 127] and KD restored mitochondrial function in the valproic acid exposure model of autism [77]. It has been shown that gut microbiota signal to the mitochondria of mucosal cells, including epithelial cells and immune cells and, further, that this signaling alters mitochondrial metabolism [128]. If this happens in ASD models, however, has not yet been explored.

Potential of Microbially Mediated Therapies in ASD

With mounting evidence for a role of the brain-gut-microbiome axis in ASD, microbially mediated therapies hold significant potential for improving the quality of life for these individuals. We are at the cusp of better understanding the effect of diet on human health and behavior; however, dietary factors are significant modulators of the gut microbiome and thus dietary interventions may be considered a microbially mediated therapy for subsets of individuals with ASD. Gluten- and casein-free (GFCF) diets are commonly utilized in the ASD population even though small yet properly controlled studies fail to show significant improvement [129, 130]. Studies in other parts of the world, however, have shown potential

benefit of a GF diet on GI symptoms and behavior in ASD [131] as well as global improvements while on a ketogenic diet [132]. The most recent study took a more comprehensive approach in randomizing children and adults with ASD to a gluten-, casein-, and soy-free (GFCFSF) diet along with a variety of supplements, and after 1 year, the treatment group showed significant improvement in the core symptoms of ASD and developmental age compared to the control group [133]. Larger, well-controlled studies that take into account the clinical heterogeneity in ASD may help to target which individuals may benefit from diets like these.

Clinical trials that evaluate the use of probiotics for the treatment of a variety of GI disorders are ongoing, with many already showing highly favorable results [134]. While probiotics containing a single beneficial bacterial strain have been successful in IBS, formulations containing multiple strains across more than one genera may be more effective in the ASD population; recent trials in ASD using a multi-strain probiotic [135] and a combination of a single strain (*B. infantis*) and colostrum [136] have provided evidence of positive benefits and support the need for larger studies.

Although the initial vancomycin trial in pediatric ASD provided compelling evidence for the potential of antibiotics for treatment of the core symptoms of ASD [24], very little progress has been made on this front. More recently, minocycline as an adjuvant to risperidone was shown to decrease irritability and hyperactivity/noncompliance, but further work is needed to determine if these improvements are sustained [137]. Conversely, a pilot study evaluating the effect of D-cycloserine on core social deficits in ASD showed no improvement in the drug treatment group but improvement in the entire cohort that was attributed to the social skills therapy program [138].

The potential for microbially mediated therapy in general may be best assessed by fecal microbiota transplantation (FMT). Vancomycin is commonly prescribed prior to FMT, as was the case in the only study to investigate the potential efficacy of FMT in children with ASD. An open-label trial of 18 children with ASD included 2 weeks of vancomycin followed by 8 weeks of FMT [139]. Improvement in GI symptoms were seen during FMT treatment and persisted at 8 weeks following treatment cessation. Improvements were also seen in behavioral symptoms of ASD, and increases in Bifidobacterium, Prevotella, and Desulfovibrio in the gut microbiome were seen in parallel. Two years following treatment, improvements in GI symptoms remain as well as improvement in behavioral symptoms and sustained abundances of Bifidobacterium and Prevotella [140].

An expanded study that includes not only a placebo group but also a vancomycin only group is needed to accurately assess the impact of FMT plus vancomycin versus vancomycin alone. It is also important to consider that individuals with ASD continue to develop albeit at a delayed pace in most cases, so the inclusion of a control group for comparison with regard to GI and behavior will be needed to evaluate long-term outcomes. It will also be important to characterize the gut microbiome in parallel with these positive changes to potentially identify microbial profiles of individuals who were responders to FMT as well as determine the functional microbiome changes that are associated with alleviation of symptoms.

Future of Brain–Gut–Microbiome Axis Research in ASD

There has been a rapid advancement in the knowledge base of the gut microbiome and the technologies used to study it. Despite this vast increase in information, the many factors that confound the current gut microbiome studies in ASD make them impossible to interpret as a whole. With the increased understanding that many factors that impact the microbiome are present in ASD, such medication exposure and GI comorbidities, the inclusion criteria that relies solely on a "diagnosis of ASD" is no longer acceptable. Future studies must thus incorporate the clinical characteristics that comprise complex phenotypes in ASD. These studies will likely result in the stratification of multiple cohorts rather than "one ASD." Along these lines, it is likely that interventions that have anecdotally shown promise in individuals with ASD but have failed to show statistically significant improvement in clinical trials may have favorable outcomes when targeted "ideal groups" are identified. Significant effort is also needed in the investigation of the brain-gut-microbiome axis in adults with ASD, as almost all research in this area has been focused on the pediatric population. In conjunction with clinical studies, basic or translational studies are also critically needed to determine the effects of specific microbiota and their impact on host physiology in health and disease. Given all of the data supporting a role for microbial modulation of CNS development, behavior and cognition, it seems a worthwhile goal to pursue these aims as a way of developing novel microbially mediated treatments for this exceptionally hard to treat set of disorders.

Biography

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Key Points

- There is significant heterogeneity within autism spectrum disorder, including varying levels of severity with regard to behavior, cognitive ability, and medical comorbidities.
- Gastrointestinal symptoms can be difficult to recognize in individuals with ASD with limited communication abilities.
- Preclinical models of ASD have illustrated significant crosstalk along the brain-gut-microbiome axis.
- Complex phenotypes based on in-depth clinical characterization are vital to conducting robust research and clinical trials.
- Microbially mediated therapies are worth further exploration in specific complex phenotypes of ASD.

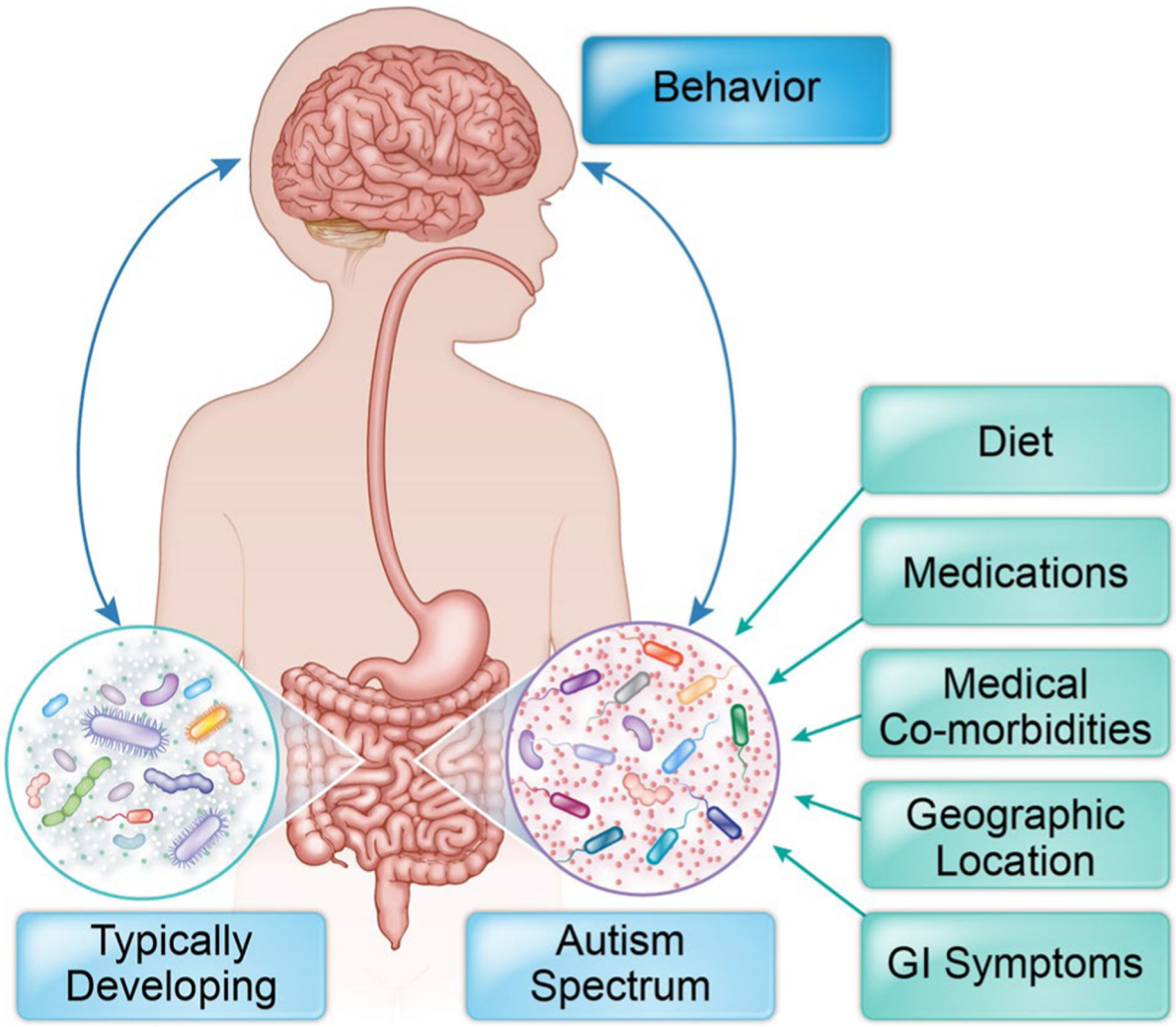

Fig. 1.

The brain-gut-microbiome axis plays an important role in autism spectrum disorder. Differences exist in the gut microbiome of typically developing children compared to children with autism spectrum disorder. However, these changes to the microbial community are affected by differences in diet, medication regimens, medical comorbidities, geographic location, and both acute and chronic GI symptoms. In addition, the brain-gut-microbiome axis is bidirectional with regard to changes in both the gut (GI symptoms and microbial profile) and behavior