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The link between autism spectrum disorder and gut microbiota: A scoping review

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Abstract

Gut dysfunction and microbial dysbiosis comorbidities are of particular interest in recent autism research, as gastrointestinal distress is present in up to 90% of autism spectrum disorder cases and therefore may play a key role in the pathogenesis of this disorder. This scoping review aims to integrate the results of studies conducted in the past 6 years examining the association between gut microbiota and autism spectrum disorder, specifically with regard to the characterization of autism spectrum disorder microbiota and potential therapeutic interventions. Studies related to the gastrointestinal microbiome of subjects with autism spectrum disorder were identified through PubMed, SCOPUS, PsycInfo, and Google Scholar databases. Studies were screened and selected based on defined inclusion and exclusion criteria; 19 studies were included. Research continues to report differences between microbiota of individuals with autism spectrum disorder and controls; however, the types and abundances of bacteria present remain inconsistent. Promising treatment interventions for autism spectrum disorder, including special diets, dietary supplementation, and of particular interest, microbiota transfer therapy, are also being explored. Research regarding the link between gut microbiota and autism spectrum disorder renders exciting results; however, it is still in its infancy of investigation. Rigorous methodologies are required to support and strengthen the reliability of existing results, and to further our understanding of the pathogenesis of autism spectrum disorder.

Lay abstract

Gastrointestinal distress and gut microbial imbalances are commonly found in children with autism spectrum disorder, and therefore may play a key role in the development of the disorder. This scoping review aimed to examine the extent, range and nature of research conducted in the past 6 years that focused on furthering our understanding of autism spectrum disorder and its association with gut microbiota. A literature review was performed with predetermined key words. Studies were screened and selected based on defined inclusion and exclusion criteria. A total of 19 studies were included for final analysis. While there are continuous reports of differences in gut microbiota between autism spectrum disorder and neurotypical individuals, knowledge about the consistency in the presence and abundance of bacterial species, as well as metabolites, remains deficient. Treatments such as special diets, vitamin, prebiotic, probiotic, and microbiota transfer therapy show promising therapeutic potential, yet are in their infancy of investigation. Overall, further research with rigorous methodologies is required to support and strengthen the reliability of existing findings. Future research should aim to increase sample sizes, eliminate biases, and subgroup autism spectrum disorder groups to help accommodate for inter-individual variation. As increasing evidence of a unique autism spectrum disorder microbiome and metabolome is acquired, autism spectrum disorder-specific biomarkers can be identified. These biomarkers have great implications in terms of elucidating the molecular mechanisms of autism spectrum disorder, preventing the onset of autism spectrum disorder, and improving treatments for individuals with autism spectrum disorder.

Keywords

autism spectrum disorder, autism spectrum disorder behavior, gastrointestinal disorders, gut microbiota, microbiota–gut–brain axis

Autism spectrum disorder (ASD) is a heterogeneous group of complex neurodevelopmental disorders that impair social interactions and communication and lead to restricted, repetitive, and stereotyped patterns of behavior, interests, and activities (World Health Organization, 2004). Commonly diagnosed in childhood, this disorder manifests

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four times more often in males than in females (Baio et al., 2018; Kantarcioglu et al., 2016). ASD is one of the fastest growing neurodevelopmental disorders in the industrialized world (Dietert et al., 2011) and currently the most commonly diagnosed neurological disorder in Canada (Ofner et al., 2018).

The exact etiology of ASD remains unclear; however, it appears to be multifactorial, involving a complex interplay of genetic and environmental factors (Dominguez-Bello et al., 2010; Watts, 2008). It is estimated that *de novo* mutations, common variants, and single nucleotide polymorphisms are overall responsible for approximately 50% of the disorder (Gaugler et al., 2014; Iossifov et al., 2015). Environmental triggers, such as pre- or postnatal exposure to chemicals and drugs, air pollution, stress, maternal infection, dietary factors, and associated medical comorbidities, contribute to the core neurobehavioral symptoms of the disorder (Kantarcioglu et al., 2016; Vuong & Hsiao, 2017). Biological processes that have been associated with ASD include impaired neuronal connectivity, immune dysfunction, mitochondrial abnormalities, and microbiome dysregulation (Chaste & Leboyer, 2012; Frye, Rose, et al., 2015; Rossignol & Frye, 2012).

Gut dysfunction and microbial dysbiosis comorbidities are of particular interest in recent autism research, as gastrointestinal (GI) distress in present in up to 90% of ASD cases and therefore may play a key role in the pathogenesis of this disorder (Liu et al., 2016).

Objective

This scoping review examines the extent, range, and nature of research activity throughout the past 6 years that focused on furthering our understanding of ASD and its association with gut microbiota.

GI disorders in ASDs

Various studies reveal a close association between ASD and GI function (Luna et al., 2016). Coincident with ASD, many children and adults experience significant GI disturbances, such as constipation, diarrhea, abdominal pain, and gastric reflux (Emanuele et al., 2010). In addition, deficient integrity of the gut epithelium and increased intestinal permeability (IP) are also commonly experienced (Emanuele et al., 2010). Such GI symptoms appear to be partly due to dysbiotic gut microbiota (Krajmalnik-Brown et al., 2015) and perhaps their missing roles on modulating metabolites that affect GI function and neurodevelopmental disorders, like ASD (Bravo et al., 2011; Hsiao et al., 2013).

Increased anxiety behaviors is also commonly reported in children with ASD who experience abdominal pain, constipation, or diarrhea when compared with ASD cases without GI symptoms (Mazurek et al., 2013); it has been noted that untreated GI symptoms may increase behavioral problems in children with ASD (Mazefsky et al., 2014). Collectively, findings suggest that ASD and GI disorders are so closely associated that children with ASD should be screened for GI disorders and children with particular GI disturbances should be screened for ASD (Luna et al., 2016).

Microbiota–gut–brain axis and ASD

Role of microbiota–gut–brain axis

The microbiota–gut–brain (MGB) axis is the multidirectional signaling between the central nervous system (CNS), the autonomic nervous system, the GI tract, and trillions of microorganisms that inhabit the gut (El Aidy et al., 2016; Luna et al., 2016). These highly integrated interactions and communications have been shown to play an important role in healthy brain function (Montiel-Castro et al., 2013). The axis comprises 200–600 million neurons (Furness, 2006) distributed among several pathways such as the vagus nerve, gut hormones, microbial metabolites, and the immune system (El Aidy et al., 2016).

MGB axis and immune system interplay. Intestinal microbes play a role in stimulating the host's immune system via release of pro-inflammatory cytokines or through the production of metabolites such as short chain fatty acids (SCFAs; Borre, Moloney, et al., 2014). Increased gut permeability can lead to translocation of gut bacteria from the intestine into the mesenteric lymphoid tissue provoking proinflammatory cytokine release (Yarandi et al., 2016). High levels of cytokines in the CNS is significant as cumulating evidence reveals that ASD pathogenesis may involve brain inflammation (Theoharides et al., 2013). Moreover, SCFAs can cross the blood–brain barrier to influence neural signaling, the production of neurotransmitters, and overall behavior (as reviewed in Yarandi et al., 2016).

MGB axis and HPA axis interaction. Cytokine release, IL-1, and IL-6 in particular, also have the ability to cross the blood–brain barrier and activate the hypothalamic-pituitary-adrenal (HPA) axis. HPA axis activation leads to cortisol release, which further activates the stress system (Dinan & Cryan, 2017). Since the CNS and GI tract are so closely related, stress may significantly impact GI physiology. This in turn impacts the presence and abundance of certain microbes, and thus results in stress-induced alteration of the microbiome structure (Gur & Bailey, 2016).

Overall, the MGB axis allows for gut microbiota to influence the host's neurodevelopmental status in a multifaceted fashion (Cryan & Dinan, 2012), and thus is suggested to be involved in ASD (Santocchi et al., 2016). The GI disturbances in ASD may be associated with gut dysbiosis, a disruption characterized by intestinal inflammation and altered intestinal function and permeability, commonly referred to as a "leaky gut" (Borre, O'Keeffe, et al., 2014).

Potential methods of developing a distinctive ASD microbiome

Mode of delivery

The method of delivery alters the microbial composition of newborn babies (Dinan & Cryan, 2015). Vaginal born babies have a microbiota that resembles vaginal microbiota composition of the mother with dominance of *Lactobacillus, Prevotella*, and *Snethia* species (Dinan & Cryan, 2015), while the microbial composition of neonates born by caesarian section (CS) resembles that of the mother's skin with dominance of *Staphylococcus, Corynebacterium*, and *Propionibacterium* species (Dinan & Cryan, 2015; Mohajeri et al., 2018). Further research suggests a higher risk for developing ASD in children who were born by CS (Berding & Donovan, 2016; Curran et al., 2015). In addition, an infant's gestational age at birth seems to have a significant effect on intestinal microbiota and health status (Penders et al., 2006). A preterm neonate not only has a gut microbiome that differs from a full-term neonate, but also has a higher risk for developing ASD (Di Mauro et al., 2013). This difference in microbiota profile is primarily due to the routine use of sterile formula and antibiotics in the neonatal intensive care unit (Di Mauro et al., 2013).

Diet

Feeding patterns may lay the foundation for an abnormal gut from birth. Feeding patterns work toward establishing bacterial colonization in newborns, as seen in breast-fed infants differing in composition and density of gut microbiota when compared with formula-fed infants (as reviewed in Liu et al., 2016). Breast-feeding appears to have been absent, or present in much shorter duration, in children with ASD (Kang et al., 2017; Schultz et al., 2006). Later in childhood, food selectivity and other feeding challenges greatly influence gut composition (as reviewed in Liu et al., 2016), especially since ASD children have strong preferences for nutrient-poor starchy, processed foods, and reject nutritious options such as fruits, vegetables, and proteins (Field et al., 2003).

Antibiotic use

Antibiotic treatment during the first 3 years of life can have harmful long-term effects on the normal microbial establishment by decreasing microbial diversity (Knight et al., 2017). Recovery of the microbiota to pretreatment state remains incomplete even several months after concluding treatment (Bik, 2016). Changes in the composition of the microbiota could lead to increased passage of metabolites, such as lipopolysaccharides or SCFAs, or enteric pathogens, such as *C. difficile* infections, through intestinal barriers (Gonzalez et al., 2011; Ng et al., 2013). In addition, Atladóttir et al. (2012) found an increased risk of ASD when pregnant mothers were treated with various antibiotics. In particular, the use of sulfonamide containing antibiotics and penicillin during the second and third trimesters increased the risk of ASD by approximately 50% (Atladóttir et al., 2012).

Maternal immune response

Infection during pregnancy, such as rubella or influenza virus, can create an inflammatory immune environment and result in the production of maternal cytokines (Meltzer & Van de Water, 2017). These cytokines can directly affect the placenta, and may cross the placenta to enter the fetal compartment and hold enduring effects on the development of the fetus. In addition, some women may produce anti-brain autoantibodies that can access the developing fetal brain, bind to fetal proteins, and thereby alter the course of neurodevelopment (Meltzer & Van de Water, 2017). Therefore, maternal immune response (MIA) during fetal development is a significant factor in the etiology of ASD (Meltzer & Van de Water, 2017). MIA during pregnancy may lead to ongoing postnatal immune dysregulation which persists in children with ASD (Meltzer & Van de Water, 2017). Throughout childhood, children with ASD may have endogenous anti-brain autoantibodies, which correlate with aberrant behaviors and impaired development (Meltzer & Van de Water, 2017).

Current research focuses for ASD

Therapeutics

Research evidence suggests that both GI issues and altered gut microbiota could render a child with a genetic predisposition for ASD, more likely to express the autistic characteristics or increase the severity of behavioral symptoms (Adams et al., 2011; De Theije et al., 2011). Therefore, interest in rebalancing human gut microbiota as treatment is expanding.

Dietary interventions. It is well understood that diet can have a major influence on the composition and metabolic products of the gut microbiota (David et al., 2014). Currently, little is known about the association between special diets and the gut microbiota in cases of autism (Liu et al., 2016). Many of the suggested special diets for children with ASD center on carbohydrate intake, such as the Atkin's diet, as subjects appear to be lacking in intestinal digestive enzymes responsible for carbohydrate digestion (Brudnak et al., 2002; Williams et al., 2011). Other documented diets focus on being either gluten-free or casein-free. Overall, the results from these diets remain inconsistent (Whiteley et al., 2013).

Prebiotics. Prebiotics, such as insulin-type fructans and galacto-oligosaccharides, are components of natural foods that stimulate growth and activity of specific microbial

strains in the gut that promote health in the host (Liu et al., 2016; Martinez, 2014). While some believe fermented foods and raw milk contain prebiotics and thus can positively alter gut flora, the available evidence seems to be mainly anecdotal (Al-Ayadhi & Elamin, 2013; Liu et al., 2016).

Probiotics. Probiotics are mixtures of living microbial strains that are ingested and believed to colonize the gut to promote host health (Liu et al., 2016). These bacteria might prevent the growth of pathogens through mechanisms that may involve producing vitamins, antioxidants, and removing toxins (Roberfroid et al., 2010). Probiotics are widely used for children with ASD. However, more research on the ASD microbiome and its response to different probiotic strains is needed to determine the most appropriate mixtures of probiotics to produce effective clinical results (Liu et al., 2016).

Fecal microbiota transplant. Fecal microbiota transplant (FMT) involves transferring samples of fecal microbiota from a donor to a recipient, aiming to replace a dysbiotic gut microbiome with healthy gut flora (Liu et al., 2016). Use of this intervention in conditions associated with distorted gut flora, such as ASD, is currently in experimental stages (Rossen et al., 2015). With the growing evidence for the role of gut bacteria in ASD, clinical trials are underway to improve our understanding of this connection (Frye, Slattery, et al., 2015).

Methodology

PubMed, SCOPUS, PsycInfo, and Google Scholar databases were used to retrieve all available articles addressing the research topic. Only articles produced in the past 6 years, 2013–2018, were considered for this review. Simple searches with predetermined key words were performed. The search terms employed were all-in-title: autism AND ("gut microflora" OR "gut bacteria" OR "gut flora" OR "gut microbiome" OR "gut microbiota" OR gastrointestinal flora" OR "gut"). If the article titles met this search criteria, the abstract was examined to determine its relevance to the scoping review, and if the full article should be read thoroughly.

Inclusion and exclusion criteria

All included studies (a) addressed gut microbiota composition, the MGB axis, or important pathways involved in the MGB axis in children with ASD; (b) had a cohort of children with ASD, or utilized an animal model of ASD; and (c) were primary source literature. Studies that were deemed to lack relevance to the research focus were primarily editorial pieces or research highlights, or were focused on developing theoretical models, and thus, were excluded. In addition, duplicate reports, unavailable full text, and literature that heavily relied on secondary sources were excluded. For details of the identification process of studies included in this scoping review, see Figure 1.

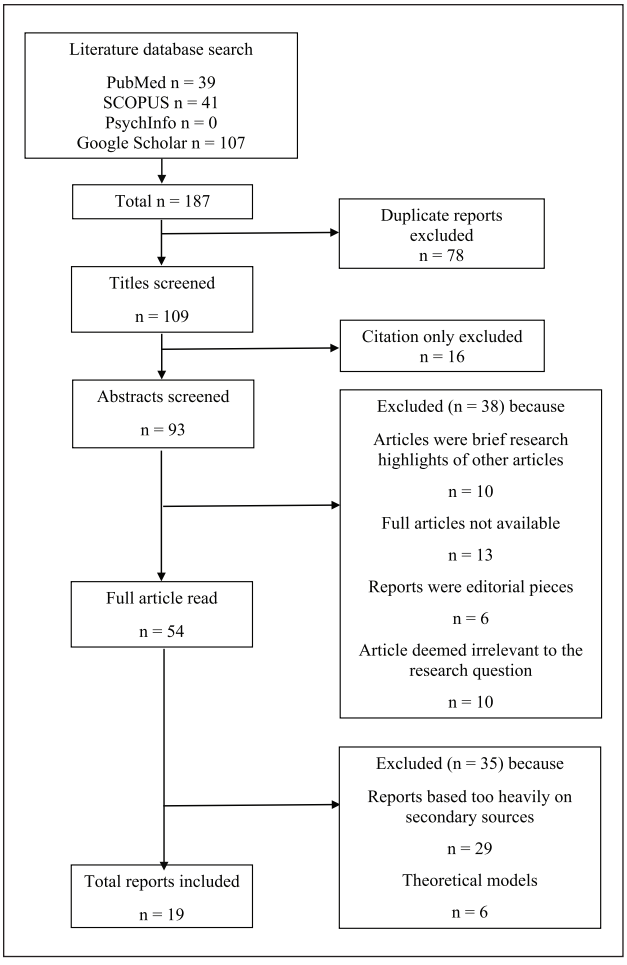

Figure 1. Flow diagram for the scoping review study selection process.

Results

Final analysis of this scoping review was based on the results of 19 studies; these are summarized in Table 1.

Therapeutics

Diet. More attention is given to the role of diet in treatment of ASD as ailments of the GI system have been shown to significantly affect the occurrence and severity of neurological symptoms of ASD. One interview-based study aimed to demonstrate the differences in behavioral habits and food consumption between children with ASD and controls (Siwek et al., 2017). While there was no statistically significant difference determined between the two groups of children in terms of frequency of consumption of products

Table 1. Details of studies included in this review.

Author	Item	Location	Study design	Sample size	Subjects	Main objectives	Key findings
Agarwala et al. (2018)	Diversity of gut microbiota in autism reveals differential abundance of *Prevotella* and *Akkermansia species*	Karnataka, India	Experimental/ instrumental design with fecal sample analysis	*N* = 60	Children with ASD, age range unspecified	Aim to better understand the degree to which gut microbiota of ASD children differs from controls and to identify bacterial species present exclusively in ASD.	*Akkermansia species* and *Prevotella species* were significantly more abundant in children with ASD compared to controls.
Coretti et al. (2017)	Sex-related alterations of gut microbiota composition in the BTBR mouse model of autism spectrum disorder	Naples, Italy	Experimental/ instrumental design Animal model	*N* = 48	BTBR mouse model of autism	Parallel gut microbiota profiles, behavioral characteristics, intestinal integrity and immunological features of colon tissues in BTBR mice, and investigate sex differences in this animal model.	BTBR mice of both sexes presented a marked intestinal dysbiosis, alterations of behavior, gut permeability and immunological state when compared to controls. Sex-related differences were clearly detected. *Bacteroides, Parabacteroides, Sutterella, Dehalobacterium*, and *Oscillospira* genera were key drivers of sex-specific gut microbiota profiles.
Coretti et al. (2018)	Gut microbiota features in young children with autism spectrum disorder	Naples, Italy	Experimental/ instrumental design with fecal sample analysis	*N* = 25	Children with ASD aged 2–4 years	Comparatively evaluate the gut microbiota composition and fecal levels of short chain fatty acids (SCFAs) in young children with ASD.	The *Bacteroidetes/Firmicutes* ratio was significantly higher. A striking depletion of *Bifidobacterium longum*, one of the dominant bacteria in infant gut microbiota, was observed. There was an increase of *Faecalibacterium prausnitzii*, a late colonizer of healthy human gut and a major butyrate producer.
De Theije et al. (2014)	Altered gut microbiota and activity in a murine model of autism spectrum disorders	Utrecht, Netherlands	Experimental/ instrumental design Animal model	*N* = 19	Valproic acid (VPA) mouse model of autism	Investigate the relation between gut microbiota and autism-like behavior.	Autism-like behavior and its intestinal phenotype is associated with altered microbial colonization and activity in a murine model for ASD, with preponderance in male offspring.
Hsiao et al. (2013)	The microbiota modulates gut physiology and behavioral abnormalities associated with autism	Pasadena, CA, USA	Experimental/ instrumental design Animal model	*N* = 47	Maternal immune activation (MIA) mouse model of ASD	Uncover molecular mechanisms involved in the pathogenesis of autism-related endophenotypes in the MIA mouse model of autism.	Demonstrates that a microbe-based therapeutic can ameliorate intestinal pathology, metabolic function, and autism-related behaviors in MIA mice, which supports a role for the gut-immune-brain axis in ASD.

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Table 1. (Continued)

Author	Item	Location	Study design	Sample size	Subjects	Main objectives	Key findings
Inoue et al. (2016)	A preliminary investigation on the relationship between gut microbiota and gene expressions in peripheral mononuclear cells of infants with autism spectrum disorders	Kyoto, Japan	Experimental/ instrumental design with fecal and blood sample analysis	*N* = 12	Children with ASD aged 3–5 years	Analysis of fecal and blood samples of infants with ASD and healthy infants were to investigate the association of altered gut microbiota and ASD development.	Feces of ASD infants had significantly higher and lower abundance of genera *Faecalibacterium* and *Blautia*, respectively. DNA microarray analysis of peripheral blood mononuclear cells (PBMC) detected more highly than low expressed genes in ASD infants than in healthy infants. Results strongly suggest that altered gut microbiota in infants results from ASD development and is associated with systemic immunity dysregulation, especially chronic inflammation.
Kang et al. (2017)	Microbiota transfer therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study	Tucson, AZ, USA	Open-label clinical trial, pilot study	*N* = 18	Children aged 7–16 years with ASD	Evaluate the impact of Microbiota transfer therapy (MTT) on gut microbiota composition and GI and ASD symptoms.	Improvements in GI symptoms, ASD symptoms, and the microbiome all persisted for at least 8 weeks after treatment ended, suggesting a long-term impact for MTT.
Kantarcioglu et al. (2016)	Microbiota–gut–brain axis: yeast species isolated from stool samples of children with suspected or diagnosed autism spectrum disorders and in vitro susceptibility against nystatin and fluconazole	Istanbul, Turkey	Experimental/ instrumental design with stool samples 17-year retrospective analysis	*N* = 1555 stool specimens	Male and female individuals with ASD or suspected ASD aged 9 months to 18 years	Identify the 3-year deposited yeast isolated from stool samples of children with diagnosed or suspected ASD and to determine in vitro activity of nystatin and fluconazole against these isolates.	The gut of the majority of healthy individuals did not contain yeast. *Candida albicans* was the most commonly isolated species in 17 years. *Candida krusei* and *Candida glabrata* were fluconazole-resistant and were not found in healthy individuals.
Labe (2016)	Does gut flora change in a mouse model of autism spectrum disorders on a ketogenic diet?	Hartford, CT, USA	Experimental/ instrumental design Animal model Fecal sample analysis	*N* = 44 fecal samples	BTBR mouse model of autism	Analyze how the ketogenic diet affects the GI flora in a mouse model of ASD and determine if the benefits of a ketogenic diet are correlated with changes in the gut flora.	No significant differences in the number of bacteria species present or abundance of bacteria between pre- and post-diet conditions.

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Table 1. (Continued)

Author	Item	Location	Study design	Sample size	Subjects	Main objectives	Key findings
Lim et al. (2017)	Modeling environmental risk factors of autism in mice induces IBD-related gut microbial dysbiosis and hyperserotonemia	Seoul, Republic of Korea	Experimental/ instrumental design Animal model	*N* = 9–14	Poly I:C- and VPA-exposed mouse model of autism, males only	Analyze the gut microbiota of poly I:C and VPA-induced mouse models of ASD.	There was a distinct pattern of microbial dysbiosis that highly recapitulated those reported in clinical cases of ASD. This microbial dysbiosis led to notable perturbations in microbial metabolic pathways that are known to negatively affect the host, especially with regard to the pathogenesis of ASD. Serum level of serotonin is significantly increased in both poly I:C and VPA mice.
Liu et al. (2017)	Effect of vitamin A supplementation on gut microbiota in children with autism spectrum disorders-a pilot study	Chongqing, China	Single-blind, non-randomized intervention pilot study	*N* = 64	Children with ASD aged 1–8 years old	Investigate the role of vitamin A (VA) in the changes of gut microbiota and changes of autism functions in children with ASD.	Proportion of *Bacteroidetes*/*Bacteroidales* significantly increased and the proportion of Bifidobacterium significantly decreased after VA intervention. Significant increase in autism biomarkers. No significant changes were observed in autism symptoms.
Navarro et al. (2015)	Are "leaky gut" and behavior associated with gluten and dairy containing diet in children with autism spectrum disorders?	Houston, TX, USA	Randomized, double-blind, placebo-controlled study	*N* = 12	Male and female children aged 4–7	Explore the association between diet type, intestinal permeability (IP) ("leaky gut"), and behavior.	Results do not support an association between dietary gluten/milk, intestinal permeability, and behavioral changes in subjects with ASD.
Newell et al. (2016)	Ketogenic diet modifies the gut microbiota in a murine model of autism spectrum disorder	Calgary, Canada	Experimental/ instrumental design Animal model	*N* = 46	BTBR mouse model of autism, males only	Examine whether gut microbiome disturbances are present in the BTBR mouse model of ASD and if the ketogenic diet is capable of altering the profile.	Consumption of a ketogenic diet triggered reductions in total gut microbial counts and compositional remodeling in the BTBR mouse. Counteracted the common ASD phenotype of a low *Firmicutes* to *Bacteroidetes* ratio. Reversed elevated *Akkermansia muciniphila* content in BTBR animals.

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Table 1. (Continued)

Author	Item	Location	Study design	Sample size	Subjects	Main objectives	Key findings
Pulikkan et al. (2018)	Gut microbial dysbiosis in Indian children with autism spectrum disorders	Kerala, India	Experimental/ instrumental design with fecal sample analysis	*N* = 54	Children with ASD aged 3–16 years	Compared fecal microbiota ASD children with family matched neurotypical children from an Indian population using next-generation sequencing of 16S rRNA gene amplicon.	Prominent dysbiosis in the gut microbiome of ASD children was observed, with higher relative abundances of families Lactobacillaceae, Bifidobacteraceae, and Veillonellaceae. The gut microbiome of neurotypical children was dominated by the family Prevotellaceae. A comparative meta-analysis with a dataset from the US population revealed a significantly high abundance of genus *Lactobacillus* in ASD children from both Indian and US populations.
Santocchi et al. (2016)	Gut to brain interaction in autism spectrum disorders: a randomized controlled trial on the role of probiotics on clinical, biochemical and neurophysiological parameters	Pisa, Italy	Randomized controlled clinical trial	*N* = 100	Children aged 18–72 months with ASD	Determine the effects of supplementation with a probiotic mixture (Vivomixx®) in ASD children not only on specific GI symptoms, but also on the core deficits of the disorder, on cognitive and language development, and on brain function and connectivity.	Trial is currently underway.
Siwek et al. (2017)	Role of the gut–brain axis in the eating behavior of children with autism spectrum disorders	Kraków, Poland	Interview	*N* = 77	Male and female children aged 3–8 years	Demonstrate the differences in behavior habits, interest in nutrition, and frequency of consumption of food products between children with ASD and healthy children.	Children with ASD had a higher intake of red meat and giblets and less frequent consumption of milk and milk products compared to the control group. No significant differences between groups in terms of frequency of consumption of products, which as the source of gluten, artificial food additives-preservatives, and artificial colors.

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Table 1. (Continued)

Author	Item	Location	Study design	Sample size	Subjects	Main objectives	Key findings
Strati et al. (2017)	New evidences on the altered gut microbiota in autism spectrum disorders	Pisa, Italy	Experimental/ instrumental design with fecal sample analysis	*N* = 80	Male and female children aged 5–17 years diagnosed with ASD	Characterize the bacterial and fungal gut microbiota in a cohort of autistic individuals.	Significant increase in the *Firmicutes/ Bacteroidetes* ratio in autistic subjects due to a reduction of the *Bacteroidetes* relative abundance. Decrease in the relative abundance of *Alistipes, Bilophila, Dialister, Parabacteroides*, and *Veillonella* in the ASD cohort, while *Collinsella, Corynebacterium, Dorea*, and *Lactobacillus* were significantly increased.
Tomova et al. (2015)	Gastrointestinal microbiota in children with autism in Slovakia	Bratislava, Slovakia	Experimental/ instrumental design with fecal sample analysis	*N* = 19	Children with ASD ages 2–9 years	Elucidate changes in fecal microbiota in children with ASD and determine its role in the development of often present GI disorders.	The fecal microbiota of autistic children showed a significant decrease of the *Bacteroidetes/Firmicutes* ratio and elevation of the amount of *Lactobacillus spp.* Found a very strong association of the amount of *Desulfovibrio spp.* with the severity of autism in the Autism Diagnostic Interview (ADI) restricted/ repetitive behavior subscale score. Participants demonstrated strong positive correlation of autism severity with the severity of GI dysfunction. Probiotic diet supplementation normalized the *Bacteroidetes/Firmicutes* ratio, *Desulfovibrio spp.* and the amount of *Bifidobacterium spp.* in feces of autistic children.
Zhang et al. (2018)	Analysis of gut microbiota profiles and microbe-disease associations in children with autism spectrum disorder in China	Beijing, China	Experimental/ instrumental design with fecal sample analysis	*N* = 41	Children with ASD ages 3–8 years	Elucidate changes of fecal microbiota in Chinese children with ASD using 16S rRNA sequencing and 16S rRNA (V3V4) gene tag amplification.	At phylum level: fecal ASD fecal analysis indicated a significant increase of the *Bacteroidetes/Firmicutes* ratio. At genus level: relative abundance of *Sutterella, Odoribacter* and *Butyricimonas* significantly increased, while abundances of *Veillonella* and streptococcus decreased compared to the control group.

ASD: autism spectrum disorder; GI: gastrointestinal; PBMC: peripheral blood mononuclear cells; ADI: autism diagnostic interview; SCFAs: short chain fatty acids; VPA: valproic acid; MIA: maternal immune activation; MTT: microbiota transfer therapy; IP: intestinal permeability; IBD: inflammatory bowel disease.

containing gluten and artificial additives, one notable difference was that neurotypical children consumed more milk and dairy products compared to children with ASD (Siwek et al., 2017). In response to common claims that gluten-free and casein-free special diets improve behavior in children with ASD, one randomized double-blind, placebo-controlled study was conducted to examine the association between gluten and milk, IP, and behavior in ASD (Navarro et al., 2015). These results, however, displayed no association between dietary gluten and milk proteins, IP, and behavior in subjects with ASD (Navarro et al., 2015).

Another diet under investigation in ASD research is the ketogenic diet (KD). KD consists of high fat, adequate protein, and low carbohydrates which causes the host metabolism to mimic a fasting state, promoting ketone body production and consumption (Newell et al., 2016). One study explores whether KD can alter gut microbiome in the $BTBR^{T}+^{tf/j}$ (BTBR) mouse model of ASD. The findings show a significant decrease in total host bacterial content in cecal and fecal matter (Newell et al., 2016). KD offset the common ASD phenotype of a low *Firmicutes/Bacteroidetes* ratio, and this diet lowered the elevated *Akkermansia muciniphila* content in cecal and fecal matter of BTBR animals (Newell et al., 2016). This study demonstrates that KD consumption triggers gut microbiota remodeling in an animal model of ASD, and these findings may provide insight into the therapeutic potential of KD manipulation by influencing gut microbial composition (Newell et al., 2016). Controversially, another study which analyzed the fecal sample of BTBR mice before and after being on a KD, found no significant difference in the overall abundance of bacteria or between the number of bacterial species present pre- and post-diet (Labe, 2016).

Vitamins. One pilot study investigated the effect of high dose vitamin A (200,000 IU) oral supplementation on changes in gut microbiota and autism behaviors in children with ASD (Liu et al., 2017). The active metabolite of vitamin A, retinoic acid, plays a key role in regulation of CNS development, and promotion of intestinal immunity and epithelial integrity (Cassani et al., 2012; McCullough et al., 1999). The findings suggest vitamin A intervention significantly increased the proportion of *Bacteroidetes/Bacteroidales* and significantly decreased *Bifidobacterium*; however, no significant changes were observed in autistic behavior (Liu et al., 2017). *Bacteroidetes* intervention may improve social behaviors in autism and this beneficial microbiota increased with vitamin A. In addition, results showed that vitamin A intervention contributed to significantly increased level of two autism biomarkers: CD38 and acid-related orphan receptor alpha (RORA) (Liu et al., 2017). Research demonstrates that decreases in both CD 38, a multifunction molecule, and RORA, a transcriptional regulator, play key roles in the aberrant social behavior seen in autism (Hu, 2012; Jin et al., 2007).

Probiotics. Currently underway is a double-blind randomized controlled trial to determine the effects of supplementation with the probiotic mixture on specific GI symptoms, core cognitive and language development deficits, and brain function and connectivity in ASD children (Santocchi et al., 2016). The probiotic mixture used was Vivomixx®, which contains 450 billion lyophilized bacterial cells and belongs to eight different probiotic strains. Possible effects of probiotic supplementation on urinary concentrations of phthalates, chemical pollutants, which have been linked to ASD, will also be evaluated (Santocchi et al., 2016). This study will provide what is lacking in current research on using probiotics as a treatment for ASD, using a rigorously controlled trial (Santocchi et al., 2016). Moreover, one study conducted using a maternal immune activation mouse model of autism, demonstrated that treatment with *Bacteroides fragilis*, a commensal microbe in humans, improved deficiencies in GI barrier integrity, corrected alterations in tight junctions of the colon, and ameliorated defects in cytokine expression (Hsiao et al., 2013). Specifically, treatment with *B. fragilis* decreased MIA-associated increases in colon interleukin 6 (IL-6), a cytokine required for the development of behavioral deficits in MIA offspring (Hsiao et al., 2013; Smith et al., 2007). Furthermore, oral treatment with *B. fragilis* improved communicative, repetitive, anxiety-like, and sensorimotor behaviors in MIA offspring (Hsiao et al., 2013). Another study also found positive effects of probiotic supplementation on fecal microbiota in children with ASD. A probiotic supplement containing three strains of *Lactobacillus*, two strains of *Bifidobacteria*, and one strain of streptococcus, was found to normalize the ratio of *Bacteroidetes*/*Firmicutes, Desulfovibrio spp.*, and *Bifidobacterium spp.* (Tomova et al., 2015).

Microbiota transfer therapy. An open-label study exploring microbiota transfer therapy (MTT) provides promising results for ASD treatment. MTT involves a 2-week antibiotic treatment, a bowel cleanse, and a high initial dose of fecal microbiota transplant followed by daily, lower maintenance doses for 7–8 weeks (Kang et al., 2017). The results show an 80% reduction of GI symptoms at completion of treatment and these significant improvements lasted for the subsequent 8 weeks (Kang et al., 2017). Additional findings include a significant improvement in behavioral ASD symptoms and an overall increase in bacterial diversity (Kang et al., 2017). These findings all persisted 8 weeks after treatment, which suggests this therapeutic approach may have a long-term impact (Kang et al., 2017).

Characterizing an ASD microbiota composition

Animals. One study investigated mice that were exposed in utero to valproic acid as an animal model for ASD, and demonstrated transgenerational impact on the gut microbiota of both male and female offspring (De Theije, et al.,

2014). A decreased abundance of *Bacteriodales* was observed in VPA-exposed offspring and increased abundance of *Erysipelotrichales* was observed in VPA-exposed male offspring (De Theije, et al., 2014).

A second study employed prenatal injected VPA and prenatal injected polyinosinic:polycytidylic acid (polyI:C) mouse models of ASD to analyze gut microbiota (Lim et al., 2017). The results found that ASD mice possessed a significantly less diverse microbial community than controls and significant changes in microbial taxa in a pattern that highly matches those of clinical ASD (Lim et al., 2017). A significant increase in *Desulfovibrio* genera was observed (Lim et al., 2017). *Desulfovibrio* is a toxin-producing harmful bacteria, cited to have been more frequently found in the stool of children with autism compared to neurotypical children (Finegold, 2011). ASD mice also had a decreased abundance of *Prevotella, Faecalibacterium prausnitzii*, and *Oscillospira* species (Lim et al., 2017). *Oscillospira* is considered an enigmatic constituent of the gut microbiome, yet is positively correlated with human health (Konikoff & Gophna, 2016). Out of all microbial taxa tested, the genus *Prevotella* showed the most prominent change compared to controls (Lim et al., 2017). This study brings new insight into how prenatal risk factors of ASD may render the host's enteric environment inhospitable for the growth of *Prevotella*. This finding draws attention to the possibility of administering prebiotics to increase *Prevotella* abundance. Increased *Prevotella* has the potential to alter the inflammatory status of ASD mice or affect behavioral abnormalities (Lim et al., 2017).

Contrastingly, Coretti et al. (2017) used the BTBR mouse model of autism to demonstrate marked intestinal dysbiosis as well as key sex differences in gut microbiota. Both sexes showed a significant increase in *Bacteroides* and *Parabacteroides*, as well as a significant decrease in *Dehalobacterium* compared to controls (Coretti et al., 2017). While *Bacteroides, Parabacteroides, Sutterella, Dehalobacterium*, and *Oscillospira* genera were identified as key drivers of sex-specific gut microbiota profiles, these genera were also found to be associated with altered behavior, increased gut permeability, and a colon pro-inflammatory state (Coretti et al., 2017). In conclusion, these findings suggest the presence of sex-specific profiles of gut microbiota, and these differing microbiota signatures should be considered when assessing the role of the MGB axis in ASD (Coretti et al., 2017).

Humans. Eight studies conducted in the past 6 years aimed to further characterize the differences in microbiota observed between neurotypical and ASD subjects. One study found that fecal microbiota in autistic infants displayed a significantly higher abundance of genus *Faecalibacterium*, and a significantly lower abundance of genus *Blautia* compared to healthy infants (Inoue et al., 2016). While another study on fecal microbiota of autistic children showed a significant decrease of the *Bacteroidetes*/*Firmicutes* ratio and elevation of the amount of Lactobacillus species (Tomova et al., 2015). An additional study characterized the bacterial and fungal gut microbiota in children with ASD. The results showed a significant increase in the *Firmicutes*/*Bacteroidetes* ratio, due to a reduction of *Bacteroidetes* abundance (Strati et al., 2017). At the genus level, a decrease in quantity of *Alistipes, Bilophila, Dialister, Parabacteroides*, and *Veillonella* was observed, while *Collinsella, Corynebacterioum, Dorea*, and *Lactobacillus* were significantly increased in the ASD cohort (Strati et al., 2017).

In contrast to Strati et al. (2017) and Tomova et al. (2015), two studies found a strong increase in *Bacteroidetes* abundance and an overall significant increase in the *Bacteroidetes*/*Firmicutes* ratio in the microbiome of children with ASD (Coretti et al., 2018; Zhang et al., 2018). These conflicting data may depend on several factors such as variations in sampling cohort, techniques, and software used to identify the gut microbiota composition, and eating habits as well as living environments (Coretti et al., 2018; Zhang et al., 2018). Zhang et al. (2018) studied Chinese children with ASD, drawing attention to the fact that most studies of gut microbiota in patients with ASD have been focused on Western populations (Zhang et al., 2018). It is important to expand these studies to non-Western diet populations in order to fully understand the diversity of gut microbiota in patients with ASD (Zhang et al., 2018).

Two studies were conducted on fecal microbiota of children with ASD in India. One study found genus *Lactobacillus, Bifidobacterium, Megaspaera*, and *Mitsuokella* (Veillonellaceae) to be significantly abundant in children with ASD compared to controls. Moreover, this study also conducted a comparative meta-analysis with publicly available dataset from the US population (Pulikkan et al., 2018). The results found that while the microbiome of Indian children with ASD was abundant in genus *Megaspaera, Bifidobacterium*, and *Mitsuokella* (Veillonellaceae), the microbiota of US children with ASD was abundant in genus *Akkermansia, Ruminococcus*, and *Lactobacillus* (Pulikkan et al., 2018). These differences could be attributed to differing dietary habits and consumption of a gluten-free diet by children with ASD in the US dataset (Pulikkan et al., 2018). For example, Veillonellaceae family is important for proper carbohydrate degradation and fermentation (Williams et al., 2011), and the native diet of Indian children is considered carbohydrate-rich (Pulikkan et al., 2018). A key finding of this study, which is consistent with Strati et al. (2017), is the high abundance of genus *Lactobacillus* among Indian and US children with ASD despite contrasting diets and geography (Pulikkan et al., 2018). The second study found a lesser abundance of *Akkermansia species* in ASD samples, and a higher abundance of *Prevotella species* compared to control (Agarwala et al., 2018). In addition, in accordance to Strati et al. (2017) and Tomova et al. (2015),

the phylum *Firmicutes* was significantly higher in abundance than control samples (Agarwala et al., 2018).

Furthermore, the relative abundance of *Candida*, a fungal genus, was more than doubled in ASD subjects compared to neurotypical subjects; this finding, however, is considered only partially significant (Strati et al., 2017). Candida grows in the gut and releases ammonia and toxins which are thought to contribute to autistic behaviors (Reichelt & Knivsberg, 2009). Another study isolated yeast deposits in stool samples of children with ASD in a 17-year retrospective assessment, and found that the majority of neurotypical children did not harbor yeast in their gut (Kantarcioglu et al., 2016). The higher prevalence of yeast was found in patients with suspected or diagnosed ASD. *Candida albicans* was the most prevalent species in both groups, while *Candida krusei* and *Candida glabrata* were only isolated in ASD samples (Kantarcioglu et al., 2016).

MGB axis

Three studies within the past 6 years aimed to further the current understanding of the multidirectional nature of the MGB axis and its implicated pathways, specifically regarding microbial metabolites and the immune system. A study utilizing the MIA mouse model of autism found that MIA offspring displayed an altered serum metabolite profile (Hsiao et al., 2013). Treating naïve mice with a metabolite that is increased by MIA caused behavioral abnormalities, which suggests that gut bacterial effects on the host metabolome impact behavior (Hsiao et al., 2013).

Another animal study used VPA- and polyI:C-injected mouse models of autism to study a distinct pattern of microbial dysbiosis related to ASD (Lim et al., 2017). This dysbiosis was found to possess the possibility of directly affecting the host's health status via metabolic changes and serotonin pathways (Lim et al., 2017). This study used the Kyoto Encyclopedia of Genes and Genomes (KEGG) as a reference to identify altered metabolic pathways related to the dysbiotic gut. The results found metabolic changes including altered steroid biosynthesis and reduced degradation of the environmentally exposed neurotoxins, dioxins (Nishijo et al., 2014). Additional metabolic changes include up-regulated pathways related to lipopolysaccharide biosynthesis, and bacterial toxins (Lim et al., 2017). These metabolic changes have been previously shown to be implicated in the pathogenesis of ASD (Lim et al., 2017). With regard to the serotonin pathway, elevated serum serotonin levels were identified and could be the cause of diarrheal symptoms and even altered mental status seen in individuals with ASD (Boyer & Shannon, 2005). Overall, these results continue to suggest a multifaceted role of gut microbiota in the systemic pathogenesis of ASD (Lim et al., 2017).

Moreover, Inoue et al. (2016) examined the fecal and blood samples of children with ASD found significantly higher abundances in *Faecalibacterium* in ASD infants. Moreover, analyses found *Faecalibacterium* to be significantly correlated with greater expression levels of genes associated in interferon signaling (Inoue et al., 2016). Thus, these bacterial genera may play a role in the dysfunction of systemic immunity, especially chronic inflammation. This supports the notion of a gut–brain–immune interaction and the potential of gut microbiota to be a deteriorating factor of ASD (Inoue et al., 2016).

Discussion

Main findings

Over the 2013–2018 period, multiple studies have focused on characterizing a distinct ASD microbiome signature in both humans and animal models. In doing so, more bacterial species have been identified as being associated to ASD pathogenesis. However, consistency in the type of bacterial species present and their relative abundances compared to neurotypical subjects continues to be elusive. Some studies even reported contradicting results, specifically with regard to the *Bacteroidetes/Firmicutes* ratio (Coretti et al., 2018; Strati et al., 2017; Tomova et al., 2015; Zhang et al., 2018). Alternatively, several studies have shown steady results that support a higher prevalence of yeast species, especially *Candida albicans*, in children with ASD (Kantarcioglu et al., 2016). Interestingly, a common focus of studies conducted over the past 6 years has been to link certain species of gut bacteria to severity of ASD symptoms (De Theije et al., 2014; Inoue et al., 2016; Tomova et al., 2015). One successful study found increased severity of symptoms to be correlated with the presence of a decrease *Bacteroidetes/Firmicutes* ratio, and increased *Lactobacillu*s and *Desulfovibrio* species compared to neurotypical individuals (Tomova et al., 2015).

A large proportion of the studies selected for this review explored therapeutic interventions that centered on the role of the MGB axis in the pathogenesis of ASD. These interventions included special diets, vitamin, prebiotic, probiotic, and microbiota transfer therapy. Moreover, another main research focus in the past 6 years was in elucidating the molecular mechanisms behind the MGB axis, particularly with regard to microbial metabolite and immune system pathways (Hsiao et al., 2013; Inoue et al., 2016; Lim et al., 2017). Overall, these results continue to suggest a multidimensional function of gut microbiota in the systemic pathogenesis of ASD.

Limitations and generalizability

Low sample sizes. Given the fact that there are numerous variables included in the study of gut microbiota, rigorous research requires a large number of participants. It is key to obtain larger cohorts of individuals with ASD as well as neurotypical individuals. Increasing sample size not only

increases the statistical power of results, but it also accommodates for the significant extent of inter-person diversity (Bolte, 2015). Unfortunately, the vast majority of studies included in this review have small sample sizes, especially studies conducted using human participants. In this area of research sample sizes are very limited due to the significant cost (Basadonne, 2017). Therefore, it would be essential to create a research network willing to work together with the same procedures in order to overcome this limitation (Basadonne, 2017).

Placebo effect and selection bias. When considering the studies testing different therapies for ASD in humans, half of the studies lacked randomization or control groups. While open-label studies can provide valuable and descriptive information on the research topic, their results should be cautiously interpreted and viewed as preliminary as they are subject to placebo effects and selection bias (Kang et al., 2017). Randomized, double-blind, placebo-controlled methodology is the ideal next step for the majority of these studies. Furthermore, clinical trials with extended observation periods after treatment ends would help confirm long-term safety and benefits of treatment (Kang et al., 2017).

Animal models. Of the 19 studies investigated, 6 employed animal models of ASD to characterize the ASD gut microbiome and its link to behavioral differences. While these results can provide insight into therapeutic targets or the development of therapeutic regimens for ASD (Lim et al., 2017), it is not confirmed that findings in animal studies can be translated to humans with ASD (Luna et al., 2016). Thus, animal model research is preliminary and should be used to guide further research on human subjects.

Large heterogeneity. A main limitation of included studies is the lack of documented, uniform ASD diagnosis along with the documented rule out of ASD in comparison groups. It is crucial to consider ASD as a group of diverse cognitive behavioral phenotypes, each with specific etiologies (Basadonne, 2017; Bolte, 2015). Participants can have a range of GI issues, including constipation, diarrhea, and alternating diarrhea/constipation; however, none of these GI comorbidities are included in the diagnostic criteria of ASD; specific GI distresses could be vital for subgrouping (Lim et al., 2017). GI symptoms in both ASD and neurotypical cohorts should be screened, characterized, and subgrouped to investigate various etiologies leading to this spectrum of disorders (Luna et al., 2016).

Implications and future recommendations

Subgrouping. Since the link between ASD and gut microbiota manifests as a variety of GI issues, subgrouping participants depending on shared GI issues, ASD etiologies, ages, and so on, would provide a more homogeneous cohort. This in turn would accommodate for inter-individual variation, especially with regard to gut microbiota (Lim et al., 2017). Another important consideration to make when subgrouping, is prior antibiotic, antifungal, and antiviral use, as these medications have significant and lasting effects on the microbial community throughout the body (Luna et al., 2016). Overall, future studies should employ detailed subgrouping to strengthen the results.

Metabolome and biomarker identification. Future studies of the microbiome should consider the metabolome. The metabolome consists of the metabolites that are produced by the microbiome, including changes in amino acid and antioxidants found or secreted in the blood (Luna et al., 2016). Significant differences have already been documented in gut bacterial metabolites between ASD and neurotypical children. While collectively, published data confirm the existence of atypical ASD-associated metabolic profiles, there is little consensus on which metabolites could serve as biomarkers for the ASD subgroups with gut microbial dysbiosis (Lim et al., 2017). However, in general, the existence of metabolome differences further supports efforts to characterize a distinct ASD microbiome to gain a deeper understanding into the pathogenesis of ASD and possible therapeutic regimens (Luna et al., 2016).

Differences in metabolome also support the identification of ASD-specific biomarkers (Luna et al., 2016). Isolating biomarkers for ASD, or at least specific subgroups of ASD, not only could help clarify the molecular mechanism of ASD, but it would also provide a clearer understanding of which individuals or specific populations may be at risk for disease development (Basadonne, 2017; Luna et al., 2016). In addition to early detection of ASD, biomarkers associated with the MGB axis could provide a method to diagnose co-morbid GI conditions in individuals with ASD. Children with ASD often present with verbal impairments and as a result are unable to express their symptoms, especially abdominal pain. Furthermore, GI distress is often accompanied by a sudden change in behaviors or unprovoked behavioral outburst. Hence, identifying biomarkers associated with abdominal pain would greatly enhance the ability of medical practitioners to determine whether GI dysfunction is a potential cause of sudden behavior changes or recurring behavioral outbursts in children with ASD (Luna et al., 2016).

Conclusion

In sum, consistent evidence reveals a close association between ASD and GI dysfunction, hence supporting a role of the MGB axis in the pathogenesis of ASD. While there are continual reports of differences in microbiome between ASD and neurotypical individuals, consistency in the presence and abundance of bacterial species, as well as metabolites, remains deficient. Increasing attention has been

directed to exploring promising, yet preliminary, therapeutic interventions centered on the role of the microbiota in the pathogenesis of ASD. As increasing evidence of a unique ASD microbiomes and metabolomes is acquired, ASD-specific biomarkers can be identified. These biomarkers have great implications in terms of elucidating the molecular mechanisms of ASD, preventing the onset of ASD, and improving treatments for individuals with ASD.

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