Review

Biological Psychiatry

Emerging Roles for the Gut Microbiome in Autism Spectrum Disorder

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ABSTRACT

Autism spectrum disorder (ASD) is a serious neurodevelopmental disorder that affects one in 45 children in the United States, with a similarly striking prevalence in countries around the world. However, mechanisms underlying its etiology and manifestations remain poorly understood. Although ASD is diagnosed based on the presence and severity of impaired social communication and repetitive behavior, immune dysregulation and gastrointestinal issues are common comorbidities. The microbiome is an integral part of human physiology; recent studies show that changes in the gut microbiota can modulate gastrointestinal physiology, immune function, and even behavior. Links between particular bacteria from the indigenous gut microbiota and phenotypes relevant to ASD raise the important question of whether microbial dysbiosis plays a role in the development or presentation of ASD symptoms. Here we review reports of microbial dysbiosis in ASD. We further discuss potential effects of the microbiota on ASD-associated symptoms, drawing on signaling mechanisms for reciprocal interactions among the microbiota, immunity, gut function, and behavior. In addition, we discuss recent findings supporting a role for the microbiome as an interface between environmental and genetic risk factors that are associated with ASD. These studies highlight the integration of pathways across multiple body systems that together can impact brain and behavior and suggest that changes in the microbiome may contribute to symptoms of neurodevelopmental disease.

Keywords: Autism, Gastrointestinal tract, Gut-brain axis, Inflammation, Microbiota, Neurodevelopment

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder that is characterized by impaired social communication and the presence of repetitive, or stereotyped, behaviors. In addition to the spectrum of behavioral abnormalities in ASD, several medical comorbidities are also observed in ASD individuals, including seizures, anxiety, sleep deficiency, and metabolic impairments (1–5). Brain changes in ASD include a reported 67% more neurons in the prefrontal cortex, more than 17% increase in brain weight, and abnormal cortical patterning. Further transcriptomic analysis of postmortem brains from human ASD individuals revealed altered expression of proteins that are important for functional synaptic activity in the prefrontal cortex and cerebellum (6–9). In addition, several brain imaging studies in living patients report correlations between abnormal frontal lobe connectivity, cortical morphology, amygdala activation, and language control centers in ASD individuals compared with neurotypical control subjects (10–13).

The exact causes of ASD are unclear but are believed to involve a combination of genetic and environmental risk factors. It is estimated that the de novo mutations, common variants, and short nucleotide polymorphisms identified across numerous ASD cases altogether account for approximately 50% of the disorder (14,15). As such, many studies highlight the possibility for environmental risk factors and associated medical comorbidities to contribute to core neurobehavioral symptoms of the disorder. Immune dysregulation and gastrointestinal (GI) disturbances are of particular interest in light of numerous studies reporting ASD-associated abnormalities in the peripheral nervous system, enteric nervous system, and neuroimmune system. Postmortem brains of ASD patients show increased microglia and astroglia activation in the cerebellum and cerebral cortex, along with increased levels of proinflammatory cytokines in the cerebrospinal fluid and cortical regions of the brain (16). Moreover, there are ASD-associated genes that encode for features of the immune system, and mutations in those genes are linked with the ASD phenotype, including loss of structural and functional connectivity in brain regions important for sociocommunicative function (17,18). Parallel studies reveal greater prevalence of GI disorders and disturbances in ASD populations compared with control subjects (19,20). Comorbid GI symptoms in subsets of ASD individuals include diarrhea/constipation, abdominal pain, and gastric reflux. Deficient integrity of the gut epithelium and increased intestinal permeability are also reported (21).

These associations of ASD with greater prevalence of immune dysregulation and GI issues motivate explorations of the ASD gut microbiome, which is emerging as a key regulator of intestinal physiology, neuroimmunity, and host behavior. Many studies report dysbiosis of the gut microbiota in ASD individuals. Perhaps most intriguingly, gnotobiotic animal and

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probiotic studies demonstrate that microbiome changes can directly cause behavioral and neuropathological endophenotypes of human ASD. This avenue of research is critical for determining roles for microbiota dysbiosis and specific bacterial species that may contribute to or modify symptoms of ASD. In this review, we examine links between the microbiome and ASD symptoms, drawing on data from animal experiments showing causal effects of the microbiome on immunity, brain, and behavior. We further explore the notion that the microbiome plays an important role in mediating symptoms of ASD and may be a key consideration for understanding immune and GI dysfunction in subsets of ASD individuals.

GUT MICROBIOTA ON ASD-RELATED ENDOPHENOTYPES IN ANIMAL MODELS

The microbiota plays an important role in regulating normal host physiology, metabolism, nutrition, and brain function. Because mammals are unable to synthesize many key nutrients, the gut microbiota assumes a primary role in digestion, synthesizing essential dietary vitamins and cofactors, such as vitamin B, riboflavin, thiamine, and folate. In addition to roles for the microbiome in regulating digestion, GI physiology, and immunity, increasing research reveals the ability of the gut microbiota to signal across the so-called microbiota-gut-brain axis. Raising animals in the absence of microbial colonization results in abnormalities in a variety of complex behaviors, pointing to the possibility that the microbiota modulates behavioral outcomes in animal models of neurodevelopmental and neurological disorders. Social communication deficits and the presence of stereotyped behaviors are hallmark diagnostic features of human ASD, and other behavioral abnormalities, such as anxiety, seizures, and hyperactivity, are often comorbid. Two independent studies demonstrate that germ-free mice exhibit decreased sociability or propensity to interact with a novel mouse versus a nonsocial object, and reduced social preference to interact with an unfamiliar mouse versus familiar mouse (22,23). This is similarly seen in germ-free rats, which exhibit reduced social investigation of an unfamiliar partner (24). Germ-free mice also display differential gene expression, exon usage, and RNA editing in the amygdala, a key emotional center of the brain mediating responses to social stimuli (25). Interestingly, social-behavioral abnormalities are impaired particularly in male mice, which parallels the male bias that is characteristic of ASD. Moreover, some of the social impairments are corrected by postnatal colonization of germ-free mice with a wild-type mouse gut microbiota at weaning, pointing to the ability to reverse abnormalities in social interactions (26). This is intriguing in light of reports that risperidone, a Food and Drug Administration–approved treatment for autism, does not correct social abnormalities in human ASD or mouse models of ASD (27,28).

Modulation of the maternal environment is also of interest given the neurodevelopmental origins of ASD. Though there are numerous perinatal risk factors that influence maternal-fetal physiology including stress, infection, gestational diabetes, breastfeeding versus formula feeding, maternal age, antibiotic use, and obesity, the changes in the gut microbiota can also be a relevant risk factor. A recent study by Buffington *et al.* (29) showed that high-fat diet–induced maternal obesity alters the offspring gut microbiome and causes social-behavioral deficits that are linked to altered signaling in the mesolimbic reward system. Remarkably, transfer of the gut microbiota from control mice into offspring of high-fat diet–fed mothers completely corrected the impairments in sociability and social novelty seen in the mice, demonstrating a key role for the gut microbiome in regulating mouse social behavior. Furthermore, treatment with the gut bacterium *Lactobacillus reuteri* alone sufficiently restores social behaviors, revealing specificity of social-behavioral modulation in this model to a particular bacterial taxon. The beneficial effect of the microbiome in these studies was associated with its ability to promote hypothalamic levels of oxytocin and activation of neurons in the ventral tegmental area. This novel finding supports the promise of probiotic treatments for social behaviors. Importantly, however, we caution against use of *L. reuteri* for ASD until additional studies examine broader physiological effects of the bacterium on host biology and until such exploratory treatments are validated to be safe and effective in humans.

In addition to social interaction, there is some evidence that manipulation of the microbiome by probiotic treatment can modulate communicative and repetitive behavior in mice. In a mouse model of maternal immune activation, a principal environmental risk factor for autism, mice develop core behavioral features of ASD (impaired social communication and stereotyped behaviors), as well as several neuropathologies and comorbid GI and immunological symptoms relevant to the human disorder (30–32). Altering the postnatal gut microbiota by early life treatment with the human gut bacterium *Bacteroides fragilis* sufficiently ameliorated deficits in the frequency and quality of adult ultrasonic vocalizations and reduced stereotypic burying behavior exhibited by the ASD-like mice. Although the mechanisms underlying the ability of the gut microbiota to modulate ASD-related behaviors are unclear, improvements in GI integrity and alterations in serum metabolites could be involved. Consistent with a possible role for the microbiome in contributing to the symptoms of ASD, it would be interesting to examine the presence and severity of ASD-related behavioral and neuropathological abnormalities in ASD animal models raised on a germ-free background or depleted of gut microbes using treatment with broad-spectrum antibiotics. Such studies would enable dissection of causal mechanisms linking the microbiome to core ASD behaviors and neuropathologies.

Anxiety-like behavior is also observed in subsets of individuals with ASD and is commonly recapitulated in animal models for ASD. The microbiome modulates anxiety-like behavior in mice, as germ-free mice exhibit increased locomotor activity and decreased anxiety-like behavior in several tasks, including open field exploration, the elevated plus maze, light-dark box, and platform step-down test (25,33,34). These behavioral changes are correlated with altered expression of genes involved in second messenger pathways and synaptic transmission, including postsynaptic density protein 95 and synaptophysin in the striatum (35). Moreover, these behavioral changes can be related to learning and memory deficits seen in both germ-free and antibiotic-treated mice (36,37).

Germ-free animals also exhibit several abnormalities in brain gene expression and neurophysiology. For example,

abnormal transcriptomic profiles are observed across the frontal cortex, striatum, amygdala, and hippocampus (35), with altered expression of genes important for synaptic long-term potentiation, steroid hormone metabolism and neuronal transmission. Consistent with this, many studies report microbiome-mediated alterations in levels of brain-derived neurotrophic factor and synaptic proteins (23,26,33,34,36,38). In addition, differences in serotonergic, dopaminergic, and glutamatergic signaling are observed in germ-free mice compared with conventionally colonized control mice (26,34,35,39,40). Germ-free mice also display an exaggerated hypothalamic-pituitary-adrenal axis, with elevated corticosterone and adrenocorticotropic hormone levels in response to stress. Furthermore, germ-free mice also exhibit increased adult hippocampal neurogenesis compared with conventionally colonized control mice (41). Interestingly, several of these effects are reversed upon colonization with a conventional gut microbiota, or even specific bacterial species (Table 1), suggesting that there are dynamic interactions across the microbiota-gut-brain axis that persists through adulthood.

POTENTIAL ROLES FOR THE MICROBIOME IN ASD

Alterations in the gut microbiota are observed in ASD individuals compared with neurotypical control subjects (Table 1). Fecal bacterial profiling reveals a higher abundance of bacteria in the genus *Clostridium* in ASD patients (42–44). ASD patients also exhibited decreased *Bacteroidetes*/*Firmicutes* ratio, increased *Lactobacillus* and *Desulfovibrio* species, which correlated with ASD severity (45). ASD severity was also linked to a reduction in short-chain fatty acids, including acetate, proprionate, and butyrate (19), which are modulated by gut microbes. Bacterial genera important for carbohydrate degradation and fermentation, including *Prevotella*, *Coprococcus*, and *Veilonellaceae*, were decreased in ASD patients (46,47). On the other hand, ASD patients were shown to have elevated abundance of *Sutterella*, which regulates mucosal metabolism and intestinal epithelial integrity (20,48). Together, these studies suggest that ASD is associated with altered composition and function of the gut microbiota.

Despite these reports of microbial dysbiosis in ASD, there is little consensus on specific bacterial species that are similarly altered across separate studies. That is, no defined microbial signature has been identified for ASD, though many studies report microbiome differences within independent cohorts of ASD individuals and control subjects (Table 1). Several factors could contribute to these discrepancies, including methodological variations and inherent heterogeneity of ASD cohorts based on symptom severity, comorbid conditions, varied lifestyle, and medical history. ASD-associated alterations in eating behavior and diet are likely to play a role, as the gut microbiota can be stably altered in response to dietary changes and exposures to xenobiotics (49).

Whether alterations in the microbiota may contribute to development of ASD is unknown. Interestingly, a small clinical study of vancomycin treatment in ASD children reported some improvements in ASD behaviors, which waned when antibiotic treatment was discontinued (50), suggesting that the microbiome may contribute actively to the severity of behavioral abnormalities in ASD. Particular case studies also link antibiotic treatment to improvements in ASD behaviors and comorbid conditions (51). In addition, the antibiotics D-cycloserine and minocycline are promising in light of their ability to treat behavioral symptoms of ASD in clinical trials and animal models (52–54). Although both antibiotics are used to treat infections, their neuroprotective effects are commonly attributed to their roles as partial *N*-methyl-D-aspartate receptor agonist and microglial activation inhibitor, respectively.

LINKS BETWEEN THE GUT MICROBIOME AND ASD-ASSOCIATED GI ABNORMALITIES

GI symptoms are variably present in ASD individuals (Table 2), ranging from 9% to 90% in prevalence (55,56). Although the precise incidence varies from study to study, there is a consensus that GI problems are common in individuals with autism (57) and that they could potentiate behavioral issues (57). A large meta-analysis of autism cases versus control subjects from 1980 to 2012 reveals greater incidence of intestinal symptoms, such as diarrhea, constipation, and abdominal pain, despite high methodological variability. Consistent with this, a multicenter study of over 14,000 ASD individuals reports a higher prevalence of inflammatory bowel disease and other bowel disorders in ASD patients compared with control subjects (4). Notably, in an examination of 960 children from the CHARGE (Childhood Autism Risks from Genetics and Environment) study, frequency of abdominal pain, diarrhea, constipation, or gaseousness was associated with greater social withdrawal, stereotypy, irritability, and hyperactivity as measured by the Aberrant Behaviors Checklist (58). Autism severity was also strongly correlated to the presence of GI symptoms as measured by the Autism Treatment Evaluation Checklist and GI severity index (57).

Whether any changes in the microbiome are caused by GI symptoms or whether they contribute to the manifestation of GI symptoms in ASD is unclear. Gut microbes influence various aspects of gut physiology, including intestinal barrier integrity, epithelial cell regeneration, mucus production, and GI motility (59). Interestingly, the severity of GI symptoms in ASD has been associated with alterations in the gut microbiota in response to treatment with antibiotics, prebiotics, or probiotics (57). In light of the intricate interactions of the gut microbiome with the gut epithelium (57), it would be interesting to examine if microbiome abnormalities in ASD are enriched in or even specific to ASD individuals with comorbid GI issues. In addition, investigations into whether particular microbiome changes are associated with specific ASD-associated dietary regimens, treatments, and comorbid medical symptoms would be of significant interest.

LINKS BETWEEN THE GUT MICROBIOME AND ASD-ASSOCIATED IMMUNE DYSREGULATION

The gut microbiota exhibits important bidirectional interactions with the immune system. Many facets of immunity are dysregulated in ASD (Table 3). Alterations in circulating and brain cytokines, chemokines, and other inflammatory factors are frequently observed in ASD, as well as abnormal distributions or responsiveness of various leukocyte subtypes

Table 1. Microbiota Changes in ASD Patients, Mouse Models With Behavioral Abnormalities, and Links to Immune and GI Abnormalities

Subject	Behavior	Description	Microbiota	Immune	GI	Reference
Children (Ages 43–84 Mo)	Regressive-onset autism	Broad-spectrum antibiotic use was linked to chronic diarrhea followed by loss of language, play, and social skills (n = 11).	X		X	(50)
Children	Regressive-onset autism	ASD (n = 13) children all had GI symptoms (diarrhea and constipation), had more clostridial species, and significant amount of non–spore-forming anaerobes and microaerophilic bacteria compared with control subjects (n = 8).	X		X	(42)
Children	Autism	ASD children had elevated levels of *Clostridium boltea* as well as *Clostridium* group I and XI.	X			(43)
Mice	Stress response	GF mice have elevated stress response as well as reduced BDNF in the cortex and hippocampus. GF colonization with *Bifidobacterium infantis* reversed stress response.	X			(40)
Children (Ages 3–16 Years)	Autism	ASD patients (n = 58) had taken antibiotics (34.5%), had GI complaints (91.4%), and were taking probiotics/prebiotics (53.4%). ASD patients had higher *Clostridium* clusters I and II compared with control subjects (n = 22).	X		X	(44)
Children (Average Ages 11–12 Years)	Regressive autism (n = 24) Nonregressive autism (n = 32)	ASD patients (n = 56) used significantly more antibiotics.	X			(114)
Mice	Visceral hypersensitivity	*Lactobacillus paracasei* NCC2461 normalized visceral sensitivity.	X			(115)
Children (Ages 6.1 ± 2.2 Years)	Autism	ASD children (n = 15) had significantly higher use of oral antibiotics during first 12 mo of life.	X			(116)
Rats	Depression-like behavior	Probiotic *B. infantis* treatment did not change behavior but decreased IFNγ, TNFα, and IL-6 cytokines.	X	X		(117)
Mice	Anxiety-like behavior	Colonic inflammation induced anxiety-like behavior, decreased hippocampal BDNF mRNA, and increased circulating TNFα and IFNγ. Probiotic *Bifidobacterium longum* restored behavior and BDNF.	X	X	X	(118)
Rats	Depression-like behavior	Probiotic *B. infantis* treatment in a maternal separation stress model normalized IL-6 levels, increased swim behavior and reduced immobility in the forced swim test, and restored basal noradrenaline levels in the brainstem.	X			(119)
Rats	Visceral hypersensitivity	Probiotic *B. infantis* 35624 reduces visceral pain.	X			(120)
Children (Ages 2-13 Years)	Impaired social, language, and verbal skills; repetitive stereotypical behaviors	ASD patients (n = 33) had varying GI symptoms. More severe autism had higher *Desulfovibrio*, *Bacteroides vulgatus*, and *Bacteroidetes*. *Firmicutes* was higher in control subjects (n = 15).	X		X	(121)
Mice	Motor activity and anxiety-like behavior	GF mice have increased motor activity and decreased anxiety. Changes in PSD-95 and synaptophysin expression in striatum.	X			(35)
Mice	Anxiety- and depression-related behaviors	Probiotic *Lactobacillus rhamnosus* treatment of mice in a stress model reduced stress and increased GABA receptor expression in prefrontal cortex.	X			(122)
Mice	Anxiety-like behavior	Chemical colitis mouse model treated with probiotic (*B. longum*) had normalized anxiety-like behavior.	X	X	X	(123)
Rats and Adults (Average Age 42 Years)	Anxiety, depression, and stress	Probiotic (*Lactobacillus helveticus* and *B. longum*) reduced anxiety-like behavior in rats and reduced psychological stress in patients.	X			(124)
Children (Onset at 13.4 ± 5.4 mo)	Autism	ASD patients with GI symptoms (n = 15) had a decrease in disaccharidases and hexose transporters, and they also had decreases in *Bacteroidetes,* increase in *Firmicutes/Bacteroidetes* ratio, and increase in *Betaproteobacteria* compared with patients with only GI (n = 7).	X		X	(47)
Mice	Stress-induced corticosterone, anxiety- and depression-related behavior	*L. rhamnosus* increased cortical GABA(B1b) receptor expression and decreased GABA(Aα2) expression in prefrontal cortex and amygdala, but increased in hippocampus. *L. rhamnosus* reduced stress, anxiety, and depression behavior.	X			(122)
Mice	Anxiety-like behavior	The chronic colitis model has increased anxiety. *B. longum* normalized behavior, but there was no change in BDNF expression.	X		X	(123)
Adults (Average Age 42 Years)	Anxiety and depression	*L. helveticus* R0052 and *B. longum* R0175 decreased hospital anxiety and depression (n = 10 treated).	X			(125)

Table 1. Continued

Subject	Behavior	Description	Microbiota	Immune	GI	Reference
Children (Ages 2–18 Years)	Autism, Asperger syndrome	ASD children (n = 58) had GI symptoms and decreased fecal SCFAs, lower levels of *Bifidobacterium* and higher levels of *Lactobacillus.*	X		X	(19)
Children (Average Age 123 Mo)	Autism	ASD children (n = 23) had elevated fecal SCFAs.	X			(126)
Mice	ASD-like behaviors	MIA mice have decreased GI barrier, increased IL-6, decreased cytokine/chemokine, and gut microbiota dysbiosis; autism-related behaviors that were restored following colonization with *B. fragilis*.	X	X	X	(32)
Mice	Social preference and repetitive behaviors	GF mice had deficits in social avoidance, social novelty, and social investigation. GF mice also had increased repetitive self-grooming.	X			(22)
Mice	Social behavior	Maternal high-fat diet induced social deficits in offspring are restored following colonization with *Lactobacillus reuteri.*	X	X		(29)

ASD, autism spectrum disorder; BDNF, brain-derived neurotrophic factor; GABA, gamma-aminobutyric acid; GF, germ free; GI, gastrointestinal; IFNγ, interferon gamma; IL, interleukin; MIA, maternal immune activation; mRNA, messenger RNA; PSD-95, postsynaptic density protein 95; SCFA, short-chain fatty acid; TNFα, tumor necrosis factor alpha.

(16–18,60). These particular ASD-related immune abnormalities are the subject of several recent reviews (17,18,61). Many of the immunophenotypes seen in ASD are consistent with elevated proinflammatory status, as indicated by an increase in cytokines and chemokines, including interferon gamma, interleukin (IL)-β, IL-6, IL-12p40, tumor necrosis factor alpha, monocyte chemoattractant protein-1, transforming growth factor-β, and chemokine (C-C motif) ligand 2, as well as a hyperactive cellular immune responses (62–65). However, ASD patients demonstrate varying immune abnormalities, including differential changes in their immune/cytokine profiles, as well as the degree of changes (66), making it difficult to pinpoint direct links between immune and microbiota alterations in ASD individuals. In addition, confounding factors such as patient-to-patient variability in diet, lifestyle, and genetics can also modify immune activity. Nevertheless, subsets ASD individuals exhibit aberrant immune activation. Many of the immunophenotypes observed involve factors and pathways that are known to be influenced by the gut microbiota, raising the question of whether ASD-associated microbial dysbiosis can contribute to the widespread immune dysregulation seen in ASD individuals.

The Microbiome and Neuroimmune Abnormalities of ASD

Both elevated microglial activation and altered microglia to neuron spatial distribution patterns are seen in the cerebral cortex and cerebellum of postmortem ASD brains (16,67–69) and surrogate markers of increased microglial activation are observed by positron emission tomography imaging of living ASD individuals (70). Interestingly, Erny *et al.* (71) demonstrate that the microbiome is required for proper development and function of adult brain microglia. Microglia from germ-free mice exhibit altered transcriptomes, including downregulation of cell activation genes (e.g., *Mapk 8*, *Fcgr2β*, *Hif1α*), reduction of genes for type 1 IFN receptor signaling (e.g., *Jak3* and *Stat1*), and upregulation of microglia transcription and survival factors (e.g., *Sfpi1* and *Csf1r*), as compared with those isolated from conventionally colonized control mice. Microglia from germ-free mice also exhibit altered morphology, with longer processes and increased branching. Following exposure to bacterial or viral challenge, microglia from germ-free mice maintain altered morphology and reduced inflammatory responses compared with those from conventionally colonized mice. Remarkably, recolonization of adult gnotobiotic mice with a conventional gut microbiota or supplementation with short-chain fatty acids, the primary products of bacterial fermentation, sufficiently corrects these deficiencies in microglial activation (71). These findings suggest that indigenous gut microbes reversibly modulate microglial function, and further motivate the identification of specific bacterial species from the gut microbiota that confer these neuroimmunomodulatory effects.

Peripheral Immune Regulation and the Microbiome

Various systemic immune abnormalities observed in ASD may also be influenced by the microbiota. For example, specific bacterial species from the gut microbiota regulate differentiation of T lymphocyte subtypes. Colonization with segmented filamentous bacteria stimulates the accumulation of inflammatory IL-17–producing Th17 cells via the acute phase protein serum amyloid A, which predisposes to symptoms of autoimmune disease in animal models (72,73). In contrast, both *Bacteroides fragilis* and a particular consortium of clostridial species upregulate levels of IL-10–producing T regulatory cells. By this mechanism, *B. fragilis* and the clostridial consortium sufficiently correct symptoms of intestinal disease and multiple sclerosis in animal models (74,75) and continue to be tested for clinical translation into patient populations. The interplay between the gut microbiome and immune system could be relevant to the immune dysregulation observed in ASD, where abnormal distributions and functions of various leukocyte subtypes are observed. For example, deficiencies in regulatory T cells and other T helper cell subtypes are reported in ASD individuals compared with control subjects (76). In addition, peripheral blood monocytes and macrophages from ASD individuals are hyperresponsive to stimulation as compared with those isolated from neurotypical control subjects, and the microbiota fundamentally regulates systemic myeloid development and differentiation (77,78).

Table 2. GI Abnormalities in ASD Patients and Links to Microbiota and Immune Changes

Subject	Behavior	Description	Microbiota	Immune	GI	Reference
Children (Ages 4–16 Years)	Infantile autism	43% (n = 9 of 21) of ASD children had abnormal intestinal permeability.			X	(127)
Children (Ages 2.6–16 Years)	Social interaction, communication, interests	Patients with celiac disease (n = 120) did not show autistic-like behaviors.			X	(128)
Children (Ages 3.5–16.3 Years)	Autism	Children with ASD (n = 21) and bowel symptoms had increased basement membrane thickness, mucosal gamma delta cell density, CD8 (+) density, and intraepithelial lymphocyte numbers compared with patients with only inflammatory bowel diseases.		X	X	(129)
Children (Average Age 6.2 Years)	Regressive autism	ASD children with GI symptoms (n = 20 of 25) show autoantibody binding to epithelial cells and colocalize with complement proteins in the intestinal mucosa.		X	X	(130)
Children (Ages 1–10 Years)	Autism	ASD children with GI symptoms (diarrhea and constipation) (n = 75) showed increased production of TNFα/IL-12 upon stimulation with cow's milk protein.		X	X	(56)
Children (Age >1 Year)	Autism	ASD children (n = 3325) had elevated link with family members with gastrointestinal autoimmune diseases such as celiac disease, Crohn's disease, and ulcerative colitis.		X	X	(131)
Children (Average Age 7.4 ± 5.1 Years)	Autism	36.7% of ASD patients (n = 33 of 90) had abnormal intestinal permeability and GI symptoms (constipation, diarrhea, and abdominal pain).			X	(132)
Children (Ages 3–10 Years)	Autism	ASD children (n = 12 of 23) with GI symptoms had elevated levels of *Sutterella* compared with control subjects (n = 9 of 9) with GI symptoms. There was also IgG or IgM antibody reactivity to *Sutterella wadsworthensis* in ASD-GI children.	X	X	X	(20)
Human	Autism	Higher rates of GI disorders in ASD patients, GI disorders in ASD children is 9–91%, abdominal pain is 2–41%, constipation is 6–45%, and diarrhea is 3–77%.			X	(133)
Children (>4 Years Old)	Autism	ASD patients (n = 88) had more impaired intestinal permeability and increased antibodies against food antigens.		X	X	(134)
Children (Average Age 7.8 ± 2.9 Years)	Autism	ASD patients (n = 37) had higher levels of the IgG antibody to gliadin and correlated with GI symptoms, but was not associated with celiac disease.		X	X	(135)
Children (Ages 10–14 Years)	Regressive, atypical autism	There was no difference in small intestine permeability between ASD (n = 103) and special needs (n = 30) children.			X	(136)

ASD, autism spectrum disorder; CD8, cluster of differentiation 8; GI, gastrointestinal; IFNγ, interferon gamma; IgG, immunoglobulin G; IgM, immunoglobulin M; IL, interleukin; TNFα, tumor necrosis factor alpha.

Overall, this research raises the fascinating question of whether microbial dysbiosis can contribute to the immune dysregulation seen in ASD, such as microglial activation and T regulatory cell deficits, and whether manipulations of the microbiota can ameliorate ASD-related immune abnormalities. Although parallel studies of immune problems in ASD and effects of the microbiome on the immune system are revealing some converging pathways, additional preclinical studies are required to determine whether microbiome changes in ASD can sufficiently cause any of the immune abnormalities seen in the disorder. Moreover, it will be important to determine whether existing animal models for ASD, which display core behavioral and neuropathological symptoms of the disorder, also exhibit immune abnormalities and microbiome changes seen in ASD. Such associations have been reported in a few mouse models of ASD environmental risk factors (32,79), but information for additional environmental and genetic models is currently lacking.

THE MICROBIOME AS A POTENTIAL MEDIATOR OF RISK FACTORS IN ASD

Although there is evidence that ASD-associated microbial dysbiosis could modulate corresponding immune, GI, and even behavioral symptoms, whether microbiome alterations contribute to the etiopathogenesis of ASD is unclear. Idiopathic ASD is thought to be a result of a combination of several genetic and environmental factors that each contributes a fraction of disease risk. The strong concordance of ASD in monozygotic twins compared with dizygotic twins reveals an ASD heritability rate of about 50% (80,81). Several genetic factors increase ASD risk, including single nucleotide polymorphisms, copy number variants, and de novo mutations in genes involved in synaptic transmission and neuronal activity (82–84). Interestingly, some ASD susceptibility genes encode components of the immune system (17). In addition, several environmental risk factors have been identified to increase risk for autism.

The microbiota is well positioned at the intersection between genes and environment, as its composition and function are dependent on genetic background and critically shaped by environmental factors, including age, infection, diet, and xenobiotics. Moreover, early life changes in the microbiota can have lasting effects on health and disease. For example, several diet-induced host phenotypes are sufficiently mediated by changes in the gut microbiota (85–88). The microbiota also conveys lasting effects of infection to the host (89) and can regulate epigenetic modification of the host genome (90,91). Interestingly, microbiota-mediated epigenetic changes can determine host transcriptional profiles. For example, the short-chain

Table 3. Immune Alterations in ASD Patients and Links to Microbiota and GI Abnormalities

Subject	Behavior	Description	Microbiota	Immune	GI	Reference(s)
Human (Ages 3–28 Years)	Autism	46% (n = 28 of 61) of ASD patients had family members with autoimmune disorders; immediate relatives with autoimmune disorders increased prevalence of autism diagnosis from 4% to 21%; autoimmune disorders include type 1 diabetes, rheumatoid arthritis, hypothyroidism, and system lupus erythematosus.		X		(137)
Children (Ages 1–17 Years)	Autism, Asperger syndrome	LPS stimulated innate immune reaction that was stronger in ASD individuals (n = 71), leading to elevated TNFα, IL-1β, and IL-6 production.	X	X		(63,138)
Human (Ages 5–44 Years)	Autism	Postmortem brain showed increased microglia and astroglia activation. Brain and CSF showed increased proinflammatory cytokines.		X		(16)
Children (Age 5.9 ± 3.9 Years)	Autism	ASD (n = 37) patients compared with control subjects (n = 29) had elevated sera IgG and IgM BDNF levels.		X		(139)
Children (Ages 4–15 Years)	Autism, Asperger syndrome	ASD with GI (n = 18) compared with control subjects (n = 27) had enhanced proinflammatory cytokine profile; increased TNFα, IFNγ, IL-4, and IL-5; and decreased regulatory cytokine IL-10.		X	X	(140)
Children (Ages 42 ± 9.8 Mo)	Autism, early onset, regressive	ASD (n = 116), control subjects (n = 96), and developmental delays (n = 32), ASD had decreased levels of IgG and IgM subclass.		X		(141)
ASD Mothers	Autism	Maternal antibodies for fetal brain proteins were elevated in mothers of ASD children. ASD mothers (n = 61), typical mothers (n = 62), and developmental delay mothers (n = 40).		X		(142)
Children (Average Age 3.47 Years)	Autism	ASD (n = 114), control subjects (n = 96), and developmental delays (n = 31). ASD had increased levels of IgG4 subclass.		X		(143)
Children (Average Age 3.2 Years)	Autism	ASD patients had elevated autoantibodies in plasma that were directed to cerebellar protein extracts.		X		(144,145)
Children (Age >1 Year)	Autism, Asperger syndrome	3325 diagnosed children with ASD in Denmark had increased risk of ASD diagnosis when they had a family history of type 1 diabetes and rheumatoid arthritis.		X		(131)
Adult (Ages 18–44 Years)	Severe autism	ASD patients (n = 22) had elevated levels of serum endotoxin that were correlated with decreased VABS socialization scores and trend toward increase in proinflammatory cytokines IL-1β and IL-6, but was not significant.	X	X		(21)
Children (Median Age 3.6 Years)	Lethargy, stereotypy, hyperactivity, impaired communication/socialization	Elevated brain and CSF chemokine (MCP-1, RANTES, and eotaxin) in ASD patients (n = 80) was associated with higher aberrant behavior and impaired learning and social skills.		X		(146)
Children (Median Age 3.4 Years)	Nonregressive and regressive autism	ASD children (n = 97) showed higher plasma levels of IL-6 and IL-12p40.		X		(147)
Children (Ages 7–15 Years)	High-functioning autism	Increased levels of serum IL-17 in male subjects with high-functioning ASD (n = 28).		X		(64)
Children (Ages 5–17 Years)	Regressive autism	ASD children (n = 34) had decreased levels of plasma IL-23, but no changes in IL-17.		X		(148,149)
Children (Ages 24–60 Mo)	Autism	Increased production of IL-17 and IL-13 in comorbid autism (n = 45) and asthma children (n = 12).		X		(150)

ASD, autism spectrum disorder; BDNF, brain-derived neurotrophic factor; CSF, cerebrospinal fluid; GI, gastrointestinal; IFNγ, interferon gamma; IgG, immunoglobulin G; IgM, immunoglobulin M; IL, interleukin; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; RANTES, regulated on activation, normal T cell expressed and secreted; TNFα, tumor necrosis factor alpha; VABS, Vineland Adaptive Behavior Scales.

fatty acid butyrate can act as a histone deacetylase inhibitor. Histone deacetylases are involved in cell cycle progression, gene silencing, differentiation, and genotoxic responses (92).

Whether the microbiota mediates effects of genetic or environmental risk factors on the development of ASD symptoms is unclear. However, increasing evidence suggests that the microbiota is altered in response to etiological risk factors for ASD. Maternal infection is a primary environmental risk factor for ASD based on numerous epidemiological, clinical, and animal studies (93–98). Modeling maternal immune activation in mice results in global changes in the composition of the adult offspring microbiome (99,100). This microbial dysbiosis is correlated with lasting behavioral abnormalities, neuropathologies, immune dysfunction, and deficient GI integrity. Interestingly, altering the microbiome via postnatal treatment with the human commensal *B. fragilis* improved GI physiology

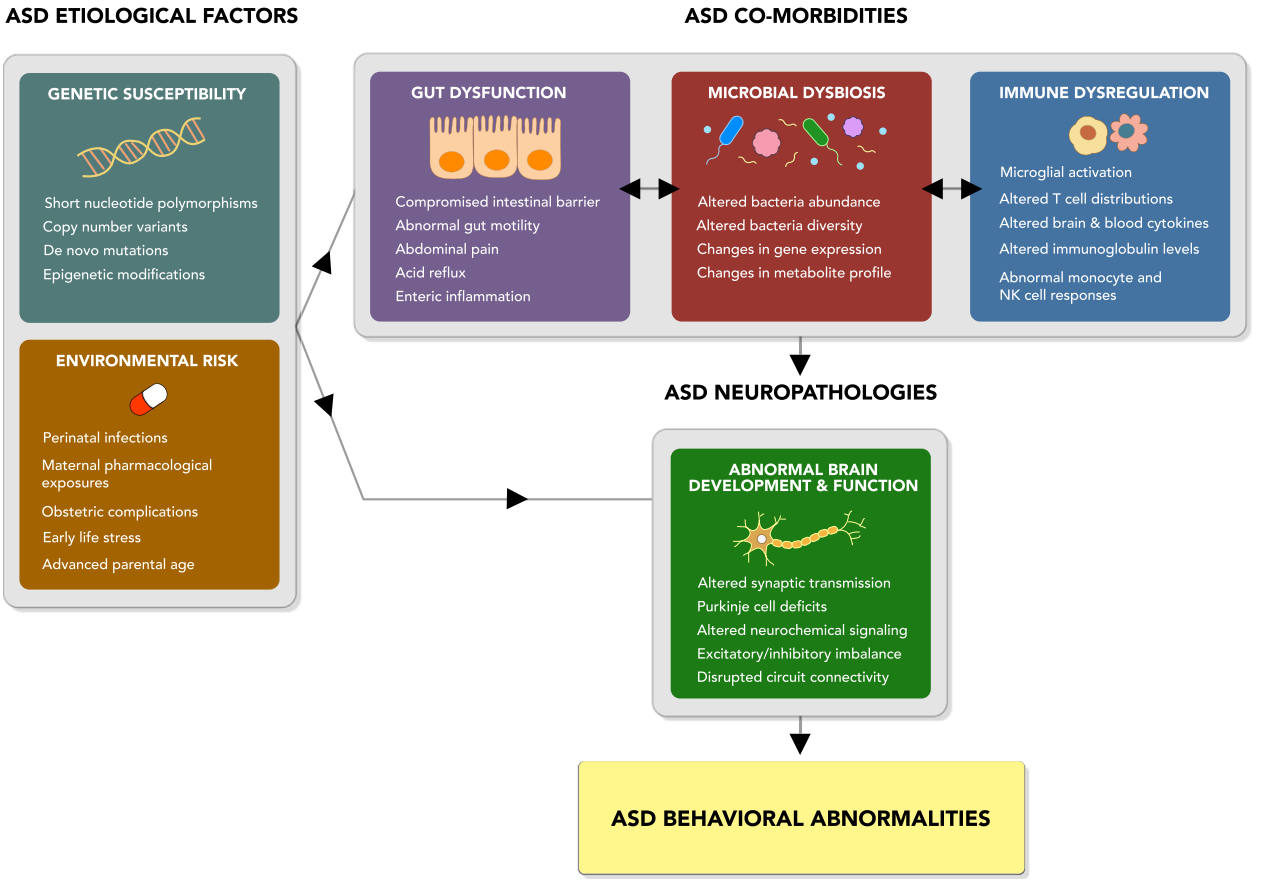

Figure 1. Model for roles of the microbiome in autism spectrum disorder (ASD). The microbiota is shaped by host genetics and environmental exposures. Select genetic and environmental risk factors for ASD could directly cause changes in the indigenous microbiota. Alternatively, the microbiota could be indirectly influenced by other medical comorbidities associated with ASD, including gastrointestinal issues and immune dysfunction. The microbiota exhibits reciprocal interactions with the gastrointestinal tract, immune system, brain, and behavior, and abnormalities in any one component of this integrated system could affect the others. In particular, dysbiosis of the intestinal microbiota, in addition to immune and gastrointestinal symptoms seen in ASD, can influence neurodevelopment, neural activity, and the manifestation of abnormal behaviors characteristic to ASD. NK, natural killer.

and performance in some tasks measuring core ASD-related behaviors. Similarly, modeling maternal exposure to valproic acid, an anticonvulsant drug that is associated with increased risk for ASD (79,101), rendered offspring with lasting changes in gut microbiota composition, as well as neuroinflammation, abnormal GI physiology, and ASD-related behavioral abnormalities (79). Whether exposures to ASD risk factors also result in microbiome alterations and whether there are any similarities across microbiota impairments across differing insults are important questions for future investigation.

Importantly, alterations in the maternal microbiome in response to environmental risk exposure or genetic risk transmission can be passed onto offspring at birth. The developing embryo is largely devoid of microbial colonization, and a preponderance of evidence suggests that mammals inherit their initial microbiome through the birthing process. Mode of birth, whether through natural birthing process or cesarean section (C-section), drives the initial seeding of the infant microbiome, such that babies delivered via the vaginal canal can be discriminated from those delivered by C-section based on their microbiome (102–104). This has significant implications for developmental disorders, including ASD, where maternal or prenatal exposures to genetic or environmental risks are believed to contribute to disease etiopathogenesis. Animal models demonstrate that microbiome changes in response to maternal stress are passed onto offspring at birth, setting in motion microbial dysbiosis that persists into adulthood (105–107). Maternal-to-offspring transmission is also believed to cause the chronic microbiota abnormalities seen in adult offspring of immune-activated mothers (18). Consistent with this, some studies report that C-section is associated with elevated risk for ASD in the offspring (108,109), though one reported no link between C-section and ASD symptoms (110). Although recent studies show offspring delivered by C-section have reduced microbial diversity compared with those by vaginal birth (111–113), there is also evidence that early life microbiome is plastic and other exposures can shape the infant microbiota. Other confounding perinatal risk factors for ASD that appear with C-section but are unrelated to the microbiome include anesthesia applied during labor, preterm birth, maternal age, and oxytocin administration. Future studies will require careful consideration of study subjects and perinatal risk factors.

FUTURE DIRECTIONS FOR INVESTIGATING EFFECTS OF THE MICROBIOME ON ASD

Emerging studies suggest the microbiota is an important regulator of GI physiology, immune function, and behavior (Figure 1). Abnormalities in each of these domains are reported in ASD, but additional characterization of comorbid medical symptoms is required to clarify the nature, strength, and reproducibility of specific associations. Evaluation of genetic background, medical history, and ASD severity, among other variables, would provide insight into whether particular symptoms are enriched in specific subtypes of ASD and would further drive hypotheses regarding possible contributions of microbial dysbiosis, GI dysfunction, or immune dysregulation to the development or persistence of ASD behaviors. Similar efforts to characterize comorbid microbiota, GI, and immune symptoms across new and existing animal models for ASD are needed, with an emphasis on identifying converging phenotypic signatures across models of different genetic and environmental ASD risk factors. Further experiments are required to determine whether microbiome, GI, or immune abnormalities can sufficiently cause primary behavioral features of ASD. Such investigations should begin with studies using gnotobiotic or xenobiotic animals to identify peripheral targets and specific brain changes for development of novel ASD therapeutics. Of particular relevance to ASD-related microbial dysbiosis studies, it would be important to determine whether fecal transplant of ASD microbiota into animals is sufficient to cause behavioral impairments, neuropathologies, and medical comorbidities seen in ASD. Moreover, well-controlled studies on the efficacy of fecal microbiota transplant in ASD patients would provide much-needed guidance for the ASD community.

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