

Review Article

Potential Etiologic Factors of Microbiome Disruption in Autism

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ABSTRACT

Purpose: The primary purpose of this article was to consider the candidate disruptors of the development of a healthy microbiome in patients with autism. The reported abnormalities in the microbiome of individuals with autism are discussed.

Methods: This selected review used data from published articles related to the assessment of microbiota in autism. Evidence-based support of factors known to affect the intestinal microbiome in individuals with autism are presented. Proposed interventions are evaluated and discussed.

Findings: Studies that have investigated the intestinal microbiome in patients with autism have reported significant differences versus unaffected controls. Increased clostridial species in autism have been reported in several studies. These differences may have resulted from a number of environmental factors. Microbiome alterations that might contribute to the development of autism include altered immune function and bacterial metabolites.

Implications: Efforts to modify microbial imbalances through a variety of interventions are addressed. Focusing on mechanisms that drive imbalances in the microbiome may affect the development of disease. Altered intestinal health may contribute to the development of autistic behaviors or autism itself. Interventions aimed at improving intestinal health may favorably affect the microbiome and autism. (*Clin Ther.* 2015;37:976–983) © 2015 Elsevier HS Journals, Inc. All rights reserved.

Key words: autism, intestinal microbiome, microbiota, pediatrics.

INTRODUCTION

Autism is a medical condition originally described by Kanner¹ in 1943. In <75 years since the recognition of the condition, the prevalence of autism has exploded from 1 in 5000 individuals to 1 in 68.2. The prevalence is 4- to 5-fold greater in boys than in girls.² For obvious reasons, there is an effort to address the potential factors that account for this rising prevalence. Although genetic factors have been closely associated with the condition, especially in families with >1 affected individual, in most cases of autism, a cause has not been identified.³ There is speculation about maternal immune factors,⁴ prenatal or environmental toxicity (reviewed by Rossignol et al⁵), and metabolic derangement⁶ contributing to the development of autism.

We should add gastrointestinal (GI) and dietary factors to the list of speculation. Interest in GI abnormalities in autism goes back to the original descriptions of affected individuals. These included remarkable differences in dietary intake and the requirement for feeding support in some patients.¹

Since then, a growing literature supports a high frequency of GI symptoms in individuals with autism. The symptoms do mirror the general pediatric community in terms of the nature of concerns such as constipation, acid reflux, and diarrhea, but consistently seem to have a greater frequency in autistic patients than in the control populations reported in

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most studies.^{7,8} Recognizing underlying GI conditions or other medical problems may be difficult in individuals with no or limited verbal capability. Pain may trigger behaviors such as aggression or self-injury in individuals with autism, and caregivers may manage these issues as behavioral without considering the underlying disease.⁹

Autism may contribute to GI issues directly because of altered eating patterns, which might change the stool pattern or microenvironment. Sensory factors that may alter feeding patterns and toileting behaviors are common, although not universal, in autism.¹⁰ Is it possible that an altered GI state could contribute to the neurologic state of autism in some individuals? This concept was suggested by Adams et al,¹¹ who reported that in patients who had GI symptoms, were identified as having more severe impairment from autism features by autism-testing tools.

We have become more capable of addressing some questions as our understanding of autism has grown and our ability to assess the GI microbiome and the metabolic gut environment, the *metabolome*, has developed. From a clinician's perspective, the discussion of intestinal microbiome and disease has been rather esoteric to this point. However, with available interventions such as probiotics and fecal microbiota transplantation (FMT) available, patients with conditions known to affect the intestinal microbiome such as autism, inflammatory bowel disease and celiac disease are asking for advice from providers how to fix these abnormalities.

MATERIALS AND METHODS

This selected review used data from published articles related to the assessment of microbiota in autism. Data from studies that compared the intestinal microbiome in patients with autism to that in healthy controls were included. The selection of articles used in this review include selected earlier literature. The primary review comes from key words, autism, autism spectrum disorders, microbiota, microbiome from 2000-present. It included prospective author initiated, retrospective and review papers in this period. All papers were considered for use without an exclusion based on the limited available literature.

RESULTS

What Is Reported Regarding Microbiome Abnormalities in Autism?

Our intestines contain tens of trillions of microbes. Although bacteria are predominant, Archaea,

Eukarya, as well as viruses are cohabitate in this complicated ecosystem of the human host. In each individual, a unique microbiome typically made up of ~1150 bacterial species is established.¹² In disease, there is a characteristic decrease in the number and diversity of these species.¹³ The development of this microbial community begins even before birth¹⁴ and continues for up to the first 3 years of life. There are differences that occur in the makeup of the colonies present based on infant nutrition; in breast-fed infants, the microbiome established is different from that in formula-fed infants.¹⁵ Between 2 and 3 years of age, there is stabilization of a child's microbiome that seems consistent with an established adult community.¹⁶ It is quite clear that dietary changes or other nutrient-based changes, such as supplementary fiber, can alter the composition of the microbiome¹⁷ in individuals of all ages.

Children are typically diagnosed with autism between ages 1 to 3 years.¹⁸ This coincidence of timing makes consideration of the developing microbiome and immune system worthy of study. There are many examples in nature of intestinal microbes altering host behavior. One such example involves the eukaryotic pathogen *Toxoplasma gondii* when it infects the rodent, causing the animal to lose its innate fear of the odor of bobcat urine. By not avoiding territory inhabited by the predator, rodents are more often caught, and *T gondii* is then excreted in the bobcat stool where it is able to infect other rodents.^{19,20} Infection with *T gondii* is also associated with behavioral changes in humans, who may exhibit poor self-control and increased high-risk behavior.²¹⁻²³ Similarly, neuropsychiatric disorders have been associated with infection with *Brucella suis*, *Leptospira* spp, *Mycobacterium tuberculosis*, and streptococci (coined the PANDAS [pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection]).²⁴⁻²⁸

In addition to these specific examples of behavioral changes caused by single organisms, there are compelling data that alterations in the entire microbial ecosystem may also affect behavior in mice. Mice that are reared in a germ-free environment have increased movement in a maze, suggesting decreased anxiety.²⁹ The metabolic product of enteric bacteria, propionic acid, has been reported to affect behavior and cognition in mice.³⁰

A recent seminal report in *Cell* describes improvement in a mouse model of autism in animals treated with the commensal organism *Bacteroides fragilis*. Not only was intestinal permeability corrected but

also communicative, stereotypic, and anxiety-related behaviors were improved.³¹ A specific polysaccharide produced by *B fragilis* interacts with the host immune system to induce proper T-cell development and correct Th1/Th2 imbalances.³²

Unique populations of microbes make up communities in various parts of the body, including the skin, mouth, GI tract, and vagina. The GI tract has the most diverse population of microbes.³³ There are remarkable differences in the microbiome of the upper GI tract and the colon, where the greatest concentrations of bacterial quantity and species are present.³⁴

Conventional culture-based microbiology techniques have significant limitations in identifying bacterial species. It has been especially difficult to culture some species or identify those present in low quantities. Culture-independent DNA sequencing of bacteria has become more cost effective and accurate, allowing for the capture of up to 50% of organisms that may have been missed by culture techniques.^{35–37}

Microbiome studies in infants and children are limited so far, but this is rapidly changing, with monthly reports forthcoming. Infants were previously believed to be born with sterile GI tracts,³⁸ but studies of meconium (first stool passed) have reported the presence of bacteria, suggesting prenatal exposure to bacteria that may initiate immune development even before birth.³⁴ Immediately at birth, there is a remarkable increase in colonization of the gut by microbes, with mode of delivery being reported to affect the types of microbes to which an infant is exposed.³⁴

Breast-fed and formula-fed infants have very different initial microbes as identified by microbiome assessment. However, with broadening of the diet, these differences are reduced over time. It is suspected, but not clear, that the early differences may influence immune system development and that breast-feeding provides protection from the development of allergy and other issues.¹⁸

The microbiome of the GI tract is dominated by bacteria of 2 phyla of organisms, Firmicutes and Bacteroidetes.¹² Using DNA sequencing, preliminary reports suggest that children with autism have greater numbers of the order Clostridiales and other alterations in the intestinal microbiome.^{11,39–42} Although our classic experience with Clostridiales has been the production of disease, including *Clostridium difficile* toxin-induced pseudomembranous colitis, *Clostridium tetani* with toxin production causing symptoms, or *Clostridium botulinum* toxin production and illness, we now know

that most *Clostridium* spp are nonpathogenic commensals that stimulate T-regulatory cells and likely help to maintain gut homeostasis. This maintenance is done in part by producing an array of metabolic byproducts.⁴³ Is it possible that these organisms could create a metabolic intestinal imbalance in individuals with autism?

Evaluating children with autism, unaffected siblings, and unrelated controls, Parracho et al³⁹ reported a greater quantity of Clostridia in the autistic group, with intermediate findings in the unaffected-sibling group, compared with that in unrelated individuals. Interestingly, in a study in healthy, unaffected, nonfamilial controls, Kang et al⁴⁴ described a diminished presence of *Prevotella* and *Coprococcus* spp known to be involved in carbohydrate fermentation in the gut. These abnormalities were identified in children with autism despite the presence or absence of GI symptoms and were not altered by dietary-intake patterns of the affected individuals. These findings may support the idea that particular microbiome imbalances could affect autism, rather than the suggestion that microbiome imbalances occur as a result of eating behaviors or dietary differences seen in autism.

Some studies in children with autism have reported metabolic disturbances such as increased levels of oxalate,⁴⁵ disordered porphyrin metabolism,⁴⁶ greater levels of homocysteine,⁴⁷ elevated *para*-cresol level,⁴⁸ disordered creatine metabolism,⁴⁹ differences in organic and fatty acid levels,^{50,51} and tryptophan deficiency.⁵² An increased level of urinary dimethylamine and lesser levels of hippurate and 4-cresol sulfate in children with autism might be associated with the growth of certain bacteria in the GI tract (*Bacteroides* and/or *Clostridia*),⁵³ and elevated levels of *para*-cresol could be the result of increased colonization of *Clostridium* spp and *Pseudomonas stutzeri*.⁴⁸ A greater level of urinary 3-(3-hydroxyphenyl)-3-hydroxypropionic acid in children with autism compared with controls has been described, and it has been suggested that the source of this compound might be multiple species of *Clostridia*.⁵⁴ Oral treatment with the antibiotic vancomycin has been associated with transient improvement in behavior in regressive-onset autistic spectrum disorders and may affect small intestine bowel overgrowth or transiently managed chronic “dysbiosis.”⁵⁵

Specific pathogenic organisms have been sought in individuals with autism. Using pyrosequencing, Finegold⁵⁶ identified *Desulfovibrio*, a gram-negative, anaerobic bacillus in the stool samples of a population

of children with autism and reported symptoms associated with this organism. The control population included siblings and other family members of the affected children that could have been a colonizing vector. His prior work suggested increases in Clostridia and increased *Bacteroidetes vulgatus* in the stool of individuals with autism.⁴⁰

Using DNA sequencing, Williams et al⁴² evaluated intestinal biopsy samples from children with autism and GI symptoms and reported the presence of *Sutterella* spp in the intestinal biopsies of 12 of 23 children with autism and not in control patients (who were unaffected with autism but were undergoing endoscopy for GI symptoms). *Sutterella* spp have been identified in individuals with inflammatory bowel disease (IBD) but have been seen in healthy individuals as well.⁴² Attempts to look for previously identified abnormalities were attempted in later publications. Kang et al⁴⁴ did not find elevated *Sutterella* spp concentrations in their population of children with autism. In fact, in their study, a stool- collection analysis, the concentration of *Sutterella* spp. was slightly less than that in unaffected controls. Differences may have been related to the method of sampling (intestinal biopsy vs. stool collection) or to population differences.

In a study that reported the microbiome-assessment findings in the duodenum of children with autism, presented in abstract form in 2014, we (Kushak et al⁵⁷) described no differences in specific disaccharidase activity between children with autism and unaffected control comparisons. Microbiota analysis at the species level demonstrated statistically significant differences in the concentrations of 8 species between children with autism and unaffected comparison subjects (Table). Subsequent analysis of these and additional data are forthcoming. We are certainly interested in why there was a decrease in *B vulgatus* in the upper GI tract in our study but a high quantity identified in the stool study by Finegold.⁵⁶ The significant reduction in *Enterobacter hormaechei* is also of interest. The presence of this organism has been associated with outbreaks of sepsis in neonatal intensive care units⁵⁸: It is known as a small-colony variant that is associated with antibiotic resistance and slow-growing, chronic infections.⁵⁹ Could an altered/reduced presence of this organism in children with autism affect the inflammatory/immune response in the gut?

Not all studies agree that there is disrupted microbiome in autism. Gondalia et al⁶⁰ looked at children

Table. Prevalences of gut species in children with autism versus unaffected controls.

Bacterial Species	Children With GI Issues and		P
	Autism	Controls	
<i>Bacteroides vulgatus</i>	↓	↑	0.05
<i>Escherichia</i> sp	↓	↑	0.01
<i>Ruminococcus gnavus</i>	↓	↑	0.01
<i>Neisseria</i> sp	↓	↑	0.02
<i>Blautia coccooides</i>	↓	↑	0.03
<i>Enterobacter hormaechei</i>	↓	↑	<0.005
<i>Burkholderia cepacia</i>	↑	↓	0.02
<i>Pedobacter</i> sp	↑	↓	0.04

with autism who had GI complaints, children with autism without GI complaints, and their unaffected, asymptomatic siblings. There were no identifiable differences in the microbiome-assessment findings between these groups. Up to this time, assessments with similar technology of fungus or other eukaryotes have not been reported.

Clearly, larger-scale population assessments and characterizing children by diagnosis, GI symptoms, and dietary status will advance our understanding of the intestinal microbiome and any association with the condition or its associated symptoms.

Proposed Mechanisms of Altered Microbiome in Autism

Many of the proposed mechanisms for an environmental contribution to the development of autism would have a potential impact on the microbiome. Perhaps the strongest candidate for an autism phenotype might be the maternal autoantibody-related autism model.⁴ Active autoimmune disease in pregnancy can allow for the transfer of immunoglobulin G autoantibodies into the fetus and alter fetal and offspring immune response. Several conditions may occur at birth that require the administration of antibiotics to the mother or neonate in the perinatal period, including maternal

infection or sepsis, group B streptococcal infection or colonization, newborn sepsis, meconium aspiration, and newborn fever. All of these circumstances set up an alteration of the microflora that the developing newborn immune system experiences. We know that after the administration of antibiotics, the microbiome is changed for at least weeks and perhaps months.⁶¹ Could this period of microflora alteration establish a longer-standing immune change in infants? Certainly many more children have antibiotic exposure in the perinatal period than those developing autism, and many of the children with autism that we follow up have not had this early exposure. This, at best, could represent an “at-risk” subset of children and needs confirmation.

In an assessment of children born to mothers who had influenza or fever during pregnancy, the frequency of autism and developmental delay was higher in those born to women who had fever. The frequency of autism and developmental delay was attenuated in children born to mothers who used antipyretics.⁶¹ Little information exists about group B streptococcal infection and its potential impact on the development of autism. In a maternal immune-activation study in which pregnant rats were exposed to group B streptococci-induced chorioamnionitis, affected offspring were exclusively male and had several features suggestive of autism, including motor impairments and social and communicative deficits.⁶²

The caesarean section mode of delivery has been a suspected factor in the development of a number of conditions in infancy, including greater risks for autism and food allergy. A large-scale epidemiologic study sought the population-attributable fraction of a number of perinatal factors, including prematurity, small for gestational age, and delivery by caesarean section. All were found to serve as an increased risk for autism; however, of the conditions evaluated, caesarean section had the greatest impact on autism.⁶³ These factors were believed to account for ~12% to 13% of cases of autism. Caesarean section changes postnatal colonization from an initial exposure to maternal vaginal flora to a more skin-based flora. There may be a protective value offered by the maternal vaginal microbiome. Children delivered by caesarean section have an increased risk for food allergy.⁶⁴ Food allergy occurs in ~8% of infants.⁶⁵ Growing data suggests a potentially higher prevalence of food allergy in children with autism over the general pediatric population. At this time, the available information

about food allergy in autism is relatively limited and more study is needed.^{66,67}

One of the remarkable functions of the intestinal microbiota is the function of carbohydrate digestion and fermentation of products that are not digested by humans. Our group and others have published that some children with autism and GI symptoms have lactase deficiency.^{68,69} The expression of genes coding for carbohydrate digestion and transport, identified in small-intestinal biopsy samples, was deficient in an autistic population compared with unaffected patients undergoing biopsy.⁶⁹ These poorly digested and poorly absorbed carbohydrate sources in the intestine may skew the quantity and makeup of the flora of Firmicutes and Bacteroidetes as microbes capable of maximizing digestion and absorption predominate.

Proposed Interventions for Altered Microbiome in Autism

At this time, clinicians' tools for managing intestinal overgrowth or imbalance include antibiotic treatments, probiotics, dietary interventions, and treatment of malabsorption if identified, such as a digestive enzyme for deficiency. The success of any of the interventions in affecting the microbiome has not been tracked significantly, although such studies are in progress in celiac disease and IBD. These approaches likely have short-term effects, and stopping the treatment will bring a gradual return to a baseline, pretreatment microbiome state. These treatments may have value if used in properly identified patients.

FMT has in recent years become a popular treatment of conditions including refractory *C difficile* infection, obesity, IBD, and autism. There is current support for FMT as effective in helping to treat refractory *C difficile* infection. It appears to be more effective than antibiotic protocols.⁷⁰ The effectiveness of FMT in treating more chronic conditions has not been reported. One study evaluating the effectiveness of FMT treating individuals with *C Difficile* offers a glimpse at treating chronic disease. Three of 10 individuals with *C Difficile* had concomitant IBD. Although the *C Difficile* was eradicated by FMT in 2 of 3 individuals with IBD, there was no reduction in chronic IBD symptoms.⁷¹ Current studies are focused on FMT specifically for inflammatory bowel disease. In a meta-analysis evaluation of the literature, clinical remission of IBD occurred in 45% of individuals, there was a suggestion that younger individuals may have a higher response rate.⁷²

The speculation that FMT could have therapeutic merit in autism is founded in published studies that have suggested that probiotics can affect brain function. Using functional magnetic resonance imaging, Tillisch et al⁷³ reported an impact of fermented milk supplemented with probiotics on the brain's emotional centers and pain centers. The idea that FMT could replace deficient microbiota such as *B fragilis* or other balancing bacteria has driven families to attempt this treatment currently. At this time, the concept is being studied. Factors such as appropriate candidates, symptoms to monitor, donor source, and frequency of dosing are not at all clear. Findings from 1 study of the durability of a single transplant suggests a significant impact on the microbiome for up to 24 weeks.⁷⁴ For the treatment of chronic conditions, it may be determined that repeated dosing will be necessary for the treatment to be effective.

Discussion

It is clear that we are at the very early stage of understanding the effect of the microbiome on individuals with autism. Technology has just begun to allow assessment of individual species in patient populations as well as quantification of species. We are still learning the function and value of particular intestinal bacterial communities in health and disease. As with other chronic medical conditions such as inflammatory bowel disease, there is growing support that alteration from a "typical" microbiota seen in healthy individuals including loss of diversity of the flora, loss of lactose fermenters and potential predominance of atypical microbes may promote disease.

CONCLUSIONS

The abnormalities of the microbiome are of great interest in autism research currently. The preliminary information in this area provides evidence for the contribution of microbiome disruption to GI symptoms, and perhaps direct neurologic impact, in autism.

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CONFLICTS OF INTEREST

The author has indicated that he has no conflicts of interest with regard to the content of this article.

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