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Gut Health in Children with Autism Spectrum Disorder- A Systematic Review

By Rachel Rynearson

Advisor: Elizabeth Klingbeil
Date: December 10, 2021

Submitted in partial fulfillment of
The requirements for the University Honors Scholar Designation
at Johnson & Wales University

Table of Contents:

Abstract:

***Introduction*:** There have many reported differences in the gut microbiota composition in children with autism spectrum disorder (ASD) compared to typically developed (TD) healthy cohorts (HC). This study aims to review existing literature and current studies regarding the role of the gut microbiota in the development of ASD. Additionally, this study aims to determine if there is a correlation between gut microbiome dysbiosis and an increased severity in ASD symptoms. ***Methods*:** An extensive literature search was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) system. A total of 7 databases including PubMed and MEDLINE were used and a total of 591 records were screened and assessed for eligibility. ***Results*:** A total of 10 papers were included. The studies ranged from 6-143 participants with ASD who were between the ages of 2 to 13 years old and were from various geographical locations throughout the world including China, the United States, and Slovakia. Also included in this study is one study that includes an animal model of 7-week-old BTBR and B6 mouse models. All the studies included collected fecal samples. Studies reported to be significantly higher in abundance in autistic children included *Bacteroides* spp., *Firmicutes,* and *Prevotella* spp. A significant decrease in the abundance of *Streptococcus* and *Lactobacillus* was also observed in the studies. The findings are inconsistent across studies as research is limited on the gut microbiome in children with ASD and the role of gut dysbiosis on the severity of ASD and related behavioral symptoms. ***Conclusion*:** A clear connection between the diversity of the gut microbiome and the severity of ASD symptoms in children has been demonstrated through review of the research. It can be determined that a greater diversity of gut microbiota is associated with improved behavioral and gastrointestinal (GI) outcomes in children with ASD. Gut microbiota is altered in children with ASD; however, further exploration is needed to

determine whether this is a cause or effect of the condition. Additionally, further trials are needed to determine the effectiveness of pre- or probiotics as well as alterations to the diet in reducing autistic behaviors and improving GI symptoms.

Objectives:

Those with autism spectrum disorder, commonly referred to as ASD, often experience a myriad of health issues in relation to ASD; however, the most common is gastrointestinal issues. Commonly seen in children with ASD, gastrointestinal issues like irritable bowel syndrome (IBS), irritable bowel disease (IBD), and Crohn's disease often accompany ASD. Since gastrointestinal disorders are so common in those with ASD, it brings up an interesting thought, that perhaps those with ASD have unique gut microbiomes due to the presence of autism spectrum disorder. This systematic review aims to analyze current studies and research surrounding the gut microbiome of children with autism spectrum disorder to determine interpret current research findings of the interaction between ASD and gut microbiota composition.

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Introduction:

The concept of a connection between the overall health of gastrointestinal health or gut health as related to brain function is a new area of study, despite the idea of a potential gut-brain connection dating back to the 1700s (Alhadeff, 2021). The United States, among other countries throughout the world, is facing a mental health crisis with cases of various mental and neurological illnesses on the rise every minute (Dawson et al., 2016). As microbiologists, neurologists, and even scientists of nutrition have conducted further research into the gut-brain connection, they have found that seemingly separate organ systems interact very closely with one another and can even affect one another (Alhadeff, 2021). In the past decade, various areas of research on the gut-brain connection have discovered that the influence of gut microbiota on mental and physical wellbeing is exponential. Research of this newfound connection has exploded in the science field as more and more scientists are jumping on board to prove just how vital this gut-brain connection is to our entire population's well-being.

Autism spectrum disorder (ASD) is a severe neurodevelopmental disorder that is primarily defined and categorized by a range of abnormal behavioral symptoms; symptoms such as restricted interests, impairment in social interactions, and stereotyped behavior (Dan et al., 2020). ASD diagnoses have doubled in a ten-year period, 2000-2010, from 6.7 to 14.7 per 1000 children (Berding et al., 2016). In 2010, nearly one in sixty-eight children were diagnosed with some form of ASD that ranges in severity. Until recently no one thought to examine the correlation between gut microbiomes and the severity of ASD symptoms, but doing so unlocks a whole new world into possible treatment of ASD symptoms.

Autism spectrum disorder refers to a variety of complex neurodevelopmental disorders that first occur during early stage of life (Zhang et al., 2018). ASD encompasses a variety of

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conditions including autistic disorder, Asperger syndrome, and pervasive developmental disorder (Berding et al., 2016). ASD is typically defined by a presence of restrictive behaviors or repetitive behaviors that are usually fixed behaviors, as well as the presence of deficits in social and communication skills (Berding et al., 2016). ASD encompasses a wide variety of symptoms, which can have a broad range of severity and can be unique between individual cases of the disorder (Berding et al., 2016). In the past ten years there has been a steady incline in the number of diagnoses of ASD that occur each year, although, the exact cause of the development of ASD is unknown and is very difficult to study (Berding et al., 2016). Researchers have dwelled on the exact cause of ASD and have been unsuccessful in pinpointing a cause, but it is likely that environmental and genetic factors play a role in the development of this disorder. ASD is a myriad of a variety of symptoms that can range in severity from patient to patient. Many patients, especially pediatric patients, can experience non-neurological symptoms of ASD such as gastrointestinal (GI) disorders, picky eating behaviors, unusual sleep patterns, and very rigid-compulsive behaviors (Zhang et al., 2018). Recently, it has been hypothesized that the gut microbiome of children may play a significant role in ASD development and the severity of symptoms (Berding et al., 2016). ASD is a highly complex neurodevelopmental disorder that is very difficult to diagnosis and treat due to the variation among patients. For years, there has been research conducted to determine new treatments for ASD; however, until recent, there was very limited to no research exploring the effects that the gut have on the symptoms of ASD. Due to the increasing prevalence of the disorder, nearly 1 out of every 68 children in the United States being diagnosed with ASD, it is critical now more than ever to determine exactly what is contributing to the development of this disease (Dan et al., 2020). A combination of factors contributes to the development and severity of the disorder including environmental and genetic

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factors and gut dysbiosis that contributes to GI issues, increased behavioral issues, and a dysregulation of associated metabolism activities that can affect the metabolic network of vital neurotransmitters (Dan et al., 2020). It is very important to note that through the findings of this review it can be determined that there is not a sole contributing factor to the development and severity of ASD as shown in the current research, and rather there are many factors that contribute to the development and severity of the disorder. A decreased gut microbiota diversity is correlated to alterations in behavior and GI abnormalities (Coretti et al., 2018). This leads us to this question: is a decreased gut microbiome diversity the cause of ASD and to blame for GI and behavioral issues or does ASD cause a decreased gut microbiome diversity leading to the alterations in behavior and GI abnormalities that ASD is defined by?

Methods:

The PRISMA method was used to conduct and guide this systematic review to provide a clear means of reporting information (Page et al., 2021). Inclusion criteria to be considered for this systematic review were as follows: studies that included sample size cohorts that included patients with ASD as well as neurotypical patients of the same age group; all patients were under the age of fourteen years old; peer-reviewed scholarly studies and journal articles; studies and research that were conducted within the last ten years. Studies that were eliminated for not having a valid sample size either did not contain autism spectrum disorder patients compared to a neurotypical cohort, or the sample size was less than five patients total. To conduct research, the Johnson & Wales University Library Academic Complete database search was used as well as PubMed database search. There were no filters or limiters applied to searches to gather the maximum possible results. These databases were last consulted October 22, 2021. Seven databases were used to collect results (MEDLINE, PubMed, Johnson & Wales Univeristy Complete Academic Database, Food Science Source, E-Journals, and Education Research Complete) and the following search terms were used to find results; *gut microbiome; gut microbiota; pediatrics; children; autism spectrum disorder.* Other systematic, literature, or narrative reviews were removed in addition to duplicates from each of the database searches. For analyses, the data was grouped based on human subjects, animal subjects, and peer-reviewed journal articles relevant to the topic. Studies and articles were screened and determined for potential bias, and there were determined to be no known bias.

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Results:

The selection process for the included studies is shown in Figure 1. The initial search conducted identified 591 records from seven databases including Medline, PubMed, and Johnson & Wales University complete academic database search. These, 579 articles and studies were excluded due to ineligibility. Articles and studies that were ineligible were other systematic reviews, periodicals, studies or articles of irrelevant subject matter, meta-analyses, narrative and literature reviews, addendums, additional duplicates (not previously removed before screening), descriptive reviews, re-prints, invalid sample sizes (less than 5 subjects), non-English articles and studies, and abstracts. In the end, a total of 10 articles that reported on the gut microbiota in children with and without ASD were included in this systematic review. Table 1 demonstrates the type of bacteria that was discovered in the 10 included studies as well as the sample size, sample collected, age group of the patients, and any interventions tested or pre-experiment preparations used.

Figure 1:

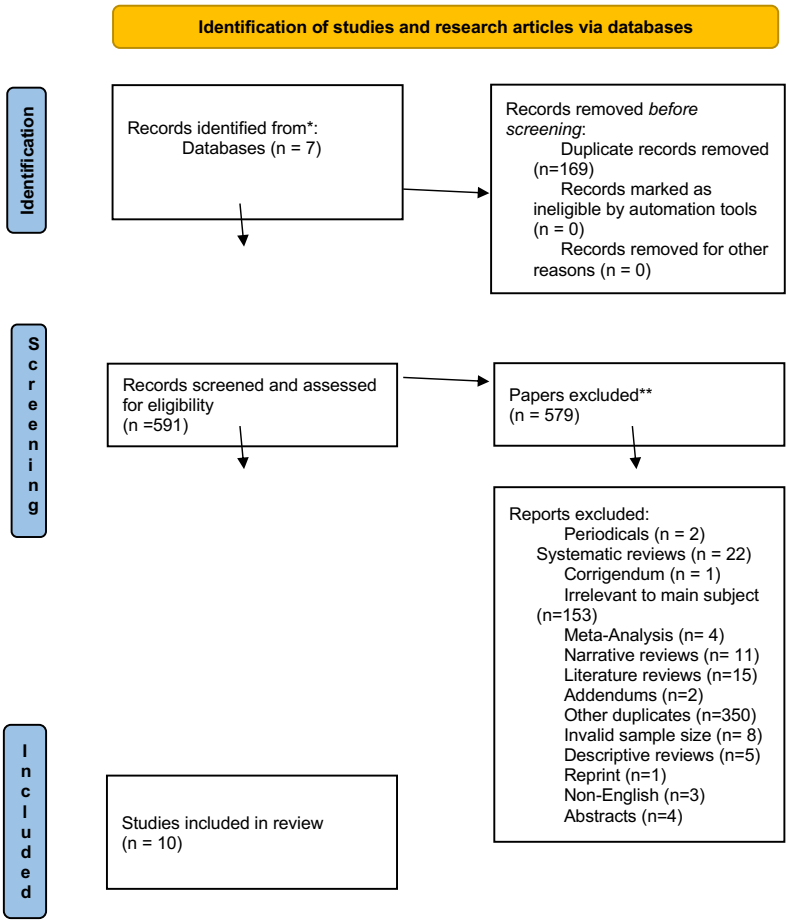
Identification of studies and research articles via databases
Identification
Records identified from*:
Databases (n = 7)
Records removed *before screening*:
Duplicate records removed (n=169)
Records marked as ineligible by automation tools (n = 0)
Records removed for other reasons (n = 0)
Screening
Records screened and assessed for eligibility (n =591)
Papers excluded**
(n = 579)
Reports excluded:
Periodicals (n = 2)
Systematic reviews (n = 22)
Corrigendum (n = 1)
Irrelevant to main subject (n=153)
Meta-Analysis (n= 4)
Narrative reviews (n= 11)
Literature reviews (n=15)
Addendums (n=2)
Other duplicates (n=350)
Invalid sample size (n= 8)
Descriptive reviews (n=5)
Reprint (n=1)
Non-English (n=3)
Abstracts (n=4)
Included
Studies included in review
(n = 10)

Table 1:

Author, Year	**Age, Years**	**Specimen Type**	**Children w/ ASD**	**TD[1]/NT[2]**	**Observed Trends in the Gut Microbiome of Children w/ ASD**	**Interventions / Pre-Experiment Measures**
Coretti et. al 2018	2-4	Fecal	11	14	↑ Firmicutes: Bacteroidetes ratio, *Faecalibacterium prausnitzii,* Proteobacteria, and *Oscillospira* ↓Actinobacteria, Actinomycetaceae, Coriobacteriaceae, Bifidobacteriaceae, Gemellaceae, and Streptococcaceae	No intervention used
Dan et. al 2020	2-13	Fecal	143 (52 w/ constipation, 5 w/ diarrhea	143	↓*Sutterella, Prevotella, and Bacteroides.* Additionally, dysregulation of metabolic activities in neurotransmitter network	All participants did not take antibiotics, pro- and prebiotic interventions, or other medications 3 months prior to the start of the study to prevent any influence on gut microbiota composition
Ding et. al 2020	2-7	Fecal	77	50	↑ *Lachnospiraceae, Clostridiales, Erysipelotrichaceae, Dorea, Collinsella,* and *Lachnoclostridium* ↓*Bacteroides, Facalibacterium, Parasutterella,* and *Paraprevotella* Presence of unitendified *Erysipelotrichaceae, Faecalibacterium,* and *Lachnospiraceae* correlated with ASD severity	All participants did not take antibiotics, pro- and prebiotic interventions, or other medications 1 month prior to the start of the study to prevent any influence on gut microbiota composition.
Grimaldi et. al 2018	4-11	Fecal	30 (divided into 2 groups, exclusion diet/ non-restricted	0	↑*Faecalibacterium prausnitzii* and *Bacteroides* spp. ↓*Bifidobacterium* spp. and Veillonellaceae family	Intervention with B-GOS® prebiotic (no exclusion diet). This was compared to a control group on an exclusion diet
Inoue et. al 2019	4-9	Fecal	13	0	↑Genra *Blautia* and *Acidaminococcus* ↓*Streptococcus, Odoribacter,* and *Eubacterium* (belonging to the family *Erysipelotrichaceae)*	Intervention with a partially hydrolyzed guar gum supplementation (a form of probiotic). This was compared to a control group with no type of intervention
Newell et. al 2016	7wks	Fecal	25 (BTBR mice)	21 (B6 mice)	↑Bacteroidetes (*Bacteroides/Prevotella* spp.) ↓Firmicutes (*Clostridium coccoides, Clostridium leptum, Clostridium* clusters XI and I and *Roseburia*	Intervention with BTBR mice on a ketogenic diet. This was compared to B6 mice on a regular chow diet

					spp., and *Lactobacillus* spp)	
Sun et. al 2019	3-12	Fecal	9	6	↑Ruminococcaceae ↓Bacteroidetes, Selenomonadales, Prevotellaceae	No intervention used
Tomova et. al 2018	2-9ASD/ 5-17 non ASD siblings/ 2-11 TD	Fecal	10	10TD/ 9 non-ASD siblings	↑*Clostridia* cluster 1, *Desulfovibrio* ↓ Firmicutes: Bacteroidetes ratio	Intervention with "Children Dophilus" probiotic supplementation with 60% *Lactobacillus* (3 strains), 25% *Bifidumbacteria* (2 strains) and 15% *Streptococcus* (1 strain) for four months
Zhang et. al 2018	3-8	Fecal	35	6	↑ Firmicutes: Bacteroidetes ratio, *Sutterella, Odoribacter,* and *Butyricimonas* ↓*Veillonella, Streptococcus,* and butyrate/ lactate producers	All participants did not take antibiotics, pro- and prebiotic interventions, or other medications 1 month prior to the start of the study to prevent any influence on gut microbiota composition
Zou et. al 2021	2-6	Fecal	29	31	↑*Candida sake, Saccharomycetaceae, Saccharomyces* ↓*Candida parapsilosis, Trichocomaceae, Asperigillus*	All participants were not a special diet or medications like probiotics or antibiotics prior to the start of the study

[1]TD- Typically Developed
[2]NT- Neurotypical

Discussion:

Through review of the research, it was determined that dysbiosis of the gut microbiome does in fact increase the severity of ASD symptoms like increased behavioral issues, as well as altered GI issues. An increase in particular bacterium types demonstrated a connection to an increased prevalence of behavioral issues associated with ASD, as well as an increase of negative GI issues like constipation and diarrhea. It was found that an imbalance of the gut microbiota structure is caused by a shift in the colonization of beneficial bacteria species in early childhood. Most of the research included in this review has determined that there is a decreased diversity of beneficial bacteria in the ASD groups as compared to a typically developed (TD) healthy cohort. Dysbiosis of gut microbiota contributes to the overall severity of ASD; the greater dysbiosis of bacteria, the more severe the level of ASD has shown to be (Ding et al., 2020), (Sun et al., 2019), (Zhang et al., 2018). Numerous types of bacteria on the phylum and family levels were analyzed in most of the studies; however, the most notable finding as demonstrated in Table 1 was the variation in the findings of an increase or decrease in the Firmicutes: Bacteroidetes ratio. Zhang et al., (2018) and Coretti et al., (2018) observed an increase in the Firmicutes: Bacteroidetes ratio, whereas Tomara et al., (2018), Sun et al., (2018), Newell et al., (2016), Ding et al., (2020), and Dan et al., (2020) observed a decrease in the Firmicutes: Bacteroidetes ratio. Those that determined that ratio to be increased additionally observed that the increased ratio could be positively correlated with an increase in the severity of symptoms in children with ASD (Zhang et al., 2018), (Coretti et al., 2018). Adversely those researchers that did not observe an increase in the ratio did observe that an increase in diversity of bacteria is correlated with less severe ASD symptoms. They observed different increases in different varieties of bacteria (Tomara et al., 2018), (Sun et al., 2018), (Newell et al., 2016),

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(Ding et al., 2020), and (Dan et al., 2020). Other types of dysbiotic microbiota profiles were also shown to increase ASD symptoms; however, the Firmicutes: Bacteroidetes ratio is particularly interesting because it changes throughout the life cycle (Mariat et al., 2009).

The Firmicutes: Bacteroidetes ratio evolves throughout the life cycle in a TD healthy cohort (Mariat et al., 2009). Mariat et al., (2009), observed that Firmicutes: Bacteroidetes ratio is the highest in adults and there is no difference between infants and the elderly in the ratio. Although the aforementioned study was not conducted on individuals with ASD, it maintains validity as it was observed in this study that in TD healthy cohorts, the diversity of microbiota in the gut microbiome increases with age; however, in those with ASD it does not (Dan et al., 2020). A variety of gut microbiota was observed in the TD healthy cohort and significant differences throughout the varying ages of the TD children were observed (Dan et al., 2020). In the ASD group, diversity did not increase with age and decreased amounts of beneficial bacteria were observed (Dan et al., 2020). This significant finding could demonstrate a change in bacteria composition occurs in early childhood may influence the gut ability to increase beneficial bacteria throughout life in children with ASD. In addition, this limitation may affect the severity of ASD symptoms. The next step in this hypothesis is to determine whether this change is driven by the presence of ASD or firstly by the gut microbiota dysbiosis.

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While further research needs to be conducted to determine if the lack of diversity in the gut microbiota is due to ASD or vice versa, it was determined that by increasing the diversity in gut microbiota, children with ASD saw a relief in previously debilitating GI symptoms (Inoue et al., 2018). One study explored the impact that partially hydrolyzed guar gum (PHGG) supplementation in the form of a probiotic was effective at alleviating constipation and diarrhea symptoms in children with ASD in this region of Japan (Inoue et al., 2018). Not only did PHGG

supplementation improve related GI symptoms, but it also decreased the level of inflammatory cytokines in the body. These further improved symptoms of leaky-gut syndrome and vastly improved symptoms of behavioral irritability (Inoue et al., 2018). Inside of our stomach, we have more than 4,000 square feet of intestinal lining, and when fully functioning, it forms a tight barrier that controls everything that gets absorbed into the bloodstream (Campos, 2021). When the gut is unhealthy, it may have large cracks and holes that can allow partially digested food, bugs, and toxins to absorb into the issues beneath it (Campos, 2021). This can cause change in the gut microbiota composition and can trigger inflammation (Campos, 2021). PHGG supplementation decreased the load of endotoxins from the intestine, resulting in a decreased production of the serum inflammatory cytokines involved in leaky-gut syndrome (Inoue et al., 2018). PHGG supplementation affected behavioral symptoms by improving constipation in children with ASD and potentially blocked neuroinflammation in the central nervous system (Inoue et al., 2018). Other studies also found that probiotic and prebiotic supplementation interventions, like the PHGG, increased the diversity of gut microbiota and decreased the Firmicutes: Bacteroidetes ratio. However, further research is needed to determine if pre- and probiotics are an effective way to treat and manage ASD symptoms (Tomova et al., 2015). To date pre- and probiotic supplementations are the interventions shown to improve and manage symptoms of ASD in children (Inoue et al., 2018). Additionally, diet modification to the ketogenic diet (KD) has been shown to improve ASD related symptoms (Newell et al., 2016).

Although Newell et al., (2016) did not test on human groups of children with ASD; they did use BTBR T+ Itpr3tf/J (BTBR) mice to test the implication of the diet. BTBR mice have been proven an effective model for human ASD without the spontaneous seizure activity (Newell et al., 2016). B6 mice were used at the control cohort or the TD healthy cohort and were

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fed a normal chow diet, whereas the BTBR mice were fed a KD chow. In BTBR mice it was found that the Firmicutes: Bacteroidetes ratio decreased with implication of the diet and increase obligate anaerobes and beneficial gut microbiota further down the GI tract (Newell et al., 2016). The KD diet increased the gut microbiota diversity in the BTBR mouse model and improved the overall ASD symptoms. Interestingly, KD diet alone altered the microbial phenotype, which may be the connection between the KD and improved ASD symptoms (Newell et al., 2016. These findings are rather critical as they could unlock the potential therapeutic power of the KD diet in treating the symptoms of ASD. This research while highly promising has only been conducted on an animal model and needs to be conducted in a human ASD population to prove further validity.

Future Directions and Recommendations:

Current research regarding the development of ASD and the gut microbiome of children with ASD is limited. To further establish if there is a link between the development of ASD and dysbiosis in the gut, there needs to be an increase in the number of reputable studies and clinical trials conducted to further support the link between the two. Additionally, these clinical trials need to be conducted with an increased sample size. The greatest sample size that was found in this review was 143 ASD and TD children with the fewest being 9 and 6 respectively (Dan et al., 2020), (Sun et al., 2019). Future researchers should also consider expanding the areas of research across many geographical locations. The current research that has been conducted is limited to parts of China and the United States. Therefore, research applied to greater geographical locations could further analyze the distinctions between ASD and TD children. Future studies should focus on further investigation of the potential connection between diversity of the gut microbiota and severity of ASD symptoms. It is vital for future research to establish if dysbiosis in the gut is caused by ASD or if ASD is influenced by dysbiosis in the gut. Current studies utilizing animal models are limited due to ethical concerns regarding the treatment of animals for the purpose of experimentation. Animal models can only provide a model for ASD symptoms and are not completely accurate in the presentation of the disorder, which limits results. Additionally, when using human models to study ASD and the gut microbiota, results can be limited when they require patient participation like collecting fecal samples, which relies solely on the accurate collection of the patient.

Conclusion:

ASD is a highly complex neurodevelopmental disorder that varies greatly from case to case. There is limited research on the development of the disorder and effective therapeutic treatments are limited. ASD is difficult to treat because it affects behavioral, physical, and mental processes. This systematic review highlights potential links between decreased diversity in the gut microbiome and severity of ASD symptoms. It can be determined that a lack of diversity in the gut microbiota is associated with increased severity of both behavioral and gastrointestinal ASD symptoms. Overall, improved diversity of beneficial gut microbiota may improve related symptoms of ASD, therefore, improving the overall quality of life. When the gut microbiota diversity is improved through pro- or prebiotic supplementation or diet modification, an increase in microbiota diversity is observed along with a decrease in the Firmicutes: Bacteroidetes ratio. Furthermore, current research supports the connection between gut microbiota diversity and ASD development. However, further research must be conducted to determine the pathophysiology of this connection. While more research is needed to find an effective treatment for ASD, there is a growing body of research to support the role of gut microbiota in the severity of ASD.

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