Exploring the Gut Microbiome-Autism Spectrum Disorder Connection: Implications for Therapeutic Interventions and Future Directions

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Abstract

The gut microbiome, a diverse community of microorganisms in the gastrointestinal tract, is vital for human health and has a symbiotic relationship with the host. Autism spectrum disorder (ASD), a complex condition affecting social interaction, speech, and behavior, manifests early in life and affects 1 in 36 children globally. Research shows a significant link between the gut microbiome and ASD symptoms, with dysbiosis observed in individuals with ASD compared to neurotypical populations. Pathogenic gut microbiota can produce toxins that increase gut permeability, impair the intestinal barrier, and activate the immune system. The vagal nerve, influencing central nervous system (CNS) activity, can release peripheral cytokines inducing depression-like behaviors. Thus, dietary changes and treatments targeting the gut microbiota, such as Microbiota Transfer Therapy (MTT), offer promising methods for treating ASD symptoms. Current research focuses on dietary therapies like gluten-free and casein-free diets, ketogenic diets, and probiotics/prebiotics supplementation to modify the gut microbiota and enhance ASD treatment outcomes. However, more research is needed to fully understand the gut microbiota-ASD connection and establish evidence-based interventions. Individualized approaches are crucial for the safety and effectiveness of therapeutic strategies for ASD. Exploring the gut microbiome’s role in ASD offers promising avenues for novel treatments and improved understanding of the disorder.
1. Introduction

Autism Spectrum Disorder (ASD) is a complex developmental disability characterized by distinct neurobiological variations in the brain. While the exact causes of ASD are not yet fully understood, some cases are associated with specific genetic conditions. ASD is characterized by a diverse array of behavioral and developmental symptoms, which typically manifest early in childhood, often before the age of three years. The term "spectrum" encapsulates the wide range of symptoms and the varying degrees of impairment that individuals with ASD may exhibit, making each case unique with its own set of challenges and strengths. Two primary categories of symptoms are commonly observed in individuals with ASD: difficulties in social communication and engagement, and the presence of restricted and repetitive behaviors. In the realm of social communication, individuals with ASD often struggle to interpret and respond to social cues, which can include challenges in maintaining eye contact, recognizing facial expressions, and understanding nonverbal gestures. These difficulties can hinder their ability to initiate and maintain meaningful interactions with others, leading to potential feelings of isolation and social disconnect. Additionally, individuals with ASD may exhibit a wide array of restricted and repetitive behaviors. These behaviors can manifest as repetitive movements, such as hand-flapping or body-rocking, intense and narrow interests in specific topics or objects, and a strong preference for adhering to rigid routines. These behaviors may serve as coping mechanisms for individuals with ASD, providing them with a sense of comfort and predictability in an otherwise overwhelming world. It is crucial to recognize that ASD is a spectrum disorder, encompassing a broad range of symptoms and levels of functioning. Some individuals with ASD may have exceptional abilities in certain areas, often referred to as "splinter skills," while facing significant challenges in others. The degree of impairment can vary widely, and early intervention, therapy, and support can play a crucial role in fostering developmental progress and enhancing the quality of life for individuals with ASD. As the understanding of the disorder continues to evolve, ongoing research efforts are working to elucidate the underlying causes and identify effective interventions. By recognizing and embracing the unique strengths and challenges of each individual with ASD, it is...
possible to foster a more inclusive and compassionate society that promotes understanding for all.

Due to its impact on health and disease, the human gut microbiome is a dynamic and complex ecosystem that has attracted significant interest. Numerous studies have shown that the biome plays an important role in a wide range of physiological functions, including immunological, metabolic, nutrition, and even neurological function. The gut microbiome is a colony of trillions of bacteria, viruses, fungi, and other microbes. The most prevalent and well-studied of the microbes are bacteria. However, due to a complex interaction of variables including genetics, nutrition, age, geography, lifestyle, and exposure to environmental effects, the variety and relative abundance of microbial species can differ significantly from one person to another. In a symbiotic connection with the human host, the gut microbiome plays a crucial part in digestion and nutritional absorption. It allows for the breaking down of fibers and complex carbohydrates that our systems cannot digest on their own, which supports energy absorption and the creation of nutrients and vitamins. Additionally, the gut microbiome supports a healthy immune system by defending against dangerous infections and fostering immunological tolerance to advantageous microbes and food antigens. A bidirectional communication connection between the gut and the brain has been revealed by recent studies, which have also given light on the gut brain axis (GBA) (Figure 1). The gut microbiota can affect not only physical health but also mental health and cognitive performance, according to this complex connection. Changes in the gut microbiota have been linked to a number of neurological and psychiatric illnesses, including anxiety, ASD, and depressive and phobic disorders. However, dysbiosis—disturbances in the delicate equilibrium of the gut microbiome—have been connected to a variety of health issues and disease states. Antibiotic usage, dietary changes, infections, stress, and other environmental effects are some factors that may cause dysbiosis. Dysbiosis itself can result in inflammation, metabolic problems, and an elevated risk of infection (Figure 3). Therefore, the gut microbiome plays a crucial role in human health, explaining the growing interest in researching therapeutic approaches that target the gut microbiota to enhance general health and treat a variety of medical diseases. Healthy gut microbiota can
be restored and maintained through a variety of methods, including probiotic supplementation, prebiotic intake, and dietary changes. It is expected that as research on the gut microbiome develops, so will understanding of how the biome affects human health and disease. Utilizing this knowledge can result in cutting-edge customized medicine strategies and the creation of targeted medicines to treat a variety of health issues related to dysbiosis, opening up a promising, new area for enhancing human health and well-being.

The central and enteric nerve systems communicate in both directions through the GBA, which connects the brain’s emotional and cognitive regions to peripheral intestine processes (Figure 1). The GBA is a complex and dynamic network of communication that is extremely important in controlling different facets of our physical and mental health (Figure 1). The investigation of the connection between the gut microbiome and ASD is driven by several compelling rationales. Each highlights the potential significance of the GBA and its impact on neurodevelopment and behavior in individuals with ASD. A high proportion of individuals with ASD experience gastrointestinal (GI) issues, such as chronic constipation, diarrhea, abdominal pain, and inflammation. These GI disturbances suggest a potential connection between gut health and ASD, prompting investigations into the role of the gut microbiome in contributing to or exacerbating these symptoms. Studies have revealed that individuals with ASD often exhibit alterations in the composition and diversity of their gut microbiota compared to neurotypical individuals. These differences have sparked curiosity about whether these changes could influence ASD symptoms. The gut microbiome is known to influence neurodevelopment and brain function through its impact on neurotransmitters and immune responses (Figure 1). Given the neurological and behavioral nature of ASD, exploring the potential link between the gut microbiome and ASD has become a significant area of interest and could pave the way for personalized therapeutic strategies. If specific gut microbiome patterns and biomarkers are associated with certain ASD symptoms, it could lead to targeted interventions tailored to individual needs, potentially improving treatment outcomes. The emerging research in this field holds promise for
developing more effective and individualized therapies for individuals with ASD, providing new avenues for enhancing their health and quality of life.

2. Gut Microbiome in ASD

The GBA is a bidirectional communication network that links the CNS with the enteric nervous system (ENS) of the gastrointestinal (GI) tract (Figure 1). This sophisticated and dynamic axis plays a crucial role in regulating various aspects of our physical and mental health. The GBA’s relevance to ASD has garnered increasing attention in recent years.⁸

![Figure 1: Gut-Brain Axis Communication Network](image)

Many individuals with ASD also experience GI disturbances, such as chronic constipation, diarrhea, and inflammation, suggesting a potential link between gut health and ASD. The gut microbiota, a crucial component of the GBA, plays a significant role in modulating brain function and behavior through the production of neurotransmitters, microbial metabolites, and immune signaling molecules.¹⁴ This suggests that disruptions in the gut microbiome could influence neurodevelopment. The GBA is involved in the regulation of immune responses, inflammation, and neural signaling, which are all processes implicated in ASD pathogenesis.⁹ The bidirectional communication between the gut and the brain can impact neurodevelopmental processes, social behaviors, and cognitive functions, which are frequently affected in individuals with ASD.¹
Understanding the relevance of the GBA to ASD holds immense potential for the development of new therapeutic strategies. Targeting the gut microbiome through interventions like probiotics, prebiotics, and dietary modifications may offer novel approaches to managing ASD-related symptoms and improve the overall well-being of affected individuals. Overall, the GBA represents a complex and promising area of research in the context of ASD. Investigating the interactions between the gut microbiota and the CNS may pave the way for innovative treatments and personalized interventions.\textsuperscript{15}

\textit{Figure 2: Blood-Brain-Barrier and Gastrointestinal Barrier}

Two separate physiological barriers in the human body, the blood-brain barrier (BBB) and the GI barrier, play crucial, protective roles (see Figure 2). The intestinal mucosa is where the intestinal barrier is in the digestive system.\textsuperscript{5} It controls the entry of beneficial molecules, poisons, and
pathogens into the body while regulating the transit of nutrients and water, among other molecules, into the bloodstream.\(^5\) The lining consists of mucous membranes that trap hazardous chemicals, epithelial cells that provide a protective barrier, and tight junctions that close cell gaps. The intestinal barrier supports immune defense, promotes nutrition absorption, and preserves healthy gut microbiota.\(^5\) Moreover, the BBB is a highly selective protective barrier that regulates the passage of substances between the bloodstream and the brain tissue to maintain a stable and controlled environment within the CNS.\(^7\) Endothelial cells, astrocytes, and pericytes play a role in maintaining the integrity of this barrier, which safeguards the brain by controlling the passage of chemicals across the BBB.\(^7\) The BBB protects the brain from toxins and keeps the brain’s environment steady for healthy neuronal activity.\(^7\) It may, however, make it more difficult to transport medications to the brain. The intricacy of the body’s defense mechanisms is shown by the importance of both barriers for maintaining general health and protecting the brain from harm.

Unidentified *Erysipelotrichaceae*, *Faecalibacterium*, and *Lachnospiraceae* have been shown to be positively linked with ASD severity.\(^10\) Notably, three microbial indicators (*Faecalitalea*, *Caproiciproducens*, and *Collinsella*) were found in a random forest model as potential biomarkers that distinguish between healthy controls and individuals with ASD based on gut microbiome profiles. This current study provides evidence for a link between gut microbiota and ASD, with the data implying that gut microbiota may play a role in symptomatology.\(^15\) As a result, modulating the gut microbiome can serve as a novel therapeutic method for ASD.\(^10\)

## 3. Mechanism of the Gut Microbiome and ASD

Certain compounds have been the center of attention in studies aiming to uncover a correlation between the gut microbiome and the onset of ASD. Vast amounts of evidence connecting changes of maternal gut microbiome profiles to the differences present in brain development patterns of offspring have been found through experimental methods.\(^16\) Specifically, research involving the influence of Cesarean section deliveries, and the use of mice as animal models, hint at these links.\(^17\) Multiple studies have found
that newborns delivered through Cesarean sections have a higher risk of developing ASD in their lifetime. Since it has been proven that the composition of the microbiota is different amongst newborns delivered through a vaginal delivery compared to those born through Cesarean delivery, the influence of the microbiota is a potential theory for the development of ASD. This theory is strongly supported by the fact that 70-80% of individuals with ASD experience GI symptoms (constipation, bloating, diarrhea, and nausea, among others).

Multiple theories relating the effects of maternal microbiome environments on fetus’ development have also been studied with mice. One particular study focused on brain metabolomic profiles and gene expression. This study demonstrated that mice that were induced with maternal obesity factors (EX) produced offspring with social behavioral deficits linked to the mesolimbic reward system. Moreover, subsequent transfer of gut biome from the study’s control mice into the offspring of the induced mothers completely corrected these deficits. Thus, this was a clear sign that the gut microbiome played a significant role in influencing the neurodevelopmental pathways of offspring, and that it can be influenced by the surrounding mother’s gut biome.

3.1 Effect of Short-Chain Fatty Acids on Neurodevelopment

Short-Chain Fatty Acids (SCFAs), primarily acetate, propionate and butyrate, have been found to play a role in the development of ASD. SCFAs are produced by the breakdown of dietary carbohydrates and amino acids. To study whether there is a clear difference in the level of microbiome SCFAs in individuals with ASD compared to individuals without ASD, Wang et al. analyzed concentrations of SCFAs in participants’ fecal samples. To prevent diet from interrupting results as a confounding variable, all subjects were given similar diets with equivalent amounts of protein, sugar, starch, and fiber. It was found that the total SCFA (primarily propionic acid, acetic acid, and butyric acid) concentration was significantly higher in the ASD group (136.6 mmol/kg compared to 111.1 mmol/kg). Providing further evidence for the role of altered SCFA levels on the onset of ASD-like symptoms, studies with rats have shown that the administration of propionic acid induced ASD-like
behaviors and produced neuropathological changes reported with ASD in the rats.\textsuperscript{21} Moreover, the previous studies have discovered that increased intestinal permeability is reported in ASD cases. Since acetic acid is suggested to play a role in the gut epithelial layer, there is a link that has appeared between the altered acetic acid levels and ASD symptoms.\textsuperscript{21,22} Subsequent analysis also found that ammonia levels were highly elevated in the fecal samples of those with ASD compared to the samples of those without ASD (42.7 mmol/kg compared to 32.3 mmol/kg).\textsuperscript{21} Thus, this study indicates that there is a clear elevation in these microbiome compounds in individuals with ASD, emphasizing an association between the onset of ASD and altered microbiome SCFAs.

3.2 Effect of Lipopolysaccharides on Neurodevelopment

Another focus of scientists working to establish the link between the microgut and the onset of ASD is lipopolysaccharides (LPS), an endotoxin that can mimic a gram-negative bacteria infection.\textsuperscript{23} A study using Wistar rats between the age of 12 and 14 weeks highlighted the specific effects of induced LPS solution in the pregnant rats.\textsuperscript{23} Although this research group previously found that prenatal exposure to LPS induces behaviors similar to those caused by ASD. These symptoms were measured with T-maze spontaneous alternation tests, which were impaired in mice that had prenatal LPS exposure.\textsuperscript{23,24,25} These tests are designed to measure social deficits, repetitive behaviors, and cognitive inflexibility in the ASD-context.\textsuperscript{23,24,25} In addition to these results, the researchers identified reduced maternal levels of magnesium, selenium, manganese, and zinc following inducement of LPS.\textsuperscript{8} More interestingly, prenatal zinc treatment prevented offspring from demonstrating such autistic-like behaviors.\textsuperscript{23,24,25} A follow-up study by the same group of researchers illustrated the immune and inflammatory effects of lowered LPS.\textsuperscript{23} LPS was shown to significantly increase the production of proinflammatory cytokines in the offspring.\textsuperscript{23} These cytokines sequestered zinc and caused maternal as well as fetal hypozincemia.\textsuperscript{23} Another study highlights a theory for the alteration of lipopolysaccharide (LPS) levels within the biome of individuals with ASD by demonstrating that 36.7% of children with ASD exhibit modified intestinal permeability, a marked contrast to the change observed in less than 5% of non-ASD children.\textsuperscript{26}
3.3 Effect of Serotonin on Neurodevelopment

Serotonin has also been shown to be associated with the development of ASD due its connection with the gut-microbiome axis. The fact that serotonin is observed to have intense activity during the early stages of development gives the neurotransmitter great importance when discussing neurodevelopmental disorders. SCFAs are able to cross the BBB and modulate the production of serotonin, influencing early brain development. About 90% of serotonin in the body is produced by enterochromaffin cells in the GI epithelium. Therefore, alterations in the gut microbiome can result in the deregulation and changes in serotonin production. The main mechanism through which serotonin can play a role in mediating ASD-symptoms is by acting as a pro-inflammatory molecule. Goeden et al. discovered that increasing maternal inflammation midway during pregnancy results in an upregulation of placental tryptophan (TRP) metabolism. Since TRP is converted into serotonin, there is increased serotonin present in the fetal forebrain, linking the maternal microbiome composition to the fetal biome, disrupting some serotonin-dependent neurodevelopmental processes. Mild immune activation in the dams used in the study also presented similar results of increased serotonin in the fetal forebrain. This study offers a strong direction for uncovering the specific mechanisms through which serotonin influences fetal brain development in the early stages of life. Positron emission tomography (PET) and post mortem studies also indicate that the pattern of serotonin synthesis in non autistic children resembles closely with the profile of synaptic density in their frontal cortex, revealing a somewhat global abnormality of serotonin synthesis and synaptogenesis. Lastly, the processes of high brain serotonin synthesis and synaptogenesis during the preschool years has been shown to be highly disrupted in autistic children.

Although serotonin serves as a vital link between the gut microbiome and the development of ASD, abnormal levels of other metabolites are associated with ASD-like symptoms. Mass spectrometry studies have enabled the observation of the gut composition as a whole, revealing differences in steroid hormones between individuals with and without
ASD.\textsuperscript{31,32} Specifically, many metabolites within the pregnenolone and androgen pathways were found to be elevated in individuals with ASD.\textsuperscript{32} This finding further demonstrates that pathways of downstream cholesterol metabolism are altered in individuals with ASD.\textsuperscript{32} Moreover, studies have indicated that levels of different metabolites are altered in mothers with children born with ASD compared to mothers of children without ASD.\textsuperscript{32,33} Through blood tests, it was found that carnitine conjugated molecules were present in lower levels in the mothers with ASD children.\textsuperscript{33} This finding hints at an underlying mechanism that this metabolite takes part in which can affect the presence of ASD symptoms. In addition to carnitine-conjugated molecules, B12, cis-4-decenoylcarnitine, catechol sulfate, 7-methylxanthine, and tiglyl carnitine were found to be in levels significantly different between both mother groups.\textsuperscript{33} The results of the study’s statistical analysis provides researchers with an idea of how changes in a wide variety of microbiome metabolites influence a developing baby. Instead of focusing on examining the relation of altered levels of one molecule, developing methods of analyzing how altered levels of one metabolic cell can induce changes in the levels of other molecules can provide a more inclusive view on the effects of the microbiome on the development of ASD.

4. Therapeutic Studies

There is compelling evidence from previously conducted research about a link between the gut microbiota and the nervous system. The bi-directional GBA includes signaling from the gut microbiota to the brain via neural, endocrine, immune, and humoral pathways.\textsuperscript{34} Bacteria, including commensal, probiotic and pathogenic, in the GI tract are involved in the activation of neural pathways and CNS signaling systems.\textsuperscript{35} Emerging data support the role of microbiota in influencing stress related to anxiety and depression disorders. Gut microbial dysbiosis, frequently observed in ASD patients, is associated with mood disorders and disruption of the GBA\textsuperscript{34} (Figure 3). A large registry based study showed an association between increased autism severity with higher probability of having GI problems.\textsuperscript{36}
4.1 Probiotics and Prebiotics

Probiotic and prebiotic supplements have been proven to improve gut health via host-microbe symbiosis and growth/maintenance of good bacteria. Probiotic bacteria and dietary prebiotics can cause distinct changes in the composition of the gut microbiota which can improve peripheral (gastrointestinal) and central (psychological) symptoms\(^\text{37}\) (Figure 3). In the double-blind placebo-controlled study by Schmidt et al.,\(^\text{38}\) forty-five healthy volunteers aged 18–45 years were administered one of two prebiotics (fructooligosaccharides, FOS, or Bimuno® galactooligosaccharides, B-GOS) or a placebo (maltodextrin) daily for 3 weeks. B-GOS intake was shown to significantly reduce salivary cortisol awakening response as well as increase processing of positive versus negative attentional vigilance as compared with the placebo. This indicates the potential of B-GOS supplementation in the treatment of stress-related disorders and suggests that it may modulate HPA activity. However, this study did not account for gender and no effect of prebiotics on subclinical anxiety or perceived stress were observed. Another randomized control study used a double-blind research method to divide 86 healthy, young adults into five groups as per colony-forming unit (CFU) and bacterial species count to assess the effect of probiotics on anxiety and related factors.\(^\text{39}\) The high CFU group reported to have a significant decrease in panic anxiety and worry as well as an increase in passive affect and anxiety.
control compared to the low CFU group. Probiotics were overall observed to improve panic anxiety, neurophysiological anxiety, negative affect, worry, and increase negative mood regulation. This study did not account for a diverse and large sample size and was reliant on self reported results, both of which posed limitations on the experiment. The use of over-the-counter probiotics with different compositions could have also led to skewed results. The study by Akkasheh et al.\textsuperscript{40} included 40 patients, aged 20 to 55 years, diagnosed with major depressive disorder (MDD) based on DSM-IV criteria who were randomized to receive probiotic supplementation for 8 weeks. Experimental results showed a significant decrease in Beck Depression Inventory scores, serum insulin levels, homeostasis model assessment of insulin resistance and serum hs-CRP concentrations in patients who received the probiotic supplementation as compared with the placebo, indicating that probiotic administration can have beneficial effects for MDD patients. The study’s limitations include a relatively short duration of intervention and insufficient information about the specific bacterial strain responsible for the treatment results.

4.2 Gluten

Imbalance in the gut microbiota is a common occurrence in individuals diagnosed with ASD. Incompletely digested peptides acting as opioid agonists have been hypothesized to cross the BBB by entering the bloodstream.\textsuperscript{41} Accumulation of these peptides affects brain function, specifically brain maturation, social communication, and learning, and can reduce pain sensitivity while increasing the severity of autism related behaviors.\textsuperscript{42} Elimination diets, particularly a gluten-free casein free (GFCF) diet, have been adopted to serve as an alternative treatment of ASD. A parental report of strict diet implementation involving the elimination of all foods containing gluten and casein has shown to be associated with an improvement of ASD behavior, physiological symptoms and social behaviors as compared to partial elimination interventions.\textsuperscript{43} Parents who implemented this diet for more than 6 months reported greater improvements in ASD behavior and symptoms as compared with a lesser implementation duration. These findings suggest that strict adoption, compliance, and length of diet implementation are all important factors in optimizing the effectiveness of GFCF diet in children with ASD. In a study by Knivsberg et al.,\textsuperscript{44} 15 children with ASD were recommended dietary...
interventions due to pathological urine patterns. The experiment reported that the GFCF diet helped reduce urinary peptide levels, indicating a reduction of opioid effects, which corresponded with an improvement in autistic behaviors, and non-verbal, cognitive and motor issues. However, it is important to consider the role of a placebo effect in this study due to the absence of a control group. Another randomized, controlled clinical trial found that a GFCF diet has a significant beneficial effect on autistic and related behaviors of prepubescent ASD patients at 8, 12 and 24 months of diet intervention. These results imply that adoption of the GFCF diet can have a positive effect on the developmental outcome for some children with ASD. The exclusion of a double-blind or placebo factor may have posed limitations on this study.

The nutritional deficiencies that could arise from the GFCF diet are an area of high concern. Long-term implementation of such dietary interventions can cause a lack of proper supplementation that could have adverse effects on bone health. Children with ASD commonly have picky eating behaviors associated with specific taste, colors or appearances of food due to which it is relatively difficult to implement nutrition plans with adequate macro and micronutrients. This can cause the implementation of a specific diet such as GFCF to become even more difficult. The high healthcare costs associated with this diet could also place a burden on the families. To overcome such challenges, appropriate nutrition plans should be curated for children with ASD and the families should be provided with proper information and training.

Fecal microbiota transplantation (FMT) has also been identified as a potential therapeutic approach to treat ASD (see Figure 4). FMT can be used to change the composition of the gut microbiota and improve GI and neurobehavioral symptoms in ASD patients. The fermentation of undigested food along with the production of bioactive compounds like SCFAs can affect the health of the large bowel. Protein fermentation by-products can also severely impact host health by increasing inflammatory response, tissue permeability, and colitis severity in the gut. In a study by Wang et al., researchers found higher fecal concentrations of large bowel fermentation products, specifically SCFAs and ammonia, in children with ASD as compared with controls. Since these fermentation
products can impact the health of the large bowel, the observed higher concentrations could be a reason for alterations in GI health and function in ASD patients. A clinical trial evaluating the efficacy of FMT in ASD patients performed FMT by fecal and oral routes in ASD children experiencing GI disorders. Although the study was limited by a lack of a control group and the use of participants from the same geographical location, the results demonstrated that FMT could improve GI symptoms and autism related behaviors while significantly changing the serum levels of neurotransmitters.

5. Microbiota Transfer Therapy

MTT, a modified FMT protocol, has emerged as a viable treatment option for ASD. A shotgun metagenomics study showed that the relative abundance of fiber consuming, and beneficial microbes increased after 10 weeks of MTT in children with ASD, thereby normalizing levels that were relatively low as compared with typically developing (TD) children. The findings also indicated that MTT leads to an initial improvement in the metabolic profiles of children with ASD, but that a booster or longer treatment time would be required for retention of the bacteria. A further open-label clinical trial evaluated the impact of MTT on gut microbiota composition and GI and ASD symptoms of ASD-diagnosed children, which will be later discussed.

In essence, MTT alters a recipient’s current gut microbiome by replacing it with a donor’s healthy gut microbiome. This is done by transferring fecal samples from the donor to the GI tract of the recipient (Figure 4). This method aims to have therapeutic benefits that can target a wide range of health issues. The knowledge on the scope of MTT efficacy and safety is, however, limited due to it being a newly emerging therapy.

Studies have been fully executed to test whether MTT can alleviate ASD symptoms, as MTT has already been tested with other psychiatric diseases. There is foundational evidence that MTT should aid in alleviating ASD symptoms, as children with ASD have GI problems or gut microbiota related issues, such as bloating and constipation. The gut
microbiome is also vastly different in children with ASD, as they have less beneficial bacteria like *Bifidobacterium spp.*, and instead, greater amounts of pathogenic bacteria, such as *Desulfovibrio* and *Clostridium*.\(^5^6\)

**Figure 4: Fecal Transplant Therapy Methodology**

One foundational study examined the effects of MTT on fecal metabolite profiles of children with ASD and GI symptoms, in comparison to children that are TD.\(^5^7\) The researchers analyzed 669 biochemical compounds in the children before, during, and after MTT. They found that fecal metabolite profiles of the ASD group became more similar to the TD group after treatment, suggesting the positive impact of MTT on fecal metabolites.\(^5^7\) In another clinical trial, MTT was used with 18 children with ASD and resulted in a significant reduction (approximately 80%) in GI symptoms. This persisted for 8 weeks after treatment and behavioral ASD symptoms also improved and remained after treatment.\(^5^2\) In addition, the microbiota of the donors appeared to be at least partially engrafted in the recipients which helped to shift the gut microbiota of children with ASD towards that of TD children. However, this study was limited as it had a small sample size and was also not placebo controlled, blinded, or randomized. A similar study in China also found FMT therapy to improve GI issues, ASD symptoms, and serum neurotransmitter levels after an 8-week follow-up.\(^4^7\)
In terms of greater long-term effects, there are minimal studies and still much to uncover. However, one study followed up with 18 participants with ASD two years after MTT therapy. The improvements in GI symptoms and autism-related symptoms observed during the initial treatment were largely maintained and even improved after the treatment ended. The study also found persistent positive changes in the gut microbiota, including increased bacterial diversity and the increases of beneficial bacteria abundance, such as for *Bifidobacteria* and *Prevotell*.58

There are also two prevalent ongoing clinical trials, as well, exploring FMT as an effective long term therapy. One of the trials, estimated to be completed in 2024, focuses on children with ASD and GI issues. In this trial, some children receive MTT treatment, while others are assigned to a placebo group to compare the effectiveness in alleviating GI symptoms and managing ASD symptoms. Another trial to be completed in 2024 follows the same methodology, but with adults with ASD and GI issues. They will undergo oral administration of full spectrum microbiota for a specified duration to understand longer lasting implications of MTT as a therapy.

MTT or FMT as a therapy has side effects and limitations. In general, it is safe, however, it can also lead to side effects such as GI discomfort, vomiting, nausea, diarrhea, and spotty stools. A study with a two-year follow-up shows the safety of FMT in reducing GI and neurobehavioral symptoms in children with ASD. No adverse side effects associated with FMT were found, suggesting its overall safety in both the short and long-term.

The implementation of treatments such as FMT and MTT in clinical settings involves navigating various challenges and opportunities for standardization and personalization. Barriers to standardization include regulatory hurdles, donor variability, and the need to establish consistent treatment protocols while ensuring efficacy and safety. Personalization strategies for these treatments involve microbiome profiling to tailor interventions based on individual patient characteristics, stratifying patients for personalized treatment selection, and implementing follow-up monitoring to adjust treatment protocols over time.
To understand the limitations of MTT more specifically, further studies are needed to clarify the exact contributions of each factor in MTT to gut microbiota changes in ASD. Many of the previously mentioned studies include a mixed group of participants with various GI issues, and a more homogeneous test group is needed. The open-label design of some studies also introduces potential placebo effects and longer observation periods in future trials would enhance the understanding of long-term safety and benefits.

6. Future Directions and Implications

So far, it has been established that gut microbiota imbalances have been observed in individuals with ASD and other psychiatric disorders. The gut microbiome affects the production of specific molecules, immune function, and GI integrity, all influencing ASD, and all areas where therapies can be developed.

For the future, ASD research and therapies are centered around microbial-mediated therapies, including probiotic therapy, prebiotic supplementation, and FMT. These therapies have growing potential in addressing ASD symptoms and GI issues that commonly supplement ASD. FMT therapy is gaining substantial acceptance, although there are concerns on long term impacts, standardization and infection. Due to these concerns, synthetic stool samples are being researched, which consist of predetermined bacterial populations and could be customized to enhance the personalized therapy’s efficacy.

Personalized dietary treatments, based on the gut-immune-endocrine-brain axis, have also been explored. These methods consider dietary factors, immune responses, hormonal regulation, and brain function connections. By screening for inflammatory responses to dietary proteins such as gluten and casein, health care providers can construct patient-specific dietary plans. These diets can be used in tandem with prebiotic or probiotic supplements to restore a healthy gut microbial balance, thereby alleviating ASD symptoms. Various studies have shown that specific bacterial strains, such as those of Bacteroides fragilis and
*lactobacillus* species, hold potential in reversing autistic behaviors and improving GI symptoms in both mouse models and autistic children. However, further research is necessary to optimize the formulations.66

Biomarkers have also served to assess the implementation of probiotic supplementation.13 Microbiota composition and inflammatory markers can demonstrate how to improve GI issues and thus how to manage ASD symptoms. This is also a non-invasive diagnostic tool for ASD that may be developed more and can serve as early intervention and prevention strategies. Both single strain and multi-strain probiotics have been effective in modulating these biomarkers and these findings highlight biomarker-guided probiotic therapy as a future option for managing ASD.13

These approaches show potential in improving both GI symptoms and behavioral irregularities of ASD. However, further research, standardization of procedures, and optimization of treatment regimens are necessary to fully evaluate their long-term effectiveness.63

7. Conclusion

The research on the gut microbiome-ASD connection has provided valuable insights into the way mechanisms of the gut and brain intertwine. Numerous studies have consistently shown differences in the gut microbiomes of individuals with ASD, characterized by dysbiosis and reduced microbial diversity.12,34,61 The discovery of gut-brain communication pathways has further underscored the relevance of these microbial communities in influencing brain development and ASD symptoms.14,15,34 These findings highlight the importance of ongoing research in this field, as discoveries may pave the way for novel and targeted treatments that leverage the gut microbiome to manage ASD more effectively. By exploring the intricate relationship between the gut microbiome and ASD, researchers can aim to unlock promising therapeutic avenues that could improve the quality of life for individuals living with these complex neurodevelopmental disorders.
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