Berkeley Pharma Tech
Journal of Medicine

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

By: Mayumi Schaepers-Cheu, Mansi Patel, Gizell Lien, Amaaya Arora

Correspondence:
mayumimagdalena@gmail.com

Keywords:
Gut Microbiome
Autism Spectrum Disorder
Neurodevelopment
Gut-Brain-Axis
Dysbiosis
Microbiota Transfer Therapy
Blood-Brain-Barrier

Submitted August 11, 2023
Accepted March 15, 2024
Published June 28, 2024

Full Open Access

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.[1] 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.[2] 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.[2] 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.[2] 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.[2] 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.[4] 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.[14] 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.[15] 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.[12] 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.[11] 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[3] (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.[3] 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.[3] 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]

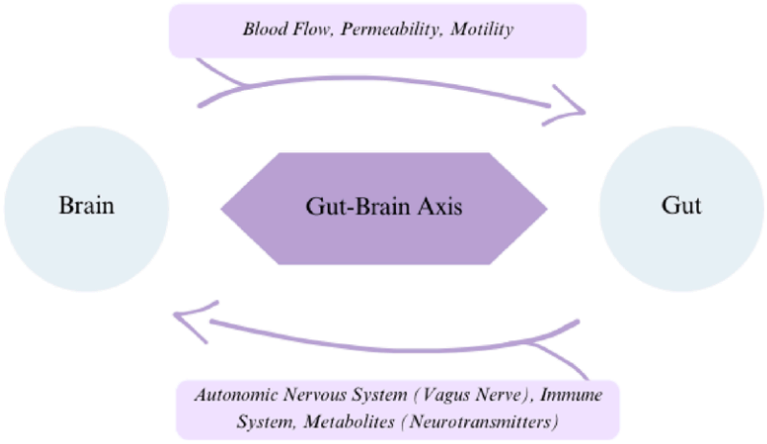

Figure 1: Gut-Brain Axis Communication Network

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.[15]

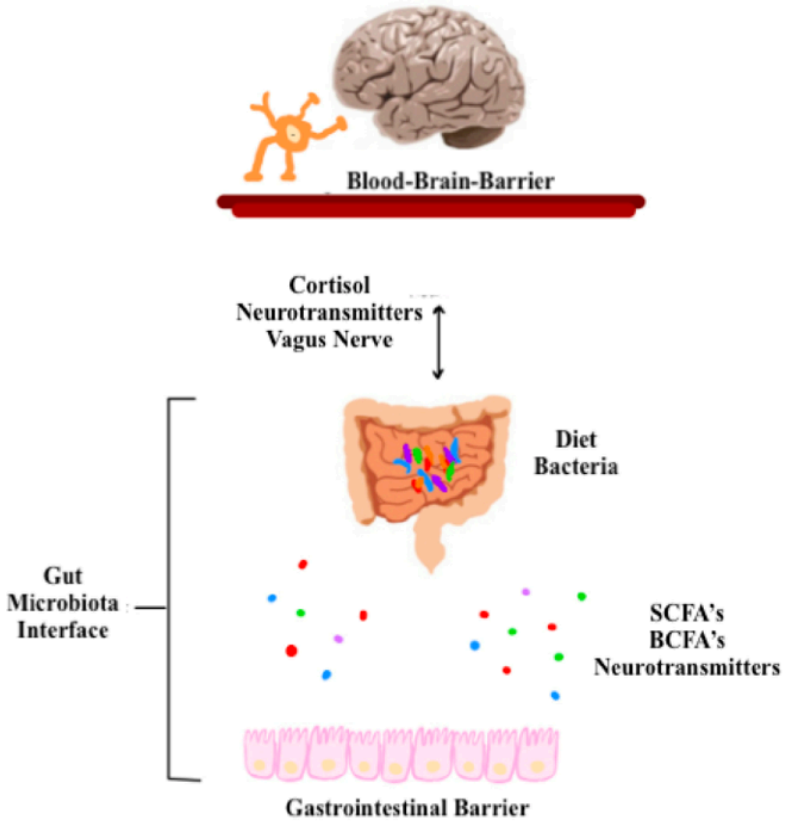

Figure 2: Blood-Brain-Barrier and Gastrointestinal Barrier

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.[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.[16] 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.[17] 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).[18]

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.[4] 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.[19] Moreover, subsequent transfer of gut biome from the study's control mice into the offspring of the induced mothers completely corrected these deficits.[19] 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.[19,20]

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.[21,22] 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.[21] 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).[21] 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.[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.[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).[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.[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.[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.[23,24,25] These tests are designed to measure social deficits, repetitive behaviors, and cognitive inflexibility in the ASD-context.[23,24,25] In addition to these results, the researchers identified reduced maternal levels of magnesium, selenium, manganese, and zinc following inducement of LPS.[8] More interestingly, prenatal zinc treatment prevented offspring from demonstrating such autistic-like behaviors.[23,24,25] A follow-up study by the same group of researchers illustrated the immune and inflammatory effects of lowered LPS.[23] LPS was shown to significantly increase the production of proinflammatory cytokines in the offspring.[23] These cytokines sequestered zinc and caused maternal as well as fetal hypozincemia.[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.[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.[27,28] 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.[28] SCFAs are able to cross the BBB and modulate the production of serotonin, influencing early brain development.[29] 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.[30] 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.[30] Mild immune activation in the dams used in the study also presented similar results of increased serotonin in the fetal forebrain.[30] 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.[28] Lastly, the processes of high brain serotonin synthesis and synaptogenesis during the preschool years has been shown to be highly disrupted in autistic children.[28]

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.[31] 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.[31,32] Specifically, many metabolites within the pregnenolone and androgen pathways were found to be elevated in individuals with ASD.[32] This finding further demonstrates that pathways of downstream cholesterol metabolism are altered in individuals with ASD.[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.[32,33] Through blood tests, it was found that carnitine conjugated molecules were present in lower levels in the mothers with ASD children.[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-4decenoylcarnitine, catechol sulfate, 7-methylxanthine, and tiglyl carnitine were found to be in levels significantly different between both mother groups.[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.[34] Bacteria, including commensal, probiotic and pathogenic, in the GI tract are involved in the activation of neural pathways and CNS signaling systems.[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[34] (Figure 3). A large registry based study showed an association between increased autism severity with higher probability of having GI problems.[36]

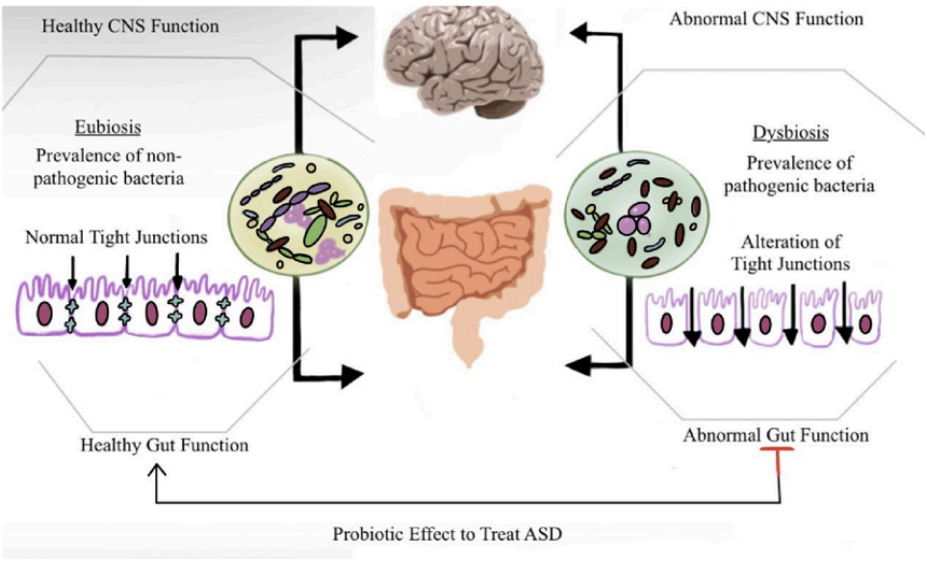

Figure 3: Eubiosis vs. Dysbiosis

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[37] (Figure 3). In the double-blind placebo-controlled study by Schmidt et al.,[38] forty-five healthy volunteers aged 1845 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.[39] The high CFU group reported to have a significant decrease in panic anxiety and worry as well 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.[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.[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.[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.[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.,[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.[45] 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.[46] 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.[47] The fermentation of undigested food along with the production of bioactive compounds like SCFAs can affect the health of the large bowel.[45] Protein fermentation by-products can also severely impact host health by increasing inflammatory response, tissue permeability, and colitis severity in the gut.[48] In a study by Wang et al.,[49] 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.[49] A clinical trial evaluating the efficacy of FMT in ASD patients performed FMT by fecal and oral routes in ASD children experiencing GI disorders.[50] 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.[50]

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.[51] 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.[51] A further open-label clinical trial evaluated the impact of MTT on gut microbiota composition and GI and ASD symptoms of ASD-diagnosed children[52] 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.[53] This is done by transferring fecal samples from the donor to the GI tract of the recipient[54] (Figure 4). This method aims to have therapeutic benefits that can target a wide range of health issues.[53] The knowledge on the scope of MTT efficacy and safety is, however, limited due to it being a newly emerging therapy.[55]

Studies have been fully executed to test whether MTT can alleviate ASD symptoms, as MTT has already been tested with other psychiatric diseases.[56] 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.[56] 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.*[56]

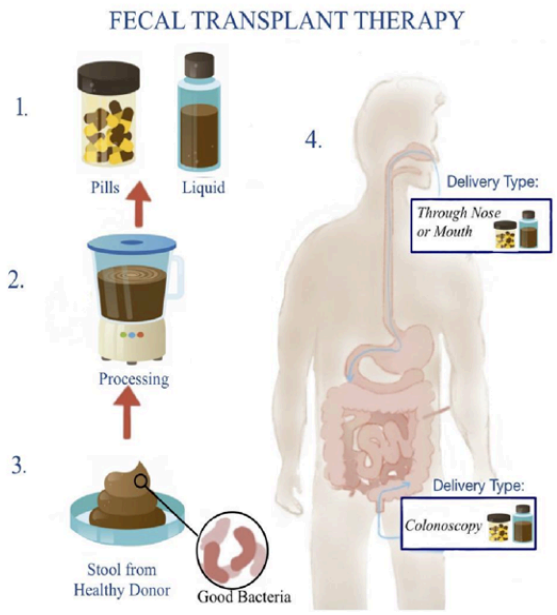

Figure 4: Fecal Transplant Therapy Methodology

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.[57] 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.[57] 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.[52] 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.[47]

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.[58] The improvements in GI symptoms and autism-related symptoms observed during the initial treatment were largely maintained and even improved after the treatment ended.[58] 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.[52] 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.[59]

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.[54] 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.[47]

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. [GR1] 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.[60] 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.[60]

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[61]. 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.[62]

For the future, ASD research and therapies are centered around microbial-mediated therapies, including probiotic therapy, prebiotic supplementation, and FMT.[63] These therapies have growing potential in addressing ASD symptoms and GI issues that commonly supplement ASD.[62,63] FMT therapy is gaining substantial acceptance, although there are concerns on long term impacts, standardization and infection.[63] 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.[64]

Personalized dietary treatments, based on the gut-immune-endocrine-brain axis, have also been explored.[64] These methods consider dietary factors, immune responses, hormonal regulation, and brain function connections.[65] By screening for inflammatory responses to dietary proteins such as gluten and casein, health care providers can construct patient-specific dietary plans.[16] These diets can be used in tandem with prebiotic or probiotic supplements to restore a healthy gut microbial balance, thereby alleviating ASD symptoms.[65,66] 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.

References

1. Fattorusso A, Di Genova L, Dell'Isola GB, Mencaroni E, Esposito S. Autism Spectrum Disorders and the Gut Microbiota. *Nutrients*. 2019 Feb 28;11(3):521. Doi: 10.3390/nu11030521. PMID: 30823414; PMCID: PMC6471505.

2. Garcia-Gutierrez E, Narbad A, Rodríguez JM. Autism Spectrum Disorder Associated With Gut Microbiota at Immune, Metabolomic, and Neuroactive Level. *Front Neurosci.* 2020 Oct 8;14:578666. doi: 10.3389/fnins.2020.578666. PMID: 33117122; PMCID: PMC7578228.

3. Osadchiy V, Martin CR, Mayer EA. The Gut-Brain Axis and the Microbiome: Mechanisms and Clinical Implications. *Clin Gastroenterol Hepatol.* 2019 Jan;17(2):322332. doi: 10.1016/j.cgh.2018.10.002. Epub 2018 Oct 4. PMID: 30292888; PMCID: PMC6999848.

4. Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, Codagnone MG, Cussotto S, Fulling C, Golubeva AV, Guzzetta KE, Jaggar M, LongSmith CM, Lyte JM, Martin JA, Molinero-Perez A, Moloney G, Morelli E, Morillas E, O'Connor R, Cruz-Pereira JS, Peterson VL, Rea K, Ritz NL, Sherwin E, Spichak S, Teichman EM, van de Wouw M, Ventura-Silva AP, Wallace-Fitzsimons SE, Hyland N, Clarke G, Dinan TG. The Microbiota-Gut-Brain Axis. *Physiol Rev.* 2019 Oct 1;99(4):1877-2013. doi: 10.1152/physrev.00018.2018. PMID: 31460832.

5. Shenoy MD, Indla V, Reddy H. Comprehensive Management of Autism: Current Evidence. *Indian J Psychol Med.* 2017 Nov-Dec;39(6):727-731. Doi: 10.4103/IJPSYM.IJPSYM_272_17. PMID: 29284801; PMCID: PMC5733418.

6. Bull MJ, Plummer NT. Part 1: The Human Gut Microbiome in Health and Disease. *Integr Med (Encinitas).* 2014 Dec;13(6):17-22. PMID: 26770121; PMCID: PMC4566439.

7. Taniya MA, Chung HJ, Al Mamun A, Alam S, Aziz MA, Emon NU, Islam MM, Hong SS, Podder BR, Ara Mimi A, Aktar Suchi S, Xiao J. Role of Gut Microbiome in Autism Spectrum Disorder and Its Therapeutic Regulation. *Front Cell Infect Microbiol*. 2022 Jul 22;12:915701. doi: 10.3389/fcimb.2022.915701. PMID: 35937689; PMCID: PMC9355470.

8. Vancamelbeke M, Vermeire S. The intestinal barrier: a fundamental role in health and disease. *Expert Rev Gastroenterol Hepatol*. 2017 Sep;11(9):821-834. Doi: 10.1080/17474124.2017.1343143. Epub 2017 Jun 26. PMID: 28650209; PMCID: PMC6104804.

9. Oh D, Cheon KA. Alteration of Gut Microbiota in Autism Spectrum Disorder: An Overview. *Soa Chongsonyon Chongsin Uihak*. 2020 Jul 1;31(3):131-145. doi: 10.5765/jkacap.190039. PMID: 32665757; PMCID: PMC7350540.

10. Forlano R, Mullish BH, Roberts LA, Thursz MR, Manousou P. The Intestinal Barrier and Its Dysfunction in Patients with Metabolic Diseases and Non-Alcoholic Fatty Liver Disease. *Int J Mol Sci.* 2022 Jan 8;23(2):662. doi: 10.3390/ijms23020662. PMID: 35054847; PMCID: PMC8775587.

11. Mayer EA, Nance K, Chen S. The Gut-Brain Axis. *Annu Rev Med.* 2022 Jan 27;73:439453. doi: 10.1146/annurev-med-042320-014032. Epub 2021 Oct 20. PMID: 34669431.

12. Nitschke A, Deonandan R, Konkle AT. The link between autism spectrum disorder and gut microbiota: A scoping review. *Autism.* 2020 Aug;24(6):1328-1344. Doi: 10.1177/1362361320913364. Epub 2020 Apr 28. PMID: 32340474.

13. Al-Ayadhi L, Zayed N, Bhat RS, Moubayed NMS, Al-Muammar MN, El-Ansary A. The use of biomarkers associated with leaky gut as a diagnostic tool for early intervention in autism spectrum disorder: a systematic review. *Gut Pathog.* 2021 Sep 13;13(1):54. doi: 10.1186/s13099-021-00448-y. PMID: 34517895; PMCID: PMC8439029.

14. Zhao Y, Wang Y, Meng F, Chen X, Chang T, Huang H, He F, Zheng Y. Altered Gut Microbiota as Potential Biomarkers for Autism Spectrum Disorder in Early Childhood. *Neuroscience.* 2023 Jul 15;523:118-131. doi: 10.1016/j.neuroscience.2023.04.029. Epub 2023 Jun 3. PMID: 37271221.

15. Ding X, Xu Y, Zhang X, Zhang L, Duan G, Song C, Li Z, Yang Y, Wang Y, Wang X, Zhu C. Gut microbiota changes in patients with autism spectrum disorders. *J Psychiatr Res.* 2020 Oct;129:149-159. doi: 10.1016/j.jpsychires.2020.06.032. Epub 2020 Jul 18. PMID: 32912596.

16. Rutayisire E, Huang K, Liu Y, Tao F. The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: a systematic review. *BMC Gastroenterol.* 2016;16(1):86. Published 2016 Jul 30. doi:10.1186/s12876-016-0498-0

17. Al-Zalabani AH, Al-Jabree AH, Zeidan ZA. Is cesarean section delivery associated with autism spectrum disorder?. *Neurosciences (Riyadh).* 2019;24(1):11-15. doi:10.17712/nsj.2019.1.20180303

18. Dinan TG, Cryan JF. Gut instincts: microbiota as a key regulator of brain development, ageing and neurodegeneration. *J Physiol.* 2017;595(2):489-503. doi:10.1113/JP273106

19. Vuong HE, Pronovost GN, Williams DW, et al. The maternal microbiome modulates fetal neurodevelopment in mice. *Nature*. 2020;586(7828):281-286. doi:10.1038/s41586020-2745-3

20. Vuong HE, Hsiao EY. Emerging Roles for the Gut Microbiome in Autism Spectrum Disorder. *Biol Psychiatry.* 2017;81(5):411-423. doi:10.1016/j.biopsych.2016.08.024

21. Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, Conlon MA. Elevated fecal short chain fatty acid and ammonia concentrations in children with autism spectrum disorder. *Dig Dis Sci.* 2012;57(8):2096-2102. doi:10.1007/s10620-012-2167-7

22. MacFabe DF, Rodríguez-Capote K, Hoffman JE, Franklin AE, MohammadAsef Y, Taylor AR, Boon F, Cain DP, Kavaliers M, Possmayer F, Ossenkopp K. A Novel Rodent Model of Autism: Intraventricular Infusions of Propionic Acid Increase Locomotor Activity and Induce Neuroinflammation and Oxidative Stress in Discrete Regions of Adult Rat Brain. *Am J Biochem Biotechnol.* 2008;4(2):146-166. doi:10.3844/ajbbsp.2008.146.166

23. Kirsten TB, Chaves-Kirsten GP, Bernardes S, et al. Lipopolysaccharide Exposure Induces Maternal Hypozincemia, and Prenatal Zinc Treatment Prevents Autistic-Like Behaviors and Disturbances in the Striatal Dopaminergic and mTOR Systems of Offspring. *PLoS One*. 2015;10(7):e0134565. Published 2015 Jul 28. doi:10.1371/journal.pone.0134565

24. Timofeeva OA, Roegge CS, Seidler FJ, Slotkin TA, Levin ED. Persistent cognitive alterations in rats after early postnatal exposure to low doses of the organophosphate pesticide, diazinon. *Neurotoxicol Teratol.* 2008;30(1):38-45. doi:10.1016/j.ntt.2007.10.002

25. Timofeeva OA, Sanders D, Seemann K, et al. Persistent behavioral alterations in rats neonatally exposed to low doses of the organophosphate pesticide, parathion. *Brain Res Bull.* 2008;77(6):404-411. doi:10.1016/j.brainresbull.2008.08.019

26. de Magistris L, Familiari V, Pascotto A, et al. Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. *J Pediatr Gastroenterol Nutr.* 2010;51(4):418-424. doi:10.1097/MPG.0b013e3181dcc4a5

27. Israelyan N, Gross Margolis K. Serotonin as a link between the gut-brain-microbiome axis in autism spectrum disorders. *Pharmacol Res.* 2019;140:115-120. doi:10.1016/j.phrs.2018.12.023

28. Zafeiriou DI, Ververi A, Vargiami E. The serotonergic system: its role in pathogenesis and early developmental treatment of autism. *Curr Neuropharmacol.* 2009;7(2):150-157. doi:10.2174/157015909788848848

29. Appleton J. The Gut-Brain Axis: Influence of Microbiota on Mood and Mental Health. *Integr Med (Encinitas).* 2018;17(4):28-32.

30. Goeden N, Velasquez J, Arnold KA, et al. Maternal Inflammation Disrupts Fetal Neurodevelopment via Increased Placental Output of Serotonin to the Fetal Brain. *J Neurosci.* 2016;36(22):6041-6049. doi:10.1523/JNEUROSCI.2534-15.2016

31. Dan Z, Mao X, Liu Q, et al. Altered gut microbial profile is associated with abnormal metabolism activity of Autism Spectrum Disorder. *Gut Microbes*. 2020;11(5):1246-1267. doi:10.1080/19490976.2020.1747329

32. Hollowood-Jones K, Adams JB, Coleman DM, et al. Altered metabolism of mothers of young children with Autism Spectrum Disorder: a case control study. *BMC Pediatr.* 2020;20:557. doi:10.1186/s12887-020-02437-7

33. Needham BD, Adame MD, Serena G, et al. Plasma and Fecal Metabolite Profiles in Autism Spectrum Disorder. *Biol Psychiatry*. 2021;89(5):451-462. doi:10.1016/j.biopsych.2020.09.025

34. Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol*. 2015;28(2):203-209. Accessed July 19, 2023. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4367209/

35. Foster JA, Neufeld KAM. Gut–brain axis: how the microbiome influences anxiety and depression. *Trends in Neurosciences*. 2013;36(5):305-312. doi:10.1016/j.tins.2013.01.005

36. Wang LW, Tancredi DJ, Thomas DW. The prevalence of gastrointestinal problems in children across the United States with autism spectrum disorders from families with multiple affected members. *J Dev Behav Pediatr*. 2011;32(5):351-360. doi:10.1097/DBP.0b013e31821bd06a

37. Saulnier DM, Ringel Y, Heyman MB, Foster JA, Bercik P, Shulman RJ, et al. The intestinal microbiome, probiotics and prebiotics in neurogastroenterology. *Gut Microbes*. 2012;4(1):17-27. doi:10.4161/gmic.22973

38. Schmidt K, Cowen PJ, Harmer CJ, Tzortzis G, Errington S, Burnet PWJ. Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. *Psychopharmacology (Berl).* 2015;232(10):1793-1801. doi:10.1007/s00213-014-3810-0

39. Tran N, Zhebrak M, Yacoub C, Pelletier J, Hawley D. The gut-brain relationship: Investigating the effect of multispecies probiotics on anxiety in a randomized placebocontrolled trial of healthy young adults. *Journal of Affective Disorders*. 2019;252:271277. doi:10.1016/j.jad.2019.04.043

40. Akkasheh G, Kashani-Poor Z, Tajabadi-Ebrahimi M, et al. Clinical and metabolic response to probiotic administration in patients with major depressive disorder: A randomized, double-blind, placebo-controlled trial. *Nutrition*. 2016;32(3):315-320. doi:10.1016/j.nut.2015.09.003

41. Reichelt KL, Knivsberg AM, Lind G, Nødland M. Probable etiology and possible treatment of childhood autism. *Brain Dysfunction*. 1991;4(6):308-319.

42. Baspinar B, Yardimci H. Gluten-Free Casein-Free Diet for Autism Spectrum Disorders: Can It Be Effective in Solving Behavioural and Gastrointestinal Problems? *Eurasian J Med.* 2020;52(3):292-297. doi:10.5152/eurasianjmed.2020.19230

43. Pennesi CM, Klein LC. Effectiveness of the gluten-free, casein-free diet for children diagnosed with autism spectrum disorder: Based on parental report. *Nutritional Neuroscience*. 2012;15(2):85-91. doi:10.1179/1476830512Y.0000000003

44. Knivsberg A, Reichelt KL, Nødland M, Høien T. Autistic Syndromes and Diet: a follow-up study. *Scand J Educ Res.* 1995;39(3):223-236. doi:10.1080/0031383950390304

45. Whiteley P, Haracopos D, Knivsberg AM, et al. The ScanBrit randomised, controlled, single-blind study of a gluten- and casein-free dietary intervention for children with autism spectrum disorders. *Nutr Neurosci*. 2010;13(2):87-100. doi:10.1179/147683010X12611460763922

46. Goday P. Whey Watchers and Wheat Watchers: The Case Against Gluten and Casein in Autism. *Nutrition in Clinical Practice*. 2008;23(6):581-582. doi:10.1177/0884533608326277

47. Li Y, Wang Y, Zhang T. Fecal Microbiota Transplantation in Autism Spectrum Disorder. *Neuropsychiatric Disease and Treatment*. 2022;18:2905-2915. doi:10.2147/NDT.S382571

48. Topping DL, Clifton PM. Short-Chain Fatty Acids and Human Colonic Function: Roles of Resistant Starch and Nonstarch Polysaccharides. *Physiol Rev.* 2001 Jul 1;81(3):1031-1064. doi: 10.1152/physrev.2001.81.3.1031.

49. Diether NE, Willing BP. Microbial Fermentation of Dietary Protein: An

Important Factor in Diet–Microbe–Host Interaction. *Microorganisms.* 2019;7(1):19. doi:10.3390/microorganisms7010019

50. Li N, Chen H, Cheng Y, et al. Fecal Microbiota Transplantation Relieves Gastrointestinal and Autism Symptoms by Improving the Gut Microbiota in an Open-Label Study. *Front Cell Infect Microbiol.* 2021;11:759435. Accessed July 25, 2023. https://www.frontiersin.org/articles/10.3389/fcimb.2021.759435

51. Nirmalkar K, Qureshi F, Kang DW, Hahn J, Adams JB, Krajmalnik-Brown R. Shotgun Metagenomics Study Suggests Alteration in Sulfur Metabolism and Oxidative Stress in Children with Autism and Improvement after Microbiota Transfer Therapy. *Int J Mol Sci.* 2022;23(21):13481. doi:10.3390/ijms232113481.

52. Kang DW, Adams JB, Gregory AC, et al. Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome*. 2017;5(1):10. doi:10.1186/s40168-016-0225-7

53. Wang JW, Kuo CH, Kuo FC, Wang YK, Hsu WH, Yu FJ, Hu HM, Hsu PI, Wang JY, Wu DC. Fecal microbiota transplantation: Review and update. *J Formos Med Assoc*. 2019 Mar;118 Suppl 1:S23-S31. doi: 10.1016/j.jfma.2018.08.011. Epub 2018 Sep 1. PMID: 30181015.

54. Antushevich H. Fecal microbiota transplantation in disease therapy. *Clin Chim Acta.* 2020 Apr;503:90-98. doi: 10.1016/j.cca.2019.12.010. Epub 2020 Jan 20. PMID: 31968211.

55. Li N, Tian H. [Current research progress and thinking of fecal microbiota transplantation for the treatment of gastrointestinal disorders]. *Zhonghua Wei Chang Wai Ke Za Zhi.* 2017 Oct 25;20(10):1104-1108. Chinese. PMID: 29130220.

56. Generoso JS, Giridharan VV, Lee J, Macedo D, Barichello T. The role of the microbiotagut-brain axis in neuropsychiatric disorders. *Braz J Psychiatry.* 2021 May-Jun;43(3):293305. doi: 10.1590/1516-4446-2020-0987. PMID: 32667590; PMCID: PMC8136391.

57. Qureshi F, Adams J, Hanagan K, Kang DW, Krajmalnik-Brown R, Hahn J. Multivariate Analysis of Fecal Metabolites from Children with Autism Spectrum Disorder and Gastrointestinal Symptoms before and after Microbiota Transfer Therapy. *J Pers Med.* 2020 Oct 2;10(4):152. doi: 10.3390/jpm10040152. PMID: 33023268; PMCID: PMC7712156.

58. Kang DW, Adams JB, Coleman DM, Pollard EL, Maldonado J, McDonough-Means S, Caporaso JG, Krajmalnik-Brown R. Long-term benefit of Microbiota Transfer Therapy on autism symptoms and gut microbiota. *Sci Rep.* 2019 Apr 9;9(1):5821. doi: 10.1038/s41598-019-42183-0. PMID: 30967657; PMCID: PMC6456593.

59. Arizona State University. Microbiota Transfer Therapy for Adults With Autism Spectrum Disorder (ASD) Who Have Gastrointestinal Disorders (MTT-ASD). ClinicalTrials.gov Identifier: NCT03408886. Last Update Posted January 9, 2023. Accessed July 15, 2023. Available at:

https://clinicaltrials.gov/ct2/show/NCT03408886

60. Jung Lee W, Lattimer LD, Stephen S, Borum ML, Doman DB. Fecal Microbiota Transplantation: A Review of Emerging Indications Beyond Relapsing Clostridium difficile Toxin Colitis. *Gastroenterol Hepatol* (N Y). 2015 Jan;11(1):24-32. PMID: 27099570; PMCID: PMC4836576.

61. Hughes HK, Rose D, Ashwood P. The Gut Microbiota and Dysbiosis in Autism Spectrum Disorders. *Curr Neurol Neurosci Rep*. 2018 Sep 24;18(11):81. doi: 10.1007/s11910-018-0887-6. PMID: 30251184; PMCID: PMC6855251.

62. Durack J, Lynch SV. The gut microbiome: Relationships with disease and opportunities for therapy. J Exp Med. 2019 Jan 7;216(1):20-40. doi: 10.1084/jem.20180448. *Epub* 2018 Oct 15. PMID: 30322864; PMCID: PMC6314516.

63. Żebrowska P, Łaczmańska I, Łaczmański Ł. Future Directions in Reducing Gastrointestinal Disorders in Children With ASD Using Fecal Microbiota Transplantation. *Front Cell Infect Microbiol*. 2021 Feb 26;11:630052. doi: 10.3389/fcimb.2021.630052. PMID: 33718277; PMCID: PMC7952982.

64. Gupta S, Allen-Vercoe E, Petrof EO. Fecal microbiota transplantation: in perspective. *Therap Adv Gastroenterol*. 2016 Mar;9(2):229-39. doi: 10.1177/1756283X15607414. PMID: 26929784; PMCID: PMC4749851.

65. Doenyas C. Novel Personalized Dietary Treatment for Autism Based on the Gut Immune-Endocrine-Brain Axis. *Front Endocrinol (Lausanne).* 2019 Aug 13;10:508. doi: 10.3389/fendo.2019.00508. PMID: 31456745; PMCID: PMC6700238.

66. Abdellatif B, McVeigh C, Bendriss G, Chaari A. The Promising Role of Probiotics in Managing the Altered Gut in Autism Spectrum Disorders. *Int J Mol Sci.* 2020 Jun 10;21(11):4159. doi: 10.3390/ijms21114159. PMID: 32532137; PMCID: PMC7312735.