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Intestinal Flora Composition and Exploration of New Therapeutic Methods in Children with Autism

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Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social communication impairments, narrow range of interests or activities, and repetitive behaviors. Early diagnosis and early intervention can achieve better prognostic effects. Many studies have shown that the occurrence and development of ASD is closely related to intestinal flora disorder, which leads to an extremely increased probability of gastrointestinal symptoms in autism children. In addition, intestinal flora disorder in early life may pose a certain threat for infants, including early colonization of microorganisms and early antibiotic administration. Meanwhile, intestinal flora disorder in autistic children also change short-chain fatty acids, amino acids, neurotransmitters in blood. This review focuses on the above points and the possible new methods for the treatment of ASD, expecting to provide new ideas for the treatment of autistic children in addition to behavioral intervention.

Keywords: *Autism Spectrum Disorder, Intestinal Flora, Gut-Brain Axis–Microecology, Intestinal Microbiota Metabolites, Treatment*

Autism spectrum disorder (ASD) is defined as a neurodevelopmental disorder and characterized by social communication impairments, a narrow range of interests or activities and repetitive behaviors (Siu et al. 2016). In recent years, the prevalence of ASD has been increasing in China and other countries. According to the latest data from the U.S. Centers for Disease Control and Prevention (CDC) in 2021, the prevalence of autism among 8-year-old is 23.0%. Recent a study has shown that the prevalence of ASD is about 1% in China(Sun et al. 2019).However, a large-scale epidemiological investigation of ASD was not held in China. The urban sample is locally representative but not

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yet nationally representative of China, which brings great difficulties to the statistics of ASD. There is a concern that the incidence rate is not the same between boys and girls, with boys significantly higher than girls, about 4.2 times(Zhou et al. 2020;Maenner et al. 2021).At present, the etiology and pathogenesis of the disease are not clear, early screening, early diagnosis, early intervention can significantly improve the prognosis of children(Sheldrick et al. 2022;Whitehouse et al. 2021).Studies have shown that intestinal flora regulates the metabolic process of nerves and endocrinology by participating in human brain-gut axis, and ultimately affects human health(Fairbrass et al. 2021).Intestinal flora and its metabolites change, affecting the brain through the nervous and endocrine systems, leading to gastrointestinal symptoms in autistic children , and will aggravate their anxiety and depression symptoms(Kurokawa et al. 2021).In addition, the transfer of maternal intestinal flora to the fetus and the use of antibiotics in early infancy are also considered to be the causes of ASD. This review focuses on the above points and the possible new methods for the treatment of ASD, expecting to provide new ideas for the treatment of autism children in addition to behavioral intervention.

1.STUDIES ON INTESTINAL FLORA AND ITS METABOLITES

1.1Research status of intestinal flora

With the deepening of the research on the intestinal flora, it was discovered that intestinal flora plays a quite major role in ASD. Although the pathogenesis is unclear, studies have demonstrated abnormalities in one or some types of intestinal flora in children with ASD.(Iglesias-Vázquez et al. 2020).Treatment to correct the disordered intestinal flora can reduce gastrointestinal symptoms in children with ASD, thereby reducing anxiety and restlessness(Zhang et al. 2022;Stewart Campbell et al. 2022).In the present stage, clinical work may start from this aspect and adjust the intestinal microbial structure of children with ASD and maintain the homeostasis of the flora for achieving the purpose of treatment.There is a great deal of research on the intestinal flora of autism in China and other countries. A study conducted a high-throughput sequencing analysis of V3-V4 region of 16S rRNA on fecal intestinal flora of children aged 2-4 years old with ASD and children in the control group, and the results showed that bacteroides and Proteobacteria were significantly increased in the ASD group, while Actinomycetaceae, Coriobacteriaceae, Bifidobacteriaceae, Gemellaceae, Streptococcaceae were clearly decreased(Coretti et al. 2019).Bifidobacterium is a vital probiotic, which has protective and nutritional effects on the human body. Bifidobacterium was significantly reduced in most children with ASD and correlated with the severity of ASD. Intestinal flora disorder is associated with severity of ASD (Golubeva et al. 2017; Ding et al. 2020). Gamma-aminobutyric acid (GABA) was altered in children with ASD, which was attributed to changes in bifidobacterium. Bifidobacterium is known to be a cardinal producer of GABA, a neurotransmitter that plays a key role in regulating nerve activation and voltage conductions(Abujamel et al. 2022).A few studies have shown elevated levels of bifidobacteria in children with ASD(Dan et al. 2020).The Chinese study conducted 16SrRNA sequencing on the intestinal flora of 48 normal children and 48 autistic children, and results showed that the intestinal flora of Prevotella, Bacteroides, Lachnospiracea_incertae_sedis, and Megamonas increase in children with ASD(Zou et al. 2020).Wang and some researchers performed 16SrRNA sequencing on stool samples from 45 children with ASD and 45 healthy children. The relative abundance of genera Lachnoclostridium, Tyzzerella subgroup 4, Flavonifractor, and unidentified Lachnospiraceae in children with ASD decreased, but clostridium increased significantly. Studies have shown that this genus is elevated in almost all studies of ASD intestinal flora(Wang et al. 2019).A study showed that Clostridium, Lactococcus, Aspergillus, Doria, and Leukopleria were part of a more stable microbiome in children with autism(Berding et al. 2019).Intestinal microbiota disorder has been confirmed in children with ASD, indicating that the intestinal microbiota is not balanced(Sanctuary et al. 2019).Intestinal flora disorder may exacerbate behavioral symptoms in children with ASD(Roussin et al. 2020).The gut microbiota varies from individual to individual and often contradicts each other, and today there is no single

microbiome composition(Fattorusso et al. 2019).Interestingly, a trial examining whether geographic location causes intestinal microbiome inconsistencies in children with ASD showed a stronger association when cross-sectional comparisons were made between cohorts of children with ASD in Arizona and Colorado(Fouquier et al. 2021).In addition, a study sequenced the stools of children with ASD and showed that it was the narrow food choices of children with ASD that led to the altered intestinal flora(Yap et al. 2021). Exploring whether there is a correlation between intestinal flora and ASD, future generations still need to continue in-depth and a large number of studies.

1.2Changes of intestinal flora and metabolites

Many diseases often have unique metabolic characteristics or specific biomarkers that can assist in disease diagnosis, predict disease course and guide treatment strategies. Autism spectrum disorders, by contrast, are based on a physician assessment of behavioral symptoms and diagnostic scales. There is currently no specific biomarker that can assist to diagnose ASD.

1.2.1 Short chain fatty acids (SCFA)

Short chain fatty acids (SCFA) are organic fatty acids with carbon atoms. It is mainly produced by gut microbes and plays an active role in maintaining energy and homeostasis (Lobzhanidze et al. 2019). SCFA can preserve the shape and function of intestinal epithelial cells. The increase and decrease of short-chain fatty acids in children with ASD are not clear. However, it is confirmed that the composition of intestinal flora and its metabolite SCFA in autism children have changed to some extent(Tran＆Mohajeri, 2021)Wang and colleagues found that fecal concentrations of other short-chain fatty acids, except caproic acid, were significantly higher in children with ASD compared with the control group(Wang et al.2012).The result showed that fecal acetic acid was low, but butyrate levels also decreased in the feces of ASD subjects, while valeric acid levels were elevated(Liu et al. 2018)Propionic acid (PPA) has some benefits for body, but it is also linked to some diseases such as ASD(Frye et al. 2017).Studies have demonstrated that injection of PAA or SCFA in the ventricles/lateral ventricles of the mouse brain causes abnormal motor and cognitive deficits.(Mepham et al. 2021).Intraperitoneal injection of propionic acid in rats also caused these changes(Shams et al. 2019).The studies suggest that propionic acid administration may cause ASD behaviors, including behavioral changes and cognitive deficits(Choi et al. 2018).Valproic acid (VPA) rat models of autism have been indicated that VPA rat intestinal flora composition is similar to that of human autism(Doroszkiewicz et al. 2021).Children whose mothers were exposed to valproic acid (VPA) had a significantly increased risk of ASD(Kuo et al. 2022).

1.2.2 Amino acids

ASD is associated with abnormal metabolism of some amino acids, and the disorder of amino acid metabolism may be one of the causes of ASD. The disturbance of intestinal flora can affect amino acids in blood and feces, such as glutamate, tryptophan, glycine, branched amino acids (BCAAs, including leucine, isoleucine,valine).

Glutamic acid is an acidic amino acid. It is a vital brain energy substance, which has the function of nourishing the brain.Due to the brain can only use glutamic acid for energy. In addition, the effect of glutamic acid on epilepsy is quite good(Zaitsev et al. 2020).Plasma glutamate/glutamine ratio was significantly higher in autism children than in the control group(Yu et al. 2020).High serum glutamic acid level in autistic children is helpful for the early diagnosis of ASD(Khalifa et al. 2018).In almost all studies, plasma glutamic acid levels were higher in autism children than in controls. Impaired glutamatergic pathways are also potential therapeutic targets for ASD (Nisar et al. 2022).Not only

do autistic children have increased levels of glutamic acid in their plasma, but also their siblings and parents(Aldred et al. 2003).Glutamic acid is an excitatory neurotransmitter that it is concentrated in the brain and usually has neuroprotective and cognitive functions. However, glutamic acid excess may produce neurotoxicity, which may play a role in the pathogenesis of ASD (Fatemi et al. 2008). Most studies have shown ascended, there is no definitive conclusion whether glutamic acid is elevated or decreased in ASD. Studies on glutamic acid levels are contradictory and inconsistent, and many trials are still needed to support this conclusion.

Tryptophan is one of the essential amino acids that cannot be synthesized in the body and supplied by the diet. It plays a vital role in the body and is a precursor for the serotonin synthesis(Kuo＆Liu, 2022)Serotonin is strongly associated with autism. Intestinal flora can directly or indirectly affect tryptophan, and the severity of symptoms of autism may be related to the consumption of tryptophan to a certain extent (Roth et al. 2021). Tryptophan is affected by pathological conditions to some extent. Currently, tryptophan and its metabolites are considered as biomarkers of ASD, which has certain scientific basis. From a therapeutic point of view, it is possible to regulate tryptophan metabolism in the gut (Agus et al. 2018).Cascio and colleagues showed that 17.5% patients had mutations in the LAT gene encoding that may affect the passage of tryptophan across the blood-brain barrier(Cascio et al. 2019).Huang showed that compared with the control group, the intestinal flora of children with ASD was deficient in tryptophan, phenylalanine, tyrosine(Huang et al. 2021).

Glycine, also known as amino-acetic acid, is a non-essential amino acid. It can synthesize endogenous oxidant glutathione and is one of the crucial amino acids of glutathione. Glycinine plays an inhibitory neurotransmitter in the central nervous system. In the early development stage, due to the relatively high concentration of chloride ions in cells, glycinine can act as an excitatory neurotransmitter, transforming into an inhibitory neurotransmitter immediately after birth. If the transformation is not timely, it may lead to the incidence of ASD (Ito, 2016) .Gly level was significantly lower than that of the control group(Yu et al. 2021).

Branched Chain Amino acids(BACC) mainly consist of leucine, isoleucine,valine. Fatigue is alleviated by lowering serotonin levels, and the branched-chain amino acid indirectly alleviates fatigue by reducing serotonin production in the brain.Most autistic children have a decreased BCCA(Tu et al. 2012).Studies have shown that one of the causes of ASD is the abnormal catabolic pathway of BCAA. Lack of expression of SLC7A5, an amino acid transporter at the BBB, resulted in significantly reduced levels of branched-chain amino acids, particularly leucine and isoleucine(Tărlungeanu et al. 2016).

1.2.3 The neurotransmitter

Serotonin(5-HT) is an indole derivative and an inhibitory neurotransmitter. Serotonin has associated to autism spectrum disorders. About one-third of ASD patients had higher 5-HT blood level (Chen et al. 2017). In addition, the level of 5-HT in blood is tightly related to the neural development of ASD offspring (Montgomery et al. 2018). A lot of studies have shown that 5-HT is the first biomarker found in autism spectrum disorders. It was confirmed and first proposed 50 years ago that 5-HT levels were rose in the blood of autistic children(SCHAIN & FREEDMAN,1961)Tanaka results add to the reliability of 5-HT levels as biomarkers for ASD(Tanaka et al. 2018).

Gamma-aminobutyric acid(GABA)is an inhibitory neurotransmitter in the central nervous system. Cortical thickness and GABA receptor density are considered to be the neurobiological basis of autism. A study has shown that GABA is positively correlated in the parietal cortex, but negatively in the frontal and occipital cortex regions of the brain (James et al. 2022). Genetic evidence suggests that several mutations in genes associated with the GABA system are

associated with the pathogenesis of autism, with abnormal excitatory/inhibitory (E/I) neurotransmission ratio(Zhao et al. 2022).

2.PATHOGENESIS AND ETIOLOGY OF INTESTINAL FLORA

2.1 Brain-gut-microbiome (BGM)

In recent years, it has been considered that intestinal flora plays a very key role in the occurrence and development of ASD. At present, scientists have conducted plenty of studies on the association between ASD and intestinal flora. These studies indicate that intestinal microbiome disturbances are closely related to ASD. There are hundreds of bacteria in the human gut, including E. coli, bifidobacteria, lactobacillus and more. Intestinal flora constitute a protective barrier of the gut, maintain the human body's homeostasis, and communicate with the brain through signal molecules derived from the gut flora and immune mediators(Chernikova et al. 2021).This system of gut microbiota interacting with the brain is called the brain-gut-microbiome.Due to the bidirectional relationship between the central nervous system and the gastrointestinal tract (gut-brain axis), changes in intestinal bacterial metabolites and neurotransmitters occur in autistic children. This affects the brain through the neuroimmune, neuroendocrine, and autonomic nervous systems, which contribute to the development of ASD. In addition, changes in gut microbes can break down the protective barrier of the intestinal wall, possibly leading to inflammation. This change releases a great deal of inflammatory factors, such as 5-HT acting on the central nervous system, affecting brain functions, emotional abnormalities(Garcia-Gutierrez et al. 2020).Although it is not possible to specifically analyze the composition of the gut flora of children with autism, it is clear that the gut flora of children with autism is altered compared to the control group(Wan et al. 2021).A study has shown that Lactobacillus reuteri ameliorates social dysfunction in ASD, and the data in mice support this view(Sgritta et al. 2019).A deeper understanding of the brain-gut-microbiome will help us to understand the occurrence and development of ASD. In addition, there is growing evidence that intestinal microbiota is an important regulator of gut-brain communication, and the transplantation of intestinal microbiota in children with ASD into experimental mice can induce autism behavior (Sharon et al. 2019). The number of bacteria in the human body is dozens of times that of somatic cells and germ cells, indicating that intestinal flora homeostasis is critical in human health and disease (Pulikkan et al. 2019). To understand the mechanism of brain-gut-axis, we can have a deeper understanding of the pathogenesis of ASD and find out its pathogenic factors to develop relevant specific drugs.

2.2 *Associated risk factors*

2.2.1 Transfer of maternal intestinal microorganisms

The brain is susceptible to environmental stress, and prenatal and early life are believed to be key to the development of ASD. Changes in the maternal gut microbiota increase the risk of ASD in offspring. These disturbed gut microbiotas are transferred to the fetus through vertical transmission, and a number of bacteria can be detected in the placenta, amniotic fluid, and umbilical cord blood (O' Mahony et al. 2015). In mammals, maternal microbes can be transmitted to offspring through natural birth, skin-to-skin contact, and breastfeeding. The similarity of intestinal microbiome structure between autism children and their mothers was higher than that between normal children and their mothers. In addition, specific gut bacteria shared between children with autism and their mothers have been linked to neurodevelopmental and social deficits with autism (Chen et al. 2020). The study by Li and colleagues confirms that changes in the mother's gut microbiota may increase the risk of autism in children. It is important to analyze and compare the intestinal microbiota of mother and child for early assessment of ASD risk, prevention and personalized

treatment of ASD (Li et al. 2019). Getting adequate folic acid and vitamins during pregnancy, reducing high-fat diets and avoiding prenatal infections can greatly reduce the risk of ASD (Sivamaruthi et al. 2020;Hall et al. 2022).

2.2.2 Effects of early antibiotic use on ASD

It has been reported that children with ASD receive more antibiotics than their peers, and oral antibiotics in early childhood may lead to long-term disruption of intestinal flora, resulting in gastrointestinal symptoms. This may explain the prevalence of gastrointestinal symptoms in autism children (Vargason et al. 2019).Maternal use of various antibiotics during pregnancy was also a potential risk factor for ASD, increasing the risk by about 10 percent (Holingue et al. 2020;Hamad et al. 2019).However, Siob Elise and colleagues found that early antibiotic use was associated with an increased risk of ADHD and autism spectrum disorder in twin studies in two countries. But after controlling for family environment and genetic factors, the link was significantly reduced (Slob et al. 2021).

3.CHANGES IN INTESTINAL FLORA AND GASTROINTESTINAL SYMPTOMS

Gastrointestinal symptoms are common in addition to the core symptoms of ASD, such as problems with social communication, limited interests, and repetitive behaviors (Stewart Campbell et al. 2022). Half or more of autism children reported gastrointestinal symptoms, about three times as many as normal children (Restrepo et al. 2020).This is closely related to the intestinal flora. The disorder of intestinal flora may be an important cause of the core symptoms of children with gastrointestinal symptoms, and affect the gut-brain axis-microecology (Liu et al. 2022). Many studies have shown that ASD is characterized by increased intestinal permeability and abnormal intestinal immune function. Compared with children with ASD without gastrointestinal symptoms, children with gastrointestinal symptoms had more behavioral and emotional problems, and the severity of gastrointestinal symptoms was closely related to the severity of ASD symptoms (Kittana et al. 2021). A study of 140-170 children with ASD showed that 24-63% of autism children had at least one gastrointestinal symptom. Another study of 50 children with ASD, 50 controls, and 50 children with other developmental disabilities showed that more than two-thirds of children with ASD had gastrointestinal symptoms (Ristori et al. 2019).Constipation is the most common gastrointestinal symptom, and obsessive-compulsive behavior is strongly associated with it (Marler et al. 2017;Peters et al. 2014).In addition, children with gastrointestinal symptoms also show more behavioral deficits, such as irritability, aggressive behavior, self-harm, hyperactivity, and sleep disturbances (Marler et al. 2020).The majority of children with ASD and gastrointestinal symptoms still do not receive appropriate treatment. Doctors and parents should take this problem seriously so that improving the symptoms may improve their quality of life and reduce ASD behavior.

4.NEW TREATMENTS FOR INTESTINAL FLORA

4.1 Probiotics

Probiotics have been widely used in recent years as a treatment tool for gastrointestinal symptoms in patients with ASD. Because they help restore intestinal mucosal barrier function, reduce inflammation, and improve some behavioral symptoms associated with ASD(Davies et al. 2021).Probiotics treatment positively regulated intestinal microflora and its metabolic activity in children with autism(Duque et al. 2021).It can regulate changes in immune cells, cytokines, emotional behavior, which has potential therapeutic effects. A foreign team used the probiotic mixture

developed by the team on the ASD mouse model after valproic acid treatment. They was pleasantly surprised to find that the probiotic could improve the behavioral symptoms of ASD mice (Mintál et al. 2022).But Eugene proposed a different view, showing that the administration of probiotics did not significantly change the composition and diversity of microbial communities in feces(Arnold et al. 2018).Although different research teams have proposed different views on the effects of probiotics, the combination of bovine colostrum and probiotics can alleviate some chronic gastrointestinal symptoms, which cannot be achieved by some interventions (Sanctuary et al. 2019).Compared with normal children, children with ASD had more clostridium and fewer bifidobacteria in their microbiota, and prebiotic galactooligosaccharide (B-GOS) administration significantly increased bifidobacteria populations(Grimaldi et al. 2017).Compared with other treatments, probiotics generally improved gastrointestinal problems in autism children and significantly improved prognosis.

4.2 Fecal microbiota transplantation(FMT)

The treatment is a novel attempt to treat digestive symptoms in autistic children. This emerging treatment has received little coverage. However, many researchers are interested in the treatment because it can relieve gastrointestinal symptoms and because the new treatment can alleviate behavioral symptoms of autism (Żebrowska et al. 2021). The method can help restore intestinal flora homeostasis in children with GI symptoms. It has the advantage of allowing complete exchange of gut microbes compared to antibiotic or probiotic therapies（Gupta et al. 2020). Kang used microbiome transfer therapy to change the gastrointestinal ecosystem. After treatment, gastrointestinal symptoms were reduced by about 80% .ASD behavioral symptoms were significantly improved (Kang et al. 2017). But this approach is still controversial. There is no denying the remarkable results of this therapy. FMT may become a useful tool for treating cognitive deficits in ASD in the future (Goo et al. 2020).

4.3 Diet therapy

One of the main clinical symptoms of ASD is rigid behavior. Abnormal rigid behavior will lead to a single type of food for children with ASD, thus forming special changes in intestinal flora of ASD. By regulating intestinal microbiota through dietary management, this therapy can reduce the incidence of ASD to some extent and relieve associated clinical symptoms. For example, a gluten-free/casein-free diet (GFCF) can improve gastrointestinal and related behavioral symptoms, while dietary therapy is a cheap and easy way to improve autism-related symptoms(Yu et al. 2022).When gluten and casein are digested by the gastrointestinal tract, substances called opioids can cross the intestinal mucosa, penetrate the blood-brain barrier, reach the central nervous system, and ultimately lead to autism(Jarmołowska et al. 2019).Another study showed that providing a GFCF diet to children and adolescents with ASD did not seem to be effective. In contrast, a GFCF diet may cause gastrointestinal effects and increase the gastrointestinal burden (Keller et al. 2021).Now, behavioral intervention is the main means of rehabilitation for children with ASD, and there is no specific drug. Dietary therapy brings hope for relieving gastrointestinal symptoms in children with ASD. However, the effect of dietary therapy is mixed at present, and the application of dietary therapy still needs a large number of clinical trials.

5.DISCUSSION

In summary, due to the complex pathogenesis of ASD, no unified conclusion has been reached as to which factor is the cause, and no relevant system can be formed. A large number of studies should continuously demonstrate the relationship between ASD and intestinal flora, and the mechanism of brain-gut-microbiome should still be explored in the first place. Secondly, some factors are also considered to be indispensable pathogenic causes, such as genetic factors, neurological development of the brain, living environment factors, birth asphyxia, drugs. Nowadays, intestinal flora disorder in autism children has been confirmed, which specific flora disorder remains to be verified by future generations. Only with a deeper understanding of the relationship between intestinal flora disorder and ASD can more targeted treatment methods besides intervention training be found. Alleviating gastrointestinal symptoms of children with ASD and seeking better quality of life for them has become a problem to be solved. Probiotics are currently in clinical trials and have shown good results. Other therapies, such as fecal bacteria transplantation (FMT) and dietary therapy, are expected to carry out clinical trials as soon as possible to bring good news to the treatment and prognosis of autism children.

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