
بسم الله الرحمن الرحیم

The Gut Microbiota and Autism Spectrum Disorder

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Autism

- Autism spectrum disorder (ASD) constitutes a group of brain developmental disorders, and it is defined by stereotyped behavior and deficits in communication and social interaction.

- Features of these disorders include: social deficits, communication difficulties, stereotyped or repetitive behaviors and interests, and in some cases, cognitive delays , seizures, anxiety, sleep deficiency, and metabolic impairments.

- In 2012, the estimated prevalence of ASD was 14.6 per 1,000 children aged 8 years, and the prevalence was significantly higher in boys (23.6 per 1,000) than that in girls (5.3 per 1,000).

- The heritability of ASD and autistic disorder was approximately 50% among Swedish children, suggesting that both genetic and environmental factors play important roles in the development of ASD.

- The cost of caring for a child with ASD but without an intellectual disability is £0.92 million in the United Kingdom and $1.4 million in the United States.

- Accumulating evidence demonstrates that gastrointestinal (GI) symptoms, such as abdominal pain, gaseousness, diarrhea, constipation are a common in patients with ASD.
- The prevalence of GI symptoms ranges from 23 to 70% in children with ASD .

The findings suggest that the gut plays an important role in the etiology of ASD. The gut consists of millions of microbiota, and hypothesize that the microbiota and its metabolites might be involved in the pathophysiology of ASD.

GUT MICROBIOTA

- The human gut consists of approximately 1 kg of bacteria, and the number of bacterial genes are in the gut is approximately about 9.9 million. The ratio of host DNA vs. microbiome DNA is 1:10
- Most bacterial species colonising the human GIT belong to the phyla Firmicutes and Bacteroidetes, while species of the phyla Actinobacteria, Proteobacteria and Verrucomicrobia exist in lower numbers .
- The number of bacterial species within the human intestinal microbiota has often been estimated to be in the range of 500 to over 1000 species .

- infant gut is colonized by the microbiome of the maternal vagina, anus and skin during delivery and by the environmental bacteria to which the neonate is exposed during the postpartum period.
- the first meconium of mice is not sterile, indicating that the microbiome colonizes the infant gut prior to parturition .

- Maternal factors, such as maternal diet and delivery mode, and postnatal factors, including antibiotics, breast-feeding, diet and host genetics, structure the neonatal microbiome in humans and animal models.
- Many studies have shown that a maternal high fat diet during pregnancy decreases the level of Bacteroides in human neonates and diminishes the abundance of nonpathogenic Campylobacter in primates .
- maternal obesity during pregnancy and gestational diabetes alter the gut microbiota and might be associated with ASD in humans .

- The birth mode and antibiotics also shape the gut microbiota .
- The gut microbiota of infants who were delivered vaginally resembles their mother's vaginal microbiota, which is dominated by Lactobacillus, Prevotella, or Sneathia spp., and the gut microbiota of babies who were born by Cesarean section is similar to their mother's skin microbiota, which is dominated by Staphylococcus, Corynebacterium, and Propionibacterium spp.

- composition of the microbiota of children who were treated with antibiotics during the first 3 years of life is less diverse in terms of both bacterial species and strains.

- Formula-fed infants present an increased species of Clostridium difficile compared with breast-fed infants . Breast-feeding for more than 6 months is associated with a lower risk of developing ASD .

- gut microbiota is associated with several disorders in children, such as abnormal behaviors, Crohn’s disease, obesity and inflammatory bowel disease (IBD) .

The gut microbiota plays important roles in human physiology and pathology.

RELATIONSHIP BETWEEN ASD AND GUT MICROBIOTA

- Gastrointestinal (GI) symptoms are prominent in ASD individuals. Wang et al., found more GI syndromes, including constipation (20%) and diarrhea (19%), in children with ASD.
- Patients with ASD who present GI symptoms display significant behavioral manifestations, such as anxiety, self-injury and aggression.

- A higher percentage of abnormal intestinal permeability was observed in 36.7% of patients with ASD compared with control children (4.8%).
- An increased intestinal permeability results in a higher antigenic load from the gastrointestinal tract. Lymphocytes and ASD-associated cytokines, such as interleukin-1b (IL-1b), IL-6, interferon-g (IFN-g), and tumor necrosis factor-a (TNF-a), are present in the circulation and cross the blood-brain barrier (BBB).
- Subsequently, IL-1b and TNF-a bind to brain endothelial cells and induce immune responses in the brain.

- Alterations in the composition of the gut microbiota and their metabolic products are commonly observed in patients with ASD and in animal models of ASD.
- observed gastrointestinal barrier defects and microbiota alterations in a mouse model displaying features of ASD. They found that bacteria belonging to Porphyromonadaceae, Prevotellaceae, unclassified Bacteroidales, and Lachnospiraceae.

- anti-epileptic drug valproic acid (VPA), when used by the mother during pregnancy, induces autistic-like social behaviors in the offspring accompanied by alterations in Bacteroidetes and Firmicutes .
- Compared with the gut microbiota of children without ASD, the gut microbiota of children with ASD is less diverse and exhibits lower levels of Bifidobacterium and Firmicutes and higher levels of Clostridium , Desulfovibrio, Caloramator and Sarcina.

- Children with autism who present GI symptoms have lower abundances of the genera Prevotella, Coprococcus, and unclassified Veillonellaceae.

- Fecal samples from children with ASD also have higher levels of the Clostridium histolyticum group (Clostridium clusters II and I) compared with samples from healthy children .

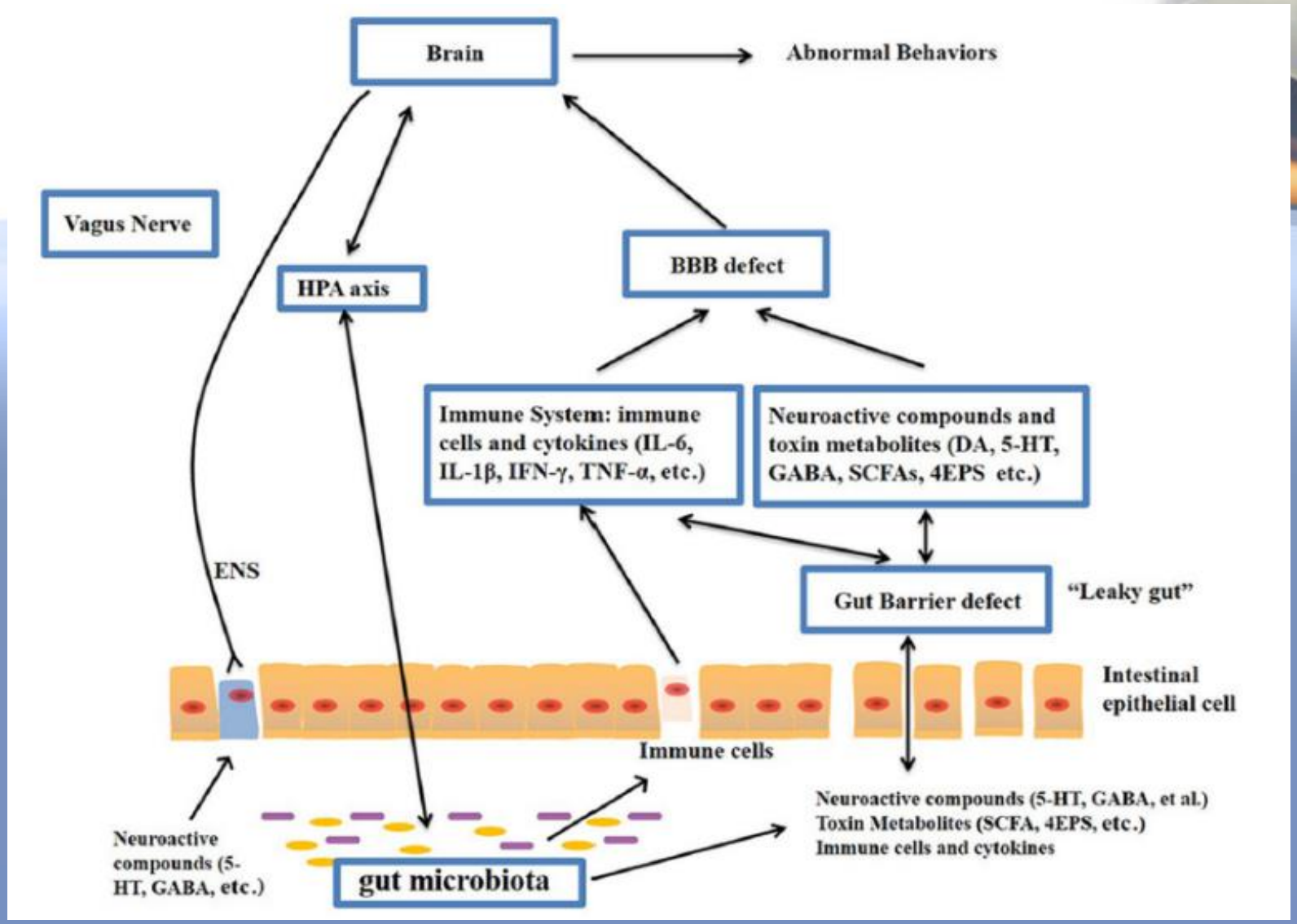
- The reduction of Clostridium yields significant improvements in children with ASD.
- children with ASD present alterations in their levels of Bifidobacterium, Prevotella, and Sutterella.
- Infants who were delivered by Cesarean section (CS) are at higher risk of developing ASD (odds ratio of 1.23) than infants delivered vaginally.
- Children with ASD have a history of using significantly more antibiotics.

early life events that can alter the composition of the microbial community, such as delivery mode and antibiotic exposure, are risk factors for ASD.

POTENTIAL RELATIONSHIPS BETWEEN THE MICROBIOTA AND ASD (THE GUT-BRAIN AXIS)

- The gut microbiota influences brain function through the neuroendocrine, neuroimmune and autonomic nervous systems and via microbiotic toxin production.

- The mucosa of the gastrointestinal tract contains millions of neurons, which constitute the enteric nervous system (ENS) and regulate gastrointestinal functions.Therefore, the gut is considered as a “second brain.”



Potential relationships between the microbiota and ASD (the gut-brain axis).

- relationships between ASD and the gut is the increased permeability of the intestinal tract of ASD individuals, referred to as a “leaky gut”
- studies have demonstrated that ASD animals present defects in the GI barrier, resulting in the entry of the toxins and bacterial products into the bloodstream, which influence brain function.
- lipopolysaccharide (LPS), components of the cell wall of gram-negative bacteria, is increased in the serum of ASD compared with healthy individuals and is associated with impaired social behavioral scores .

- integrity of both the gut barrier and the BBB were impaired in ASD individuals, as evidenced by increased levels of claudin (CLDN)-5, CLDN-12, CLDN-3, and MMP-9 in the ASD brain and decreased levels of intestinal tight junction components (CLDN-1, OCLN, TRIC) in ASD individuals compared with controls.

- Intestinal permeability, measured by the lactulose: mannitol test, has been shown to be increased in autistic children compared with healthy controls.

- germ-free (GF) mice display increased BBB permeability. Bacterial products (e.g., acetate and propionate) can enhance the integrity of the BBB .

Gut Microbiota-Mediated Metabolites

- Gut microbiota-mediated metabolites, such as short-chain fatty acids (SCFAs), phenol compounds, and free amino acids (FAA), affect ASD-like behaviors through the vagal pathways.
- SCFAs, including acetic acid (AA), proprionic acid (PPA), butyrate, isobutyric acid, valeric acid and isovaleric acid, are principal products of the gut bacterial.
- SCFAs play a critical role in patients with ASD. higher concentrations of total SCFAs and ammonia in fecal matter from children with autism compared with controls .
- PPA, a short-chain fatty acid that is mainly produced by Clostridia and Desulfovibrio, can cross the BBB and induce ASD-like behaviors.
- PPA leads to impaired social behavior in rats, likely by altering some neurotransmitters, such as dopamine and serotonin.
- FAA, derived from the hydrolysis of proteins and peptides, has also been found to be associated with ASD. the level of total FAA in fecal samples was higher in children with autism than that in heathy children.

- 3-(3-hydroxyphenyl)-3-hydroxypropanoic acid, a phenylalanine metabolite of Clostridia spp. was shown to be increased in the urine of ASD patients and is associated with autistic behaviors in animals .

The Immune System Pathways

- The gut can also communicate with the brain through immunological pathways.
- Increased levels of pro-inflammatory cytokines, such as IL-1b, IL-6, IL-8, and IL-12 , in the plasma of ASD individuals.
- Immune responses to toxins produced by pathogenic microbiota increase gut permeability.
- impaired intestinal barrier is observed in response to infection or stress, which allows the translocation of the gut bacteria across the intestinal wall and into the mesenteric lymphoid tissue, where ,they activate the immune system through mucosal immune cells .
- The activated immune system releases inflammatory cytokines and activates the vagal system, which in turn regulates CNS activity.
- metabolic compounds, such as lipopolysaccharide (LPS) produced by gut microbiota, are absorbed into the blood through an impaired gut wall and activate Toll-like receptors in the ENS and CNS .

- An IgE mediated allergic immune response in the intestine increases the 5-hydroxytryptamine (5-HT) levels and decreases the 5- hydroxyl indole acetic acid (5-HIAA) levels in the intestine. It also reduces social communication and increases repetitive behavior.

Neuroactive Compound Pathways

- The gut microbiota produces neuroactive compounds such as dopamine (DA), 5-HT, g aminobutyric acid (GABA) and histamine, which activate or inhibit central neurons through the vagus nerve.

- Compared with specificpathogen-free (SPF)mice with a normal gutmicrobiota, SPF mice exhibit a significant elevation of monoamine neurotransmission (of compounds such as noradrenaline, DA and 5-HT), decreased levels of nerve growth factor-inducible clone A (NGFIA) and brain-derived neurotrophic factor (BDNF), increased corticosterone levels, and increased anxiety-like behaviors.

- Serotonin, which is synthesized in the intestines and brain, is important for the regulation of mood and cognition .
- association between whole-blood serotonin levels and GI symptoms in ASD individuals.

MODULATION OF THE GUT MICROBIOTA IS A POTENTIAL THERAPY FOR CHILDREN WITH ASD

- modulation of the gut microbiota is a potential therapy in children with ASD. Probiotics, prebiotics, fecal microbiota transplantation (FMT) and diet have getting considerable attention .

➢ Probiotics

- Probiotics may prevent intestinal inflammatory diseases by regulating intestinal tight junction protein expression and barrier function.

- Probiotics, such as the lactic acid-producing bacteria belonging to Lactococcin, Lactobacilli, Bifidobacteria and Saccharomycetes, are beneficial to the host when provided in adequate quantities.

- The probiotic/prebiotic can normalize the gut microbiota, enhance gut barrier and relieve the ASD-like behaviors in animal models or ASD patients.

- treatment with Bacteroides fragilis reduced gut permeability, altered the composition of the gut microbiota and decreased ASD-like behaviors in a rodent model of ASD.

- Supplementation with a probiotic containing Lactobacillus, Bifidobacteria and Streptococci normalizes the Bacteroidetes/Firmicutes ratio, and the amounts of Desulfovibrio spp. and Bifidobacterium spp. in the feces of children with ASD are similar to those found in samples from their nonautistic.

- ASD boy with severe cognitive disability was treated with VSL#3 (a multi-strain mixture of 10 probiotics) for 4 weeks. The treatment relieved the GI symptoms and improved the autistic core symptoms (Grossi et al., 2016).

- prebiotics” refers to non-digestible oligosaccharides that induce the growth of beneficial bacteria .
- The prebiotic galactooligosaccharide (B-GOS) increases the levels of Bifidobacterium spp , as demonstrated through the analysis of fecal samples from children with ASD and controls (Grimaldi et al.,2017).

Model	Behavior Tests	Treatment	Dosage and time	Effects	Limitations	References
ASD animal model	Pre-pulse inhibition, open field exploration, marble burying, social interaction and adult ultrasonic vocalizations	Probiotic:*Bacteroides fragilis*	1×10^{10} CFU every otherday for 6 days	Improved gut barrier integrity, normalized gut microbiota, reversed ASD-related behaviors, decreased 4EPS in serum	An animal study	Hsiao et al., 2013
33 ASD children	ATEC	Delpro® (containing *Lactocillus acidophilus, Lactobacillus casei, Lactobacillus delbruecki, Bifdobacteria longum, Bifdobacteria bifdum* and 8 mg of Del-Immune V powder).	1×10^{8} billion CFUs, three times daily for 6 months	88% of patients reported a decrease in total ATEC score, 48% reported a decrease in diarrhea and 52% reported a decrease in constipation	There was no control or placebo, and has a selection bias	West et al., 2013
10 autistic children, their 9 non-autistic siblings and 10 control	CARS, ADI	Probiotic containing *Lactobacillus, Bifidobacteria and Streptococci*	One capsule three times a day for 4 months.	Increased of the *Bacteroidetes/Firmicutes* ratio, normalized the amount of *Bifidobacterium* and *Lactobacillus* and decreased the TNFα levels in the feces of children with autism	Not mentioned the alternation of ASD-like behavior after probiotic treatment	Tomova et al., 2015
22 autistic children	Not mentioned	Probiotic: Lactobacillus acidophilus	5×10^{9} CFU/g twice daily for 2 months	Decreased DA/LA ratio in urine, improved some autistic symptoms (e.g., ability of concentration and carrying out orders)	No control group. The behavior tests are not clear	Kaluzna-Czaplinska and Blaszczyk, 2012

➢ Fecal Microbiota Transplantation (FMT)

- Fecal Microbiota Transplantation (FMT) is an intervention in which the fecal microbiota from a healthy individual is delivered to a patient with a dysbiotic gut microbiota.
- highly efficacious in the treatment of recurrent Clostridium difficile infections (CDI).
- using FMT to treat children with ASD. However, the safety of FMT should be considered.

➢ Other Potential Therapies (e.g., Diet and Antibiotics)

- Children with ASD tolerate a narrower range of foods and exhibit more feeding problems than children without ASD.
- Many parents complain that their children with ASD are selective eaters.

- Compared with controls, children with ASD ingest fewer fruits, vegetables, and proteins and have a significantly lower daily intake of potassium, copper, folate, and calcium.

- Decreases in carbohydrate intake decrease the levels of Roseburia spp. and Eubacterium rectale.
- participants with ASD who are treated with omega-3 fatty acids for 12 weeks exhibit significant improvements in social behaviors.

Food intake influences the composition of the gut microbiota

- A gluten-free and/or casein-free (GF/CF) diet improves ASD behaviors, physiological symptoms, and social behaviors.

- The ketogenic diet is a high fat and low-carbohydrate diet and results in reductions in the total gut microbial counts, increased sociability, decreased repetitive behaviors, and improved social communication in an ASD animal model .

- Children with regressive-onset autism who are treated with vancomycin, a broad-spectrum oral antibiotic,for a short period exhibit improvements in diarrhea and autistic behaviors .

CONCLUSION

- Multiple studies showing that an abnormal gut microbiota is related to ASD.
- relationship between the gut microbiota and the CNS.
- the role of the gut microbiota in ASD.
- Some potential therapies for modulating the gut microbiota in patients with ASD. Many recent clinical studies have shown that treatments that regulate the gut microbiota result in improvements in ASD symptoms.


Probiotics
help us digest