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Treatment of Autism with Microbiota

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Abstract

Gastrointestinal symptoms are a common problem in patients with autism spectrum disorder (ASD), but the underlying mechanisms are unknown. Autistic children often suffer gastrointestinal (GI) problems that correlate with ASD severity. Several previous studies have reported abnormal gut bacteria in children with Autism disease. The gut microbiota-Autism connection has been tested in a mouse model of Autism, where the microbiome was mechanistically linked to abnormal metabolites and behavior. Similarly, a study of children with Autism spectrum disorder found that oral non-absorbable antibiotic treatment improved GI and ASD symptoms, albeit temporarily.

In this review, Autism and their therapy with gut microbiota are summarized. These studies are among the most promising areas of research for human health; however, further studies are needed before clinical practice.

Key words: *Autism; Microbiota; Gut; Intestine; probiotics; fecal microbiota transplantation (FMT).*

Introduction

Autism spectrum disorder (ASD) is a group of developmental disorders, which generally begins early in childhood. People with Autism specrum disorder could have a wide range of symptoms and levels of disability, hence the name "spectrum" disorder. These patients have repetitive behavior, limited interests and could have difficulty communicating and interacting with other people (1). It is thought that many factors come together in the formation of the disease. However, there is no reason not yet defined as like in many other diseases. However, it is thought that the disease is caused by an anomaly in the central nervous system and a disorder in chemical communication between the brain and the cells. Genetic factors can also influence the formation of the disease. Sibling and twin studies confirm this knowledge. The possibility of autism in both two brothers in identical twins are higher than dizygotic twins (2).

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In addition to genetic factors, maternal nutritional status, drug use, smoking and alcohol use increase the risk of autism. In addition, before and after the mother's birth, folate, iron and polyunsaturated inadequacies in the use of fatty acids are also important risk factors (2-9). The first neuronal development occurs in the form of neural tube formation and brain development. This development occurs between the 3rd and 4th weeks of the gestational period of the human and continues until the age of 2.5 years.

Therefore, the mother's nutrition, microbial infections, prenatal stress and neurodevelopmental diseases such as schizophrenia, autism are closely related. Brain and neuronal development depend on factors such as the form of birth, mother's age and of breast milk and these factors continue to influence brain and neuronal development in the postnatal period (8).

At the same time, childhood and adolescence are important steps in brain and neuronal development. These periods are thought to be the initial period of schizophrenia, autism, mood disorders and many neuropsychiatric disorders. Despite these informations, the underlying cause of autism is still unknown. In addition, the inadequacy of clinical studies constitutes limitations in the understanding of the etiology of the disease.

Increased numbers of studies on the autism gastrointestinal pathology and new observations may help to take an important step in the prevention and treatment of this disease. When the intestinal microbiota changes, the function of the intestine impairs and this that it is thought that it may lead to a change in behavior.

In order to understand the etiology of autism and to provide clinical solutions to the disease, various studies were used in this review to evaluate the relationship between autism and microbiota, especially intestinal microbiota.

Human gut microbiota

Human intestinal microbiota is a complex ecosystem with various functions integrated in the host organism (metabolic, immunity, nutrient absorption, etc.). Human microbiota is formed by viruses, bacteria, yeasts, fungi, and last but not least, viruses, whose composition has not been completely described.

Human microbial populations include populations of microbial species that live on or in human body - commensal bacteria, viruses and fungi (and other single-celled animals such as protists) that call our bodies home.

Relationship between autism and microbiota

Gastrointestinal symptoms appear in ASD individuals. They have more GI syndromes, including constipation (20%) and diarrhea (19%), than in their unaffected siblings (42 vs. 23%, respectively). ASD patients with symptoms of GI may exhibit significant behavioral symptoms such as anxiety, self-harm and aggression. Many evidence suggests that the intestinal microbiota is directly or indirectly associated with ASD symptoms, in part by influencing the immune system and metabolism. A higher percentage of abnormal intestinal permeability has been observed in 36.7% of patients with ASD and their relatives (21.2%) compared with control children (4. 8%). An increased intestinal permeability has been resulted in a higher antigenic load from the gastrointestinal tract.

Lymphocytes and ASD-associated cytokines, such as interleukin-1β (IL-1β), IL-6, interferon-γ (IFN-γ), and tumor necrosis factor-a (TNF-α), are present in the circulation and has been cross the blood-brain barrier. Subsequently, IL-1β and TNF-α 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. it was observed gastrointestinal barrier defects and microbiota alterations in a mouse model displaying features of ASD.

It was found that bacteria belonging to *Porphyromonadaceae*, *Prevotellaceae*, unclassified *Bacteroidales*, and *Lachnospiraceae* were more abundant in offspring of mothers with maternal immune activation (MIA) than in control offspring, whereas *Ruminococcaceae*, *Erysipelotrichaceae*, and *Alcaligenaceae* were more abundant in the latter.

As shown in mice, the 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 *Lactobacillus, Clostridium, Bacteroidetes, Desulfovibrio, Caloramator* and *Sarcina*. In summary, the roles of gut fungal and bacteria in ASD still need more large samples studies.

Autism and Microbiota Relationship : Proposed mechanisms

In explaining the relationship between autism and intestinal microbiota, various mechanisms are suggested such as impaired bowel permeability, production of toxins, immunological and metabolic abnormalities.

Leaky Intestine Hypothesis

The "Leaky Intestine Hypothesis" is the transfer of various numbers and metabolite species from the blood-brain barrier to the circulatory system due to impaired epithelial barrier functions of the small and large intestine.

Increased intestinal permeability in autistic individuals is thought to be based on the intestinal-brain relationship in the pathogenesis of autism (3). Short chain fatty acids produced by various bacteria in the intestinal tract have been found to affect brain and behavior of the individual through brain and intestinal blood and blood-brain barrier. Propionic acid and other short-chain fatty acids produced by these bacteria, they play a role in many biochemical processes related to autism. So, they cause changes on brain, immune system, metabolic functions and behavior.

In addition, increased propionic acid and other short chain fatty acids levels cause growth retardation, seizures, metabolic acidosis and gastrointestinal symptoms.

The Gut - Brain Axis

Brain -Intestinal Axis is one of the most popular research fields of the last period from health sciences to psychology. According to this thesis, there is a second control mechanism similar to the control system of the brain that was alone until now. So the brain doesn't do everything alone. In most cases, the brain and intestines interact. From success in education to depression, from Parkinson's disease to autism, the intestines have a determining role as well as the brain. For example, while there are data showing that people with mental problems had difficulties in the digestive system in the past, today it is mentioned that those who have problems in the digestive system have mental problems. In other words, what affects what and cause-effect relationship has completely changed

direction with new data. As such, the intestines are not viewed as a passive organ as it used to be, but as an active and determinant 'second brain'.

The intestinal-brain axis is a bi-directional neuronal communication system between the intestine and the brain. This two-way communication system affects both the neuroendocrine and neuroimmune mechanisms as well as the central nervous system and the enteric nervous system. The primary components that provide the signal from the brain to the intestinal microbiota are the sympathetic and parasympathetic arms of the central nervous system. The sympathetic system is exhibited inhibitory feature in the intestine by reducing intestinal secretion and preventing intestinal motor functions. Sympathetic nervous system activates in stress situations that changes intestinal mobility (4). The hypothalamic-pituitary-adrenal axis is another mechanism by which intestinal microbiota affects the brain. When the hypothalamic-pituitary-adrenal axis activates, the release of cortisol level and proinflammatory cytokines increases. It has been observed in studies conducted in experimental animals that intestinal inflammation and corticotropin hormone levels increased and intestinal microbiota content changes, under the high level of stress. It has been found that increased corticotropin hormone levels lead to depression-like behaviors and altered intestinal microbiota content (5).

In addition, the metabolites produced by the intestinal microbiota can pass through the blood-brain barrier and affect cerebral function. For example, *Lactobacillus rhamnosus* provides gamma butyric acid (GABA) production, an important inhibitor neurotransmitter for the brain. However, the intestinal microbiota provides the production of many monoamines such as norepinealin, dopamine, and serotonin (7). Serotonin (5-hydroxytryptamine or 5-HT) is an important monomer that plays regulatory roles in many organs. Recent studies have shown a relationship between serotonin synthesis and intestinal microbiota. Studies conducted on experimental animals have shown that bacteria in the intestinal microbiota perform 5-HT biosynthesis and provide a passage between the blood and the intestine (9).

Also in the study comparing germ-free and with intestinal microbiota transplanted experimental animals; it has been found that there are significant differences in 38 metabolites in the cerebral concentration that regulate brain activity between the two groups (14).

Intestinal microbiota also plays a role in cerebral diseases by regulating immunological responses. Pathogenic bacteria and their metabolites can stimulate the proinflammatory cytokines (IL-1, IL-6) secreted from the intestinal epithelium and macrophages.

Increased cytokine levels are closely related to various neuropsychiatric disorders such as depression and anxiety.

In autistic individuals, changes in circulation of brain cytokines, 3 chemokines, and other inflammatory factors are seen in addition to impairments in the immune system, disorders of response or distribution of various leukocyte types (10).

Another mechanism is the use of neuronal circuits in intestinal-brain axis communication. The intestinal microbiota transmits its signals to brain with vagus sinus. When Bifidobacterium longum was given to vagus nerve excised experimental animals, it has been observed that chronic colitis induced anxiety development decreased significantly. (11).

Changing in intestinal Microbiota

Intestinal microbiota has also brought with it the idea of being a new human organ with functions such as enhancing mucosal barrier richness, regulation of gene expression, and postnatal intestinal maturation (12).

Intestinal microbiota often takes food items from carbohydrates. The indigestible oligosaccharides result in the synthesis of short chain fatty acids such as butyrate, propionate and acetate, which are rich energy sources for the host, which escape from proximal digestion, owing to in the colonization of colonies such as Bifidobacterium.

With the formation of these fatty acids, the pH in the small intestine and colon is reduced, and this has two effects. The first is necessary for low pH intestinal microbiota composition, and the second one prevents the development of pathogenic bacteria such as Entero bacteriaceae and Clostridia (13). When the intestinal microbiota contents of autistic individuals are examined, it is determined that they are quite different from healthy individuals.

For example, chronic diarrhea, which is the result of antibiotic use in autistic individuals, and possible sources of observed behavioral symptoms are *Clostridium* spp.

Some of these bacteria are beneficial to the gut, while the clear majority are not pathogenic organisms. However, species such as *Clostridium tetani* and *Clostridium perfringens* produce toxins and cause various infections in humans. *Clostridium difficile* is typically responsible from diarrhea associated with antibiotic use (14).

The Clostridium hypothesis proposed by Sandler and colleagues suggests that 6 weeks of treatment with vancomycin, an antibiotic known to be active against *Clostridia protozoa*, may treat children with regressive autism.

Eight of the 10 children studied showed improvement in gastrointestinal symptoms as well as neurobehavioral symptoms. After the completion of vancomycin therapy, behavioral symptoms were reestablished in all children.

It has also been observed that intestinal microbiota of autistic children has markedly decreased species of *Prevotella, Coprococcus*, and *Veillonellaceae* (15). These changes were found to be closely related to the specific diets, additional diets, and severity of gastrointestinal symptoms. These findings support the idea that "certain bacterium of intestinal microbiology affects autism" rather than the idea that dietary differences or eating behaviors affect the microbiota in autistic individuals. Another species that differs in autistic individuals and healthy individuals is Gram-negative and anaerobic bacteria Desulfovibrio spp.

3 strains of *Lactobacillus* (60%), 2 strains of *Bifidumbacteria* (25%) and 1 strain of *Streptococcus* (15%) were injected for 3 months in a study consisting of 10 autistic children, 9 non-autistic siblings and 10 healthy non-autistic individuals.

When fecal specimens were examined before probiotic reinforcement, *Bacteroidetes* / *Firmicutes* rate were found to be significantly lower compared to the control group in autistic children. *Clostridia* and *Desulfovibrio* concentrations in autistic children have been observed significantly higher than control groups.

When fecal samples were examined after probiotic treatment, that have been observed *Bacteroidetes* / *Firmicutes* rates increase and the concentration of *Desulfovibrio* that were decreased. In autistic children, a reduction in TNF-α levels, an indicator of inflammation after probiotic therapy, has also been observed. Sutterella spp. is another type of bacteria found to play a role in autism. In a study of fecal specimens of 9 control groups with 20

autistic children, 22 siblings and no autism in their family, *Sutterella* spp. and *Ruminococcus torques* were found to be high (16). There is no definite evidence between *Sutterella* and autism. It is stated that Sutterella spp. May be an indicator of infection with gastrointestinal symptoms of autistic children. Although the role of the *Sutterella* strain in autism is not as clear as *Clostridia*, His presence in the class of *Betaproteobacteria* and showing similar associations with *Neisseria gonorhoeae* and *Neisseria meningitidis*, *Bordetella pertussis* and *Burkholderia cepacian* make it more likely to play a role in autism (22).

Sulfur metabolism disorders

Sulfur is one of the abundant minerals in the body. This is not surprising given that it has a decisive role in health and takes part in hundreds of physiological processes. In autistic individuals, there are deficiencies in the metabolic pathways of transmet- ration and trans-sulphation associated with the metabolism of sulfur-containing methionine and cysteine amino acids.

In some studies conducted with blood and urine samples of individuals with ASD, some disorders were detected in transmethylation and trans-sulfuration metabolic pathways. Sulfur-containing amino acids such as methionine and cysteine It is thought to also affect the metabolism.

It is thought that deficiency in methionine synthesis may cause diseases such as autism by preventing gene expression.In studies on intestinal microbiota and genetics, it has been concluded that the genetic structure may affect the bacterial content. In addition, it has been observed that twins living in separate regions often have similar bacterial content even years later (25). These results showed that methionine deficiencies may cause changes in the intestinal microbiota content in individuals with ASD (18,19).

Relationship between intestinal microbiota and gastrointestinal symptoms

Gastrointestinal symptoms, such as diarrhea, contusion, vomiting, or abdominal pain, are frequently seen in autistic individuals due to intestinal microbiota alteration. It is also reported that most autistic individuals develop inflammatory intestinal disease.

The prevalence of gastrointestinal symptoms in infants with autism has been reported to be very high and gastrointestinal symptoms such as diarrhea, constipation, food allergy / intolerance has been reported more frequently in the first 3 years of life in these children (20). The relationship between gastrointestinal symptoms and autism symptoms; is thought to be due to an interaction between impaired mitochondrial function and intestinal inflammation. Deterioration to mitochondrial enzymes or damage to their transport are observed frequently in autistic individuals. However, also in other mitochondrial diseases, gastrointestinal symptoms are often encountered. For example, it is thought that propionate, a degradation product of intestinal microbiota, is a mitochondrial toxin that can cause neurotoxicity by affecting mitochondrial metabolism, thus causing mitochondrial disorders and triggering gastrointestinal symptoms (21).

In addition, the accompanying gastrointestinal symptoms in autistic children reduce the expression of messenger RNAs that make enzymes (sucrose, isomaltase, maltase, etc.) digesting enzymes of hexose carriers (sodium-dependent glucose transporters), glucose transporters and carbohydrates. Thus, with the increase of Bacteroidetes spp., Firmicutes spp. and Betaproteobacteria spp. in the intestinal environment, disorders of digestion and

absorption of carbohydrates occur. Because of the accumulation of undigested carbohydrates in the intestine, intestinal inflammation occurs, leading to an increase in behavioral problems in autistic individuals.

However, the nutritional arrangement of the individual changes the intestinal microbiota content and thus affects the intestinal brain axis. Problematic eating behaviors of autistic children lead to gastrointestinal symptoms, food allergies or metabolic abnormalities.

It has been determined that 90% of autistic children have food related problems, milk and dairy products, nuts and fruit allergies. While autistic children who exhibit selective eating and refusal behaviors often prefer to eat high carbohydrate foods, snacks, and processed foods, fruit, vegetable and protein content were refused to eat high nutrients.

High carbohydrate foods and snacks cause weight gain due to excessive calorie intake and increase the prevalence of obesity among autistic children (22).

Due to different dietary preferences of autistic individuals, intestinal microbiota contents show differences. When fecal specimens of autistic individuals are examined, it is seen that free amino acids are quite high. These metabolites are associated with the presence of high amounts of proteolytic bacteria (eg *Clostridium* and *Bacteroides*) in autistic children. Excess accumulation of some free amino acids such as glutamine causes neuronal cell death and leads to neuropsychiatric diseases. In fecal samples of autistic children, glutamine levels were found high; in contrast, glycine, serine, threonine, alanine, and histidine amino acids were found to be low amount in autistic children (23).

In addition, some food items have therapeutic effects on autistic individuals. There is evidence that gluten-free diets improve the symptoms of autistic individuals. It has been determined that these diets have beneficial effects on behaviors and thus help to reduce the negative effects such as social rejection, social restrictions, stigmatization the treatment of autistic individuals.

Conclusions

Current studies suggest that there is a strong correlation between the change of intestinal microbiota and the symptoms of autism. The intestinal microbiota composition can control autistic behaviors through various mechanisms. It has also been found that the probiotic supplements that are made reduce the symptoms of autism.

Based on the positive effects of probiotic treatments on autistic individuals, probiotic studies with autistic individuals may provide guidance for new information. Differences in methodology and other factors in the study population, especially in the diet, limit the studies investigating the relationship between autism and microbiota composition.

In order to better understand the relationship between autism and microbiota in this light of information, more extensive studies on nutrition and microbiota in autistic individuals are needed. Based and effect-focused future work will be useful for clarifying the relationship between microbiota and autism. Based and effect-focused future work will be useful for clarifying the relationship between microbiota and autism. The works to be done will be useful for clarifying the relationship between microbiota and autism.

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