CHAPTER 12

Effects of the Gut Microbiota on Autism Spectrum Disorder

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1 Introduction

Autism spectrum disorder is a neurodevelopmental disease that arises before 3 years of age, is characterized by disorder in social interaction and communication, and involves a narrow field of interests and recurring behaviors. Although the pathophysiology of autism is not fully known, genetic susceptibility is suggested to modify the neurological development in early childhood (Tuohy et al., 2015). Autism spectrum disorder affects 1 of every 68 children, and is seen among boys 4 times more than girls (Rosenfeld, 2015).

The prominent risk factors related to autism are advanced ages of mother and father, lower educational background of the family, male gender, presence of autism among the family, lower birth weight, lower gestational age, prenatal virus, and exposure to drugs. Autism is a defect in the function and development of the brain. The cause of this defect is not clear. Although it is known that certain genetic factors are effective in the etiology of autism, this is prevalent among a small majority of the population with autism (Van De Sande et al., 2014). Autism spectrum disorder has been reported to be associated with sleeping problems, gastrointestinal (GI) metabolic disorders, immune disregulation, homeostatic imbalance, oxidative stress, mitochondrial dysfunction, and neuroinflammation in the course of development (Reddy and Saier, 2015).

It has been shown in studies that gut bacteria may play a role in the pathophysiology of autism spectrum disorder. It has further been shown that the gut bacteria, as well as the metabolic end-products, possess surprising physiological activities, such as mood, cognitive behavior, depression, and brain development (Tuohy et al., 2015).

Such GI disorders as chronic constipation, diarrhea, stomachache, gastro-esophageal reflux, and vomiting are frequently seen in individuals with autism spectrum disorder. In various studies, children with autism have been reported to modify GI motility and increased intestinal permeability. The GI microbial population is responsible for the pathogenesis of some of the

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foregoing disorders. Microbial changes in the GI system stimulate gut permeability, leading to the leaky gut syndrome. This situation ends up with septicemia of microbial products and cytokines and gives rise to neurodevelopmental diseases, such as autism (Reddy and Saier, 2015).

It is difficult to ascertain the underlying causes of GI conditions and other medical problems because verbal communication is little if any among children with autism. Pain may trigger aggression or self-injury and caregivers, taking the underlying disease into consideration, may manage this situation and be able to lessen the behavioral disorders developing in conjunction therewith. Prebiotics, probiotics, antibiotics, various diet therapies, and other correlated treatments are applicable in treating autism spectrum disorder (Buie, 2015).

Diet plays a critical role among mammals in the configuration of such metabolic processes as gut microbiota and activities and neurochemicals' processes. Pulp and prebiotics maintain a relative increase in the *Bifidobacterium* and lactobacilli and in the generation of short chain fatty acids. *Lactobacillus* and *Bifidobacterium* are important members of the useful gut microbiota and are effective on immune function, mucosal integrity, generation of bioactive components, such as folates, and γ-aminobutyric acid (Tuohy et al., 2015).

Although it is known that environmental factors play a significant role in autism, there is very little positive evidence regarding the correlation between diet and the onset or progression of the disease. Nonetheless, recent research is concentrated on how diet configures the processes between the gut microbiome and brain axis. Diet may thereby be used to remedy some of the symptoms of autism in the near future (Tuohy et al., 2015).

This chapter focuses on the effects of gut microbiota and the products on the autism spectrum.

2 Gut Microbiota

Human gut is host to more than 100 trillion microorganisms, including no less than 1000 different bacteria. In the course of vaginal delivery, the baby is exposed to complex microbiota, thus the colonization of the gut microbiota starts at birth. The genome of the host permanently affects the diversity and function of the gut microbiota. Other environmental factors, such as infection, diet, antibiotics, stress, and disease, on the other hand, affect the natural composition of the gut microbiota (Li and Zhou, 2016).

Microbiota is a balanced composition condition that normally posesses the intestinal system. Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Verrucomicrobia, and Fusobacteria are the basic gut bacteria. Firmicutes are Gram-positive bacteria, which are comprised of clostridia and lactic acid bacteria. Lactic acid bacterium and *Bifidobacterium*, which are two important types of gut bacteria, either exist in the gut as of birth, or are obtained from digested foods. Gut bacteria play a critical role in human health. Gut bacteria contribute to the defense system of the human gut and assist in the continuation of the gut's normal function (Zhang et al., 2015).

2.1 Gut Bacteria and the Immune System

The gut inhibits pathogenic bacteria through the mechanical and immune barriers. The mechanical barrier is formed of a single layer of gut epithelial cells, mucus, and enterocytes. The immune barrier, on the other hand, is formed of intraepithelial lymphocytes, macrophages, natural killer cells, Peyer's plaques, and the mesenteric lymph node. Commensal bacteria and probiotics regulate the integrity of the gut barrier. Commenal bacteria contribute to the gut defense system by way of resisting against the pathogenic bacteria. Attacks by pathogenic bacteria are avoided by way of lowering the gut pH with the short chain fatty acids formed by commensal bacteria (Zhang et al., 2015).

2.2 Gut Bacteria Benefit the Host

While contribution to the host's defense system is not its sole benefit, the gut bacterium also assists in the continuation of the normal functions in the gut. Gut microbiota has either inhibitor or stimulant effects on the host's physiological processes. Gut bacteria assists the host also by ways of regulating the gut's motility, forming the vitamins, and absorbing the minerals. In the proximal section of the colon, organic acids are effective on bacterial growth by way of lowering fecal pH and affecting the colonic water absorption. Gut bacteria are also essential for natural compounds, such as lignans. Lignans are found in foods, such as flaxseeds, vegetables, and fruits. Lignans are protective against cardiovascular diseases; hyperlipidaemia; breast, colon, and prostate cancers; osteoporosis; and menopausal syndrome (Zhang et al., 2015).

The microbiota is effective on the intestinal function of the host by way of regulating the gut associated lymphoid tissue maturation, increasing the tissue regeneration and gut motility, and lowering the permeability of the epithelial cells in the gut. It thereby provides reinforcement of the barrier's integrity. Similarly, the gut microbiota also affects the morphogenesis of the vascular system surrounding the gut. This is associated with the increase in the glycolization of the tissue factor (TF), it leads to the division of the thrombin, and proteinase activated receptor 1 (PAR 1) is activated. TF phosphorylation also occurs and induces the expression of angiopoietin 1. It thereby brings along the increase in vascularization. The gut microbiota may affect the nervous system of the host by way of decreasing the synaptic connectivity and increasing both behaviors similar to anxiety and pain perception. It has also been shown that gut microbiota supports the host's adiposity and regulates the host's metabolism. Gut microbiota is also effective on bone homeostasis. For instance, they may lead to a decline in bone masses by way of supporting the functions of osteoclasts and increasing the number of proinflammatory T helper 17 cells (Sommer and Bäckhed, 2013).

2.3 Dietary Intake and Gut Microbiota

Diet has an important role in the generation, maturation, and protection of microbial diversity in the gut's ecosystem. In comparison to those fed by formula, babies fed by breast milk from

birth have different gut microbial species in terms of composition and intensity. Breast milk is an important source of protective factors, including commensal bacteria, which are not found in the formula. These protective factors support the GI system, pancreas/endocrine system, and the associated mucosal defenses. Breast milk is an important source of lactic acid bacterium and *Bifidobacterium* for newborns. Higher amounts of *Bifidobacterium* and *Lactobacillus* in babies fed breast milk indicate they have a healthy gut microbiota. Breast milk has numerous benefits, such as increasing resistance against diseases and decreasing the rate of occurrence of gastroenteritis, respiratory tract infections, otitis media, urinary system infections, diarrhea, and necrotizing enterocolitis. It is suggested that breast milk brings about these benefits partially by forming a healthy gut microbiome.

Colonization of the gut bacteria is affected by various factors. Anatomical structure of the host and physiology of the digestive system are among the important factors affecting the gut bacteria. In a study, rats fed a Western diet were found to have Firmucutes that were decreased 1.5 times. Fecal contents of the rats fed a Western diet were rich in the species from desulfovibrionaceae family. In the same study, the fecal contents of rats fed low-fat foods contained 4.3% *Lactobacillus gasseri* species and were rich in *Ruminococcus*, Lachnospriraceae, and Bacteroidates. *L. gasseri* species were not found in the feces of rats fed a Western diet (Tachon et al., 2014).

In a study, it was observed that in the first 24 h following the ingestion of diets containing high fat/low fiber, or low fat/high fiber, the composition of the microbiota was modified, however, it remained stable for 10 days in 10 persons fed under a control in the meantime (Wu et al., 2011). In another study, in comparison with the plant-based diet, the animal-based diet was proved to increase the species of *Alistipes*, *Bilophila*, and *Bacteroides*; significantly decrease the carbohydrate fermentation products; and increase the concentration of amino acid fermentation products (Zhang et al., 2015).

It was shown in a study that in comparison to the formula without fiber, the enteral formula enriched with fiber led to negative symptoms in the gut. Polyphenols and fiber are considered positive diet factors (Zhang et al., 2015).

It has been suggested that dietary iron may be harmful to gut bacteria. An increase in the availability of iron may increase the proliferation of gut bacteria and the permeability of the gut barrier (Zhang et al., 2015).

3 Gut–Brain Axis and the Microbiota

The evidence gathered in recent years has reinforced the suggestion that gut microbiota may regulate brain growth via the gut–brain axis, and thereby bring along behavioral phenotypes.

Communication between the microbiota–gut–brain axis clarifies how the signals incoming from gut microbiota affects the function of the brain and the brain's messages on the microbiota activity and GI physiology. This bidirectional connection takes place along both

the neuroendocrine and neuroimmune mechanisms, comprising also the autonomic nervous system and enteric nervous system (Li and Zhou, 2016).

3.1 Effect of Microbiota on the Gastrointestinal System

Microbiota have been shown to regulate gene expression and raise the mucosal barrier by way of comparing the germ-free rats with those colonized with *Bacteroides thetaiotaomicrom* (primary member of the human gut microflora). These findings indicate that commensal bacteria may affect the gene expression, and that the products of the bacteria may be effective in the nervous system (Collins and Bercik, 2009).

Germ-free rodentia have extended cecum, and this is the indicator of the presence of an intense defect in GI motility. Abnormal motility in the germ-free animals reflects mature enterendocrine system deficiency, changes in the neurotransmission, and the immaturity of the immune system. Inflammatory cells are scarce in the germ-free guts, and lymphoid structures therein are not developed. However, in lamina propria of the gut of the healthy host, there are significant number of inflammatory cells, maintaining the protection and function of the normal epithelial structure. Disruption of this balance leads to dysbiosis. The effect of dysbiosis on the host is determined by the change in the structure and size of the bacteria composition of the GI system (Collins and Bercik, 2009).

3.2 Effect of Gastrointestinal System Physiology on Microbiota

Just like microbiota is effective on the physiology of the host, the GI system is also effective on the microbiota. Under normal conditions, the GI system maintains a stable natural environment for the commensal bacteria, and thereby reinforces structural and functional integrity. Disruption of the normal GI system physiology leads to changes in the microbial composition. Occurrence of change in the bacterial composition of the GI system following the halt of the normal interdigestive motility is an example of such changes. Epithelial cell physiology may affect the microbial ecosystem in relation to mucosa at times of change in the mucus secretion and in the intestinal barrier function. The symphatetic nervous system facilitates the enteric bacterias' selective identification of the mucosal immune system. Administration of norepinefrine increases the internalization of pathogenic bacteria in the folliciles. Although the cellular mechanism underlying it has not been fully clarified, it is suggested that it involves the selective intake of pathogenic bacteria for the interaction of dendritic cells and symphatetic nerves, and for identification to mucosal immune system (Collins and Bercik, 2009).

Secretion of biological amines, such as norepinefrine, may affect the composition of intestinal microbiota. These neurotransmitters have been shown by in vitro studies to stimulate the growth of *Escherichia coli* (Collins and Bercik, 2009).

3.3 Effect of Brain on Microbiota

In a couple of animal studies, psychological stress may modify GI flora, although such studies are limited. Changes in GI floras were reported from the rats, which underwent stress by way of giving no food or water. However, unlike stress response, such environmental changes are suggested to have direct effect on microbiota. Stress arising from parting from the mother in early life has been shown to form changes in the microbiota of the descendant. This is associated with increased corticosterones and inflammatory stocins (O'Mahony et al., 2009). From another study, the rats parting from their mothers showed an increase in intestinal permeability and defenselessness in their GI systems (Varghese et al., 2006). Parting from mother leads to an increased stress response, increased intestinal permeability, and change in the bacteria composition of its GI systems in the descendant. These changes may then contribute to the sensitivity of the GI system against chemicals, because when the stressor is applied, the GI system becomes colonized, and maturation process of the immune and physiological systems of the host continues at the same time. There are several mechanisms in connection with the stress's ability to modify the bacterial composition of the GI system. These are the changes in the motility of the GI system, as well as the changes in the epithelial cell function and in the mucus secretion. Secretion of norepinephrine to the GI system during stress may stimulate selective growth of specific types of bacteria species (Collins and Bercik, 2009).

3.4 Effect of Microbiota on Brain and Behavior

Postnatal gut microbial colonization occurs in parallel with coginitive development. There are ever-increasing evidences proving the dependence of cognitive activity to microbiota, and to the metabolic activity (Dinan et al., 2015).

In patients with depression symptoms, after ingesting fructose and other sugars, profiles of their hydrogen excretion via respiration have been shown to be abnormal. Fructose malabsorption provides substrate for rapid bacterial fermentation, and this leads to change in the GI motility and microbiota profiles. In the rats, having been given *Bifidobacterium infantis* for 14 days, plasma tryptophane levels were seen to increase. It was concluded that commensal bacteria might affect the tryptophane metabolism (Desbonnet et al., 2008). The studies have shown that in response to the entry of noninvasive pathogenic bacteria in caecum, brain stem nuclei is rapidly activated, and this causes behaviors similar to anxiety also among the rats (Collins and Bercik, 2009).

Behavioral change was seen in the rats chronically injected with *Helicobacter pylori*. This infection brings about change in the gastric physiology, and it is recovered following the cure of *H. pylori*. There are also changes observed in the feeding habits. Although the mechanism mediating to the changes in the brain and behavior during and after *H. pylori* infection is not known, response to the infection requires permanent immune activation (Collins and Bercik, 2009).

Although the mechanism of the effect of microbiota on behavior is not known, it is suggested to involve immune-mediated neural and humoral mechanisms. These mechanisms involve the activation of the natural immune response in the GI system. Activation of Toll-like receptor-2, -4, and -5 in the germ-free rats is reduced, while the same is increased in the course of colonization. This is the situation that shows the interaction between these receptors and the microbiota (Collins and Bercik, 2009).

Breach of the epithelial layer and interaction with commensal bacteria stimulates the generation of immunoglobulin A and B lymphocytes. Immunoglobulin A limits the penetration of epithelium with microbiota (Collins and Bercik, 2009).

3.5 Intestinal Microbiota and Central Nervous System

Both sympathetic and parasympathetic paths of the autonomic nervous system may regulate the gut functions. For instance, such as local motility, acid secretion, bicarbonate and mucus generation, intestinal permeability, mucosal immune response. Most of these functions are affected by the sympathetic and parasympathetic effects over the enteric nervous system (Mayer et al., 2015).

Defects in the intestinal transition are associated with the increase of the microbial colonization in the small intestines. It has been reported that the number of big contractions decreases in patients with constipation, and this situation may contribute to the symptoms and constipation in the patients with inflammatory intestinal disease. The increase in the intestinal transition is characterized by the increase in the number of the big contractions, and may be seen in the inflammatory intestinal diseases dominated by diarrhea. Sleep quality, stress, and food ingestion may affect these contraction movements. Acute stress is associated with the increase in the parasympathetic activities in the small and large intestines, and with the decrease in the vagus activity in the stomach. Autonomic nervous system-mediated modulation of the mucus secretion has significant effects on the size and quality of the intestinal mucus layer found in the enteric microbiota. The autonomic nervous system also affects the epithelial mechanisms involving the activation of the immune system by the gut. This activation may occur either directly by the regulation of the intestinal immune cells, or indirectly by the modification of the lumen bacteria to the intestinal immune cells. In several respectively carried out clinical studies, it has been shown that being under stress may stimulate the increase in the permeability of the gut epithelia, facilitate the translocation of the organisms in the lumen, and may stimulate the immune response in the intestinal mucosa (Mayer et al., 2015).

Although the changes caused by the central nervous system around the gut include the cytokines, gamma-amino butyric acid (GABA), serotonin, and catecholamines, they are not solely limited to these, and they are secreted to the intestinal lumen also by the neurons and immune cells. Different types of stressors may increase the levels of the catecholamines. Metabolites

produced by the gut microbial and the cytokines are secreted during the immune response to the microbial and they give signals via the receptors on the local cells in the gut. Norepinephrine may stimulate the proliferation of some types of enteric pathogens and may escalate the lethal features of campylobacter jejunin. However, the nonpathogenic organisms and other microbial signalization molecules of the catecholamines within the gut microbiota composition, as well as the metabolic activities in the healthy individuals, are not known (Mayer et al., 2015).

3.6 Potential Mechanisms of Microbiota's Effect on the Function of Central Nervous System

3.6.1 By way of modifying the microbial composition

Administration of potential probiotic bacteria or infection agents exogenously may modify the gut microbiota composition in many ways (Cryan and Dinan, 2012).

3.6.2 Immune activation

Microbiota and probiotic agents may affect the immune system directly. Furthermore, the immune system is in a bidirectional relationship with the central nervous system, and this causes the effects of the bacteria on the central nervous system to become the primary goal. Indirect effects of gut microbiota on the natural immune system may also bring about a change in the cytokines in circulation (Cryan and Dinan, 2012).

3.6.3 Vagus nerve

Vagus nerve has a regulatory effect on multiple organ functions, such as bronchial contraction, heart rate, and gut motility. Nearly 80% of the nerve fibers are sensory and transmit information to the central nervous system regarding the status of the organs in the body. It has been shown that many of the gut microbiota's effects on the brain are associated with vagal activation. However, mechanisms independent of vagus also play a role in the microbiota–brain interaction (Cryan and Dinan, 2012).

3.6.4 Tryptophane metabolism

Tryptophane is the precursor of many biologic active agents. In many of the brain and GI system disorders, defects in the kynurenine pathway in the tryptophane metabolism are not taken into consideration. The metabolic pathway of kynurenine is catalyzed either by indoleamin 2,3-dioxygenase or tryptophane 2,3-dioxygenase enzymes. There are several studies showing that *B. infantis* may modify kynurenine concentration (Cryan and Dinan, 2012).

3.6.5 Microbial metabolites and neurometabolites

Gut bacteria regulates various metabolic reactions, such as short chain fatty acids, choline and bile acid, which are essential for the host's health (Cryan and Dinan, 2012).

Bacteria have the capacity to form neurotransmitters and neuromodulators. It has been determined that *Lactobacillus* spp. and *Bifidobacterium* spp. produce GABA; *Escherichia* spp., *Bacillus* spp., and *Saccharomyces* spp. produce noradrenaline; *Candida* spp., *Streptococcus* spp., *Escherichia* spp., and *Enterococcus* spp. produce serotonin. (Cryan and Dinan, 2012).

4 The Microbiota–Gut–Brain Axis and Autism Spectrum Disorder

The human brain has inner passages, which are critical in the configuration of gut microbiota. Prior to being turned into active excipients, ingested nutrients and chemicals pass through these passages; they are then absorbed, and enter into systemic circulation directly by the mediation of the hepatic portal vein (Tuohy et al., 2015).

Western diet, use of drugs, and excessive hygiene are regarded as the probable causes of the inexplicable increase in the various chronic diseases, including the autoimmune and brain diseases, by way of modifying the human gut microbiota. It is suggested that factors associated with microbiom are regarded as the probable causes of the increase in the prevalence of autism (Mayer et al., 2014). Since the association between gut microbiota and autism has been defined, the number of studies indicating the importance of the microbiota–gut–brain axis in the development and occurrence of autism has been on the rise (Tuohy et al., 2015).

4.1 Nutritional Problems in Autism Spectrum Disorder

More than 90% of the children with autism encounter problems associated with nutrition. Choosy eating is the most common nutrition problem associated with autism. Children with autism who also encounter the problem of choosy eating consume foods with specific tissues, shapes, and colors. While such children strongly prefer consuming starch, processed foods, and junk foods, they reject fruits, vegetables, and/or proteins. This sort of nutrition leads to an increase in the risk of occurrence of nutritional problems, and/or associated medical problems in the children with autism. Probable detrimental outcomes of this situation are weaker bone growth and vitamin/mineral insufficiencies (Mulle et al., 2013).

Etiological factors contributing to the nutrition problem seen in autism spectrum disorder have not been understood. Although constipation and encopresis are seen at a higher level among those with autism in comparison to those without autism, this situation is suggested to arise primarily not from an organic etiology, but from a neurobehavioral association. Various environmental and behavioral factors are assumed to cause the nutritional problems seen in autism. It has further been shown that choosy eating in autism arises from the mixed behaviors adopted by the children. Current theories fail to explain the tendency of children with autism to refuse consuming fruits and vegetables, and to prefer processed and junk foods (Mulle et al., 2013).

The major functions of the healthy gut microbiota (particularly those of the members of the Bacteroidetes species) are to assist in the breakdown of complex vegetal polysaccharides and other indigestible dietary foods, and to support digestion and host's health. That is why deviations in the generation and regulation of the gut microbiota in the autism spectrum disorder may bring along discomfort and pain in the digestion of vegetal nutrients. Those with autism, thus, abstain from the pain arising from the respective difficulties by refusing to consume such nutrients. This hypothesis is consistent with the studies showing that long-lasting nutrition problems are the learned behaviors depending on the function of abstaining from unpleasant nutritional experiences. Although the correlation between gut dysbiosis, diet type, and nutrition problems in the autism spectrum disorder is in need of explanation, the approach of correcting such behaviors as anxiety, irritation, and desocialization seen in autism by way of restoring the microbial balance in the gut seems logical. Indeed, it has been shown in the respectively carried out studies that probiotics are capable of correcting the behavioral problems in association with the GI disorders seen in autism (Mulle et al., 2013).

About one-third of the children with autism suffer with GI problems, such as stomachache, diarrhea, and chronic constipation. In a study focusing on the mucosal immunity in autism for the purpose of establishing the association between GI symptoms and autism, bulge, stomachache, constipation, and diarrhea were seen among 18%–40% of the patients with autism. Low disaccharides enzyme activity, increased intestinal permeability, and bacterial increase were also reported from such persons (Ashwood et al., 2004). In a study carried out among 1513 children at 20–60 months of age, flatulence, bulge, constipation, and diarrhea prevalence was seen to be 6–8 times more in those with autism in comparison to those without autism. Besides, there is a strong correlation between the intensity of autism and GI symptoms (Li and Zhou, 2016). In another study carried out among 2973 children with autism, it was seen that 24% of them had at least one of the GI problems, and that they were associated with hypersensory sensitiveness and anxiety (Li and Zhou, 2016). In a study of 111 control groups with medium-level behavioral problems, 48 control groups with severe behavioral problem with autism, and of 66 healthy control groups, the association between GI symptoms and behavioral problems were sought. In the study, behavioral disorders among children were found to be not associated with GI symptoms, intestinal inflammation, microbiota, and intestinal permeability; however, the children with autism displaying severe behavioral disorder were found to have more enterocyte damage than those with medium-level behavioral disorders than those from the healthy group (Pusponegoro et al., 2015). In another study carried out in relation to the same issue, upon comparing the feces of children with autism (22 boys, 6 girls) with those of the healthy control group without autism, no difference was found in terms of microbial composition; however, examining them individually, 1 of each 3 persons with autism were found to have abnormal gut flora (Gondalia et al., 2010).

Although the cause of the GI problems seen in autism is not clear, it is suggested that developmental deficits seen in the nervous system may lead to this. While *Choromodomain helicase* DNA-binding protein 8 (CHD8) gene is one of the important candidate genes in autism, constipation prevalence among the children with CHD8 mutation is quite higher than those without the said mutation (60 and 26%, respectively) (Li and Zhou, 2016).

4.2 Animal and Human Studies

4.2.1 Animal studies

The first evidence of the association between the gut microbiota disorders and neurobehavioral diseases has been put forth on the basis of the germ-free rats. Germ-free rats are delivered by caesarean section, and kept in a sterile gnobotic environment. These rats are deprived of all microorganisms. In this way, researchers attempt to attain information regarding how the presence or absence of the gut microbiota affects the behaviors. In a study, stress was shown to lead to an increase in the adrenocorticotropic hormone and corticosterone levels in the germ-free rats in comparison with the group not containing specific pathogen. This exaggerated response was seen to recover after *B. infantis* was administered to germ-free rats (Rosenfeld, 2015).

When pregnant rats were administered daily with PPA (as a bacterial metabolite) 500 mg/kg, or with lipopolysaccharide (LPS) 50 mg/kg via injection, this was shown to lead to behavioral disorders similar to autism spectrum disorder in the male and female descendants. In the same study, while males undergoing LPS treatment were found to be ultrasensitive in the voice test, the same sensitivity toward the same test was found to arise in the females upon being exposed to pre- and postnatal PPA (Rosenfeld, 2015).

Valporic acid is a drug used generally in the treatment of epilepsy and other neuropsychological diseases. There is an association between the intake of this decision by the mother during pregnancy and the risk of the future occurrence of autism spectrum disorder in the child the same mother delivered. It was shown that social behavior insufficiencies similar to autism and gut disbiosis were found in the rats having been exposed to VPA in uterus. It was ascertained that administration of immune stimulant polyinosinic:polycytidylic acid (Poly I:C) to mother during pregnancy led to a critical disorder in the descendant's gut–microbiota–brain axis. Administration of *B. fragilis* restores gut impermeability and microbiota population, and thereby diminishes the disorders in the communicative, stereotypic, anxiety-like, and sensorimotor behaviors (Rosenfeld, 2015).

4.2.2 Human studies

As it is known, GI system disorders are generally seen in the patients with autism spectrum disorder. Disorders in the gut microbiota form a thin line with regard to the connections between these two distinct systems. For instance, it is admitted that intestinal colonization of *Clostrum tetani* escalates the intensity and risk of autism (Rosenfeld, 2015).

In a study conducted among children from 37 to 208 months of age, 23 with autism, 22 were the normally growing brothers/sisters, and 9 were from the control groups, it was shown that, the number of *Suturella* spp. in the feces of children with autism is higher in comparison to those of the control group, and that the number of *Ruminococcus torques* was higher in those with autism having functional GI disorders than those with autism not having functional GI disorder (Wang et al., 2012). In a similar study, it was found that while the ratio of Bacteroidetes/Firmicutes in the fecal microbiotas of the autistic children decreased, the amount of *Lactobacillus* spp. increased. In the same study, it was shown that *Desulfovibrio* spp. tended to increase in the children with autism, and that the amount was strongly associated with the intensity of autism (Tomova et al., 2015). In another study, it was shown that the species of *Prevotella*, *Coprococcus*, and unclassified Veillonellaceae were found less in the children with autism (Kang et al., 2013). In numerous follow-up studies, such clostridial groups as *Clostridium bolteae* were shown to be significantly high in the autism spectrum disorder. *Desulfovibrio* is an anaerobic bacillus with different lethal factors and is found abundantly in those with autism. In a study, it was ascertained that, not only *Desulfovibrio* spp., but also *B. vulgatus* was found was found in high amounts in the feces of the intensely autistic children. On the contrary, Firmicutes was ascertained to be dominant in the feces of the control group without autism (Finegold et al., 2010).

In another study, while high amounts of *Sarcina* and *Clostridium* species, as well as *Alistipes* and *Akkermansia* species were found in the feces of the children with autism, Eubacteriaceae and *Bifidobacterium* species were found to be decreasing (De Angelis et al., 2013). It was found in another study that while acetic, butyric, isobutyric, valeric, and isovaleric acids were high in the feces of the children with autism, caproic acid decreased (Wang et al., 2012).

4.3 Disturbance in the Microbiota–Gut–Brain Axis Contributing to Autism

Numerous chemicals produced by the gut microbiota in the gut, including tryptophane, dopamine, serotonin, GABA, hydroxy butyrate, choline, taurine, acetate, succinate, lactate, acetyl-CoA, creatine, betaine, glutamate, glutamine, fatty acids, hippurate, are important for the brain chemistry. GABA and serotonin has been shown to play a critical role in the neural development in autism. Levels of both serotonin and GABA have been modified in those with autism. Both of these two neurotransmitters are provided from amino acid catabolism, serotonin from tryptophane, GABA from glutamate. Metabolites derived in the gut may directly or indirectly affect the neurological development in the brain, on the condition that they pass through the blood–brain barrier. For instance, bacterial GABA may either show its effect on the intestinal nervous system locally, or modify the plasma GABA concentration; however, it may not pass through the blood–brain barrier in healthy persons, but acetate passes through the blood–brain barrier rapidly (acetate is substrate for the cholesterol biogenesis in the cells of the mammals). On the other hand, undesired chemicals may leak to the brain through passage in the course of oxidative or inflammatory stress. It is

known that there is compromise in the passage through blood–brain barrier in autism (Tuohy et al., 2015).

The effect of gut microbiota on both the metabolite profiles and on the physiological parameters has been identified. It is seen that gut microbiotas of the patients with autism are quite different than those of their healthy peers without autism. Abnormal gut microbiota composition and activity is defined as a feature of autism. In recent studies, it has been shown that bacterial LPS triggers chronic systemic inflammation. Permanent blood–brain barrier damage, permeability, and change in behaviors have been ascertained in the rats exposed to systemic inflammation caused by LPS (Tuohy et al., 2015).

Most of those with autism have an intense history of antibiotic intake. Oral antibiotics disarrange the protective microbiota and lead to the proliferation of anaerobic bacteria in the gut. Such bacteria as Clostridia, Bacteroidetes, and *Desulfovibrio* may stimulate the GI symptoms and the behavioral problems seen in autism. These bacteria may also produce some of the metabolites, which contribute directly to the pathology of autism. For instance, *C. tetani* produces powerful neurotoxin (tetanus neurotoxin). Tetanus neurotoxin may pass the vagus nerve and follows a path through the intestinal system toward the brain. Tetanus neurotoxin disarranges neurotransmitter secretion and this ends up with various behavioral disorders observed in autism. 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA), as a metabolic product specific to *Clostridium* species, depletes the catecholamines in the brain, and leads to autism symptoms (Li and Zhou, 2016).

4.3.1 Short chain fatty acids

Short chain fatty acids are capable of affecting the brain activity directly upon passing the blood–brain barrier. The level of short chain fatty acid in the feces is affected by certain factors, such as soluble pulp ingested with diet, transit time, drug intake, and bacteria amount. There are also studies available that show that the levels of short chain fatty acids and ammonia are high in the feces of the children with autism. Increased levels of butyric acid in the feces are associated with the disorder in the social behavior of the male offspring of the rats exposed to valproic acid. The mother's physical condition may also affect the autism risk in the baby (Li and Zhou, 2016). Foley et al. (2014), in a study they conducted with the rats, showed that exposure to propionic acid in the prenatal period led to critical disorders in the social behaviors in the neonatal and adolescent periods. More than the necessary amount of propionic acid may easily enter into blood circulation, and may modify the neurotransmitter secretion and neural activity in the brain upon passing the blood–brain barrier (Li and Zhou, 2016).

Propionic acid is found in the gut together with the other short chain fatty acids, such as acetate and butyrate. All of these short chain fatty acids are the major metabolic products of the enteric bacteria. Propionic acid is found naturally in various nutrients (such as cheese), and is used as a preservative in numerous processed foods. Propionic acid is produced by

intestinal bacteria usually in the gut lumen. Propionic acid is a weak organic acid, rapidly passing the gut–blood barrier and metabolized especially in the liver. While proper levels of propionic acid have such benefits of restoring insulin sensitivity, lowering cholesterol, and lowering food intake, excessive levels have negative effects on health and behavior. Propionic acid is effective on neurotransmitter synthesis, calcium influx, lipid metabolism, mitochondrial function, and on the immune activation, and this is suggested to contribute to the behaviors observed in autism and to the biochemical findings (MacFabe, 2012).

4.3.2 Amino acid metabolism

Since amino acid metabolism plays a critical role in the biosynthesis of the metabolites, including the neurotransmitters, it is suspected to play a role also in the autism spectrum disorder. There are studies available showing that glutamate/glutamine (Glu:Gln) ratio in the blood of the persons when autism is high. In comparison with the healthy control group, it was found that plasma glutamic acid, phenylaline, asparagine, tyrosine, alanine, and lysine was higher; however, glutamine concentration was lower in those with autism and in their families. It has been ascertained that amino acid transport, and later neurotransmitter generation is modified in the fibroblasts of the individuals with autism. Fibroblasts use the same amino acid transporter with the brain, and it is therefore suggested that the amino acid transport modified in the fibroblasts also indicates the modification of the amino acids' blood–brain barrier transport (Tuohy et al., 2015). In a study conducted with 48 persons with autism spectrum disorder and 53 persons of a peer control group, glycine, serine, threonine, alanine, histidine, and glutamyl amino acids were in lesser quanitities in urine samples of those with autism as compared to those of their healthy peers. In the same study, it was observed that the profiles and oxidative stress markers of the gut microbiota metabolites were also modified (Ming et al., 2012).

Conversion of glutamate to glutamine is important for the avoidance of ammonia toxicity. That is why the increased glutamate/glutamine ratio in the blood of those with autism is the indicator of ammonia detoxification and modification in the glutamate circuit in the brain, and this situation may be effective in the behaviors of the patients. Diets containing lower pulp and higher protein may cause an increase in the systemic contribution of the ammonia, derived in the gut by proteolytic microbiota from the amino acid fermentation, and may thereby worsen the ammonia toxicity. Catabolism of the amino acids contained in the diet may either affect the amino acid availability or ends up with the production of active biologic ingredients, such as short chain fatty acids, or branched chain fatty acids (Tuohy et al., 2015).

Amino acid levels in the blood and urine are affected from numerous factors, including amino acid absorption from the digested foods, amino acid and protein degradation, protein secretion, and feces and protein excretion. Changes in the amino acid ratios are effective on the metabolic pathways requiring amino acid, including the generation and ratios of different

neurotransmitters. Most of the amino acids are produced either from diet or endogenously; however, gut microbiota, too, may be effective in the amino acid catabolism or generation. Besides, β-alanin may be formed by *Candida albicans* in the gut over propionate and ammonia reaction. β-Alanin is found in meat and hinders the uptake of GABA by the intestinal cells. HPHPA is the metabolite of *m*-tyrosine produced by some of the *Clostridia* species. *m*-Tyrosine is the oxidation product of phenylalanine and has been shown to cause symptoms similar to autism spectrum disorder in laboratory animals. HPHPA is seen among persons with *C. difficile* infection, and levels increase too much during acute psychotic periods of schizophrenic patients. Cholesterol supplementation used in baby formulas may modify the acid profile in the brain by way of increasing lysine and glycine supplementation and decreasing γ-aminobutyrate, leucine, isoleucine, methionine, alanine, β-alanine, theronine, glutamine, and glutamate concentration. DHA, too, has similar effects, other than decreasing taurine level and being ineffective on leucine and isoleucine. Although amino acid metabolism is closely associated with the biology of autism, more studies should be conducted on the relations between gut microbiota, nutrition, and amino acid metabolism in autism.

4.3.3 Lipid metabolism

Linoleic and α-linoleic acids are essential fatty acids. They are not produced by mammals, so they are to be taken with diet. These essential fatty acids and derivatives play critical roles in brain development, phospholipids generation, and membrane fluidity. In comparison with arachidonic acid (ARA) and DHA, the brain has lower linoleic and α-linoleic acid concentration. DHA has a critical structural role in the brain, and plays a role in cell signalization and cellular proliferation. Arachidonic acid is necessary for signal transduction and cell growth. It is suggested that both ARA and EPA play a critical role in the brain function. ALA and LA derivatives are converted by phospholipases to eicosanoids. Eicosanoids are thought to act as local hormones to activate the immune cells, and to initiate the platelet aggregation. On the contrary, DHA and EPA may be converted to antiinflammatory resolvins and protectins. Some of the diet intervention studies conducted by $n-3$ and $n-6$ fatty acids the symptoms were recovered in the persons with autism spectrum disorder. In 13 persons with autism spectrum disorder administered with ARA + DHA supplement, social behaviors were observed to get better. In a study, it was shown that ALA supplementation modified fatty acid profile in liver, adipose tissue, and in the brain, and that administration of bifidobacterium breve together with ALA could have modified the fatty acid profile much more (Wall et al., 2010). It was further seen in the same study that DHA was found higher and ARA lower in the brains of the animals administered with $n-3$ + probiotic in comparison to the same group administered only with ALA. In another study, while DHA and ARA were seen to increase in the brains of the rats administered only with *B. breve*, probiotics were also found as capable of modifying the fatty acid profile in the brain (Tuohy et al., 2015).

In the neurological disorders and in the developmental and degenerative disorders of the brain, such as autism spectrum disorder, depression, and schizophrenia, irregular phospholipids metabolism, fatty acid insufficiencies, or dyslipidaemia may be seen. It is suspected that both abnormal cholesterol metabolism and phospholipids metabolism play a pathological role in autism. In the persons with autism, phospholipase A2 is higher in the blood, ARA is decreased in the cell membranes, and $n-3/n-6$ ratio is probably higher (Tuohy et al., 2015).

Brain-derived neurotrophic factor (BDNF) facilitates synaptic transmission by way of modifying the secretion of the neurotransmitters. BDNF is reduced in the autism spectrum disorder. Betterment in the behaviors of the animals administered with probiotics is associated with BDNF changes in hippocampus and amygdala. However, the same result was not attained from all of the probiotic studies. In a study conducted with rats, it was shown that pulp of the prebiotic diet increased BDNF in the brain (Savignac et al., 2013). Upregulation of BDNF by probiotics may also be effective on novo lipogenesis in the brain, facilitate synaptic vesicle formation, and may thereby lead to the betterment of the brain functions in the children suffering from autism spectrum disorder. However, more studies should be conducted with both humans and animals on this issue.

5 Treatments to Modify the Gut Microbiota in Order to Recover the Symptoms in Autism Spectrum Disorder

Many treatments have specific effects on the gut microbiota, the effects of some of them on specific bacteria, and/or metabolites are at the level of presumption with long-term effects not being known.

5.1 Antibiotics

Various studies have shown that antibiotic administration is high among the children with autism. Vancomycine and metronidazole are the two antibiotics administered in the treatment of autism symptoms. Vancomycine targets Gram-positive bacteria and is not absorbed from the GI system. Metronidazole, too, targets the same gut bacteria; however, it is less reliable due to its side effects (Frye et al., 2015).

Vancomycine was administered for 8 weeks to 11 children with autism, having GI symptoms and high irritability, and it was observed that for 8 of them, vancomycine had a temporary effect. Therapeutic response to vancomycine may be associated partly or wholly with the metabolic dysfunction arising from the overproduction of short chain fatty acid fermentation products. However, more objective clinical studies should be conducted to clearly understand the effects of the antibiotics on the autism symptoms, and the underlying mechanisms.

5.2 Antifungals

Despite insufficient evidences indicating too many fungal growths in the children with autism, antifungal agents are administered in the clinical practice. A study showed that there was no difference between the children with autism and those growing typically in terms of ferment in their guts. Families state that antifungal treatment is mostly beneficial and rarely worsens the symptoms. While the toxicity of the antifungal treatments is not known, tracking of liver transaminases may be required for safety (Frye et al., 2015).

5.3 Probiotics

Probiotics show positive effects on the guts by way of modifying the immunity of the host and restoring the normal commensal bacteria on the one hand, and suppressing the pathogen bacteria, stabilizing the intestinal mucosal barrier, which lowers the absorption of harmful bacterial metabolites, because it stimulates mucine generation, and supporting the synthesis of the antioxidant substances on the other hand (Rosenfeld, 2015). It was shown that in 19 children with autism having undergone probiotic treatment, Bacteriodetes/Firmicutes ratio and *Desulfovibrio* spp. and *Bifidobacterium* spp. amount was recovered (Rosenfeld, 2015). In another study, it was reported that upon administering probiotics to autistic children with a high ratio of D-arabinitol and D-/L-arabinitol in urine, the metabolic disorder in question was ameliorated, and the children's behavioral performance was also improved (Kaluzna-Czaplinska and Blaszczyk, 2012).

Administration of oral probiotics is successfully applied in the treatment of GI problems. In a study conducted respectively, it was shown that oral administration of *L. plantarum* WCFS1 significantly increased the number of *L. enterococci*, and decreased the number of Erec482 (*Clostridium* cluster XIVa). *L. plantarum* WCFS1 treatment furthermore corrected the fecal consistency and the behavioral disorders of those with autism (Li and Zhou, 2016).

Although the mechanism of the remedying effects of probiotics are not clear, studies have shown that the probiotics target the neurotransmitters in circulation, and the neuroimmune responses within the microbiota gut–brain axis. It has been shown that myloperoxidase concentrations, which are the indicators of inflammation and oxidation, are decreased in 85 children with autism, having been administered probiotics (Russo, 2015). Also another study showed that behavioral abnormalities associated with autism, as well as the GI disorders were significantly declined by means of the *B. fragilis* treatment (Hsiao and McBride, 2013). *B. fragilis* treatment also restores the intestinal barrier integrity. In view of the foregoing reasons, probiotics are seen to maintain a promising therapy for the autism spectrum disorder.

Although the administration of probiotics to children with autism is encouraged, there are scarce number of clinical studies in relation with the quality and effectiveness of the commercial probiotics and there are certain limitations in question. One of them is

that they contain a small portion of the total bacteria within the gut. For instance, while human gut contains more than 1000 different types of bacteria, probiotics generally contain less than 10 types of bacteria. Another limitation is that they are produced from milk, and human gut microbiota is not a natural environment for such organisms (Frye et al., 2015).

5.4 Digestive Enzymes

There are defects in the some of the intestinal digestion enzymes, particularly in those responsible for the digestion of carbohydrates. Under such a circumstance, the indigested carbohydrates are transported to the large intestines, and may stimulate bacterial fermentation. However, the outcomes of the clinical studies in which digestion enzymes are administered are contradictory. In a study, 46 persons with autism were administered both carbohydrates and protein enzymes in all meals. Recovery was observed in the autism symptoms. In another study, only the protein enzymes were administered for 1 meal a day, and no recovery was observed in the autism syndromes (Frye et al., 2015). Apart from being a quite challenging field of treatment, more studies should be conducted.

5.5 Vitamins

While vitamin deficiencies may modify the composition of the gut microbiota, gut microbiata, on the other hand, may modify the specific vitamin and mineral requirements. For instance, *Lactococcus* spp. is associated with folate biosynthesis. Biotin and B_{12} are essential factors for the breakdown of propionic acid. Insufficiency of these vitamins in the diet may impair the propionic acid and carnitine metabolism, and may contribute to the mitochondrial dysfunction. In a study where all vitamin levels in the children with autism were calculated, it was found that their biotin levels were quite lower than those of their healthy peers. Biotin is a vitamin synthesized by the bacteria in the gut microbiata. Gut bacteria and the vitamin requirements and bioefficacy involve so many complex interactions that require more studies (Frye et al., 2015).

5.6 Unprocessed and Fermented Nutrients

Fermented nutrients and those like raw camel milk possess natural bacteria and prebiotics, and are theoretically capable of affecting gut bacteria by various means. In some preliminary studies, camel milk was shown to be beneficial for the children with autism. In a study during which either raw or boiled camel milk was consumed, children with autism showed significant recovery in their autism symptoms according both to their glutathione, superoxide dismutase, and myeloperoxidase calculations, and to Childhood Autism Rating Scale (CARS) index; however, cow milk has not shown any similar effect (Al-Ayadhi and Elamin, 2013). Because the boiled camel milk also became effective, the effect in question is suggested not

to arise from live bacteria. In another study, only raw camel milk was shown to bring recovery in the autism symptoms (Bashir and Al-Ayadhi, 2014). Although there are studies showing the positive effects of fermented nutrients, such as kefir, they are still inadequate (Frye et al., 2015).

5.7 Diet Treatments

Diet emulsifiers may modify composition, and proinflammatory potential of the gut microbiota. Changes brought along by diet in the microbiota may affect the serum metabolites, and modify the brain activity of the host, which is why some of the nutrients may show remedying effect on the disorders associated with autism (Li and Zhou, 2016).

In some of the studies, during which children with autism were administered a gluten- and casein-free diet, and the effect of which was observed, improvements were detected on the hyperactivity and daily life skills of those children (Whitely et al., 2010). Persons with autism are not capable of metabolizing gluten and casein fully to peptides. Due to the more porous structure of their guts, it is put forth that peptides move to the blood circulation, pass through the blood–brain barrier, and bind to the opioid receptors. This effect is described to end up with such typical autistic behaviors as monotonous body movements, introversion, looking after the parts of the objects, and overreaction toward the changes in the routines (Feucht and Ogata, 2010). In gluten- and casein-free diets, gluten-containing products and diary products are totally excluded from the diet.

A specific carbohydrate diet is another diet being administered in the treatment of autism spectrum disorder. The purpose of this diet is to recover the malabsorption symptoms and to avoid the growth of pathogenic intestinal microflora. This diet basically recommends monosaccharide intake from vegetables, particular vegetables, and from honey sources; however, it also recommends the limitation of complex carbohydrate consumption. Digestion of polysaccharides lasts longer than the digestion of monosaccharides. Nutrient residuals may lead to malabsorption upon laying the groundwork for the proliferation of pathogenic intestinal flora (Kawicka and Regulska, 2013).

A ketogenic diet is administered in the treatment of diet-resistant children with epilepsy. Canitano et al. (2005) have determined the epilepsy prevalence among autistic children at 2–3 years of age as one-third. Due to the fact that the prevalence of epilepsy is high among those with autism, it is suggested that a ketogenic diet may show a positive effect on the mechanism of the neurological diseases (Canitano et al., 2005).

In the dysfunction of the GI system, such substances as oxalate may disrupt the neurological development of the child, and may lead to abnormalities in the nervous system. In a study conducted among patients with autism, it was shown that plasma oxalate concentration was

3 times higher in comparison with the ordinary values, and 2.5 times higher in urine. That is why it is suggested that higher oxalate concentrations in blood and urine may be one of the causes of the pathogenesis of autism. It is recommended in the low oxalate diet to limit daily oxalate intake with 40–50 mg/day, and to provide supplemental support to the patients (Kawicka and Regulska, 2013).

6 Summary

Gut microbiota is effective on the host's health and on the diseases. Microbiota, or the products thereof, affect the brain by means of various mechanisms. Some of the key nutrients, normal gut development, neurobehavioral patterns, and immunological function are dependent on gut microbiota. Diversity and amount of the bacteria existing in the gut microbiota affects the host's health directly. These variations may modify other responses by ways of affecting metabolic profiles, virulence factors, immunological responses, and neurological functions.

Changes in the gut microbiota may be effective for the development of autism spectrum disorder. Frequently observed GI problems and gut microbiota disorders are considered as its indicators. Restoration of the balance in microbiotal–gut–brain axis has positive remedying effects on the autistic disorders. To ascertain the role of the microbiota on the etiology of autism spectrum disorder, more randomized double blind clinical studies should be conducted in the future.

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