The Role of Gut Microbiota in Autism Spectrum Disorder

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SUMMARY

Human microbiota are colonies of microorganisms located in different parts of the human body with diverse functions. Healthy gut microbiota comprises differing ratios of microoganisms wholly contributing to metabolic and other molecular reactions in a healthy, functioning body. After the demonstration of the bidirectional interaction between the central nervous system and gut microbiota through neuroendocrine, neuroimmune, and autonomic nervous mechanisms, investigations have been started on the microbiota-gut-brain axis in psychiatric disorders. Autism spectrum disorder (ASD), which is a neurodevelopmental disorder of early childhood, is one of these disorders. Most of such studies were cross-sectional and mainly investigated the bacterial species. Changes in gut microbiota composition and the leaky gut syndrome are some of the hypotheses proposed to explain the core symptoms and gastrointestinal (GI) symptoms of ASD. Probiotics, prebiotics, fecal microbiota transplantation, diet have been proposed as treatment options. However, the role of microbiota in diagnosis, follow-up, and treatment is not yet clear. The bidirectional interaction between central nervous system and intestinal microbiota makes it difficult to establish the cause-effect relationship. The current data on microbiota may be useful to plan patient-specific treatment in autistic children with GI symptoms. This article aims to review the results of the studies on microbiota in animal models and children and discuss the emerging clinical relationship of ASD and gut microbiota.

Keywords: Microbiota, gut, autism spectrum disorder

INTRODUCTION

Although the human body has a physical and molecular protective mechanism against microorganisms, some body parts are a natural ecosystem that allows microorganisms to live. Studies on these microorganisms have increased considerably due to the development of anaerobic culture techniques, the new generation nucleotide sequencing technique of the 16S RNA and mass spectrometry (Fouhy et al. 2012). The microorganisms colonising different ecosystems of the human body are referred to as the microbiota such as the intestinal, oral and skin microbiota and their genetic material is called the microbiome. The Human Microbiome Project was started in 2012 to investigate the microbiota and microbiome of the human body, and it is still ongoing (Human Microbiome Project Consortium 2012). Identifying the microorganisms species making up the microbiota needs to be supplemented by further investigations to explain their effects on disease and health; their relation to each other and their effects on the body. The highest concentration of the GIS microbiota, consisting mainly of bacteria, is in the colon and, hence, the bacteria colonising the colon, formerly called as the gut flora, are referred to as the gut microbiota (Savage 1977). Bacterial colonization of the human body begins with vaginal delivery when the gut bacterial flora of the new born mainly consists of the Lactobacillus genus, compatible with that of the mother's vagina, whereas the gut flora of babies delivered by caesarean section is predominated by the Clostridium genus. In the first year of life Actinobacter and Proteobacter have dominance which shifts to an adult-like profile with the predominance of the Firmicutes, Bacteroidetes ve Actinobacteria, and particularly

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the Bifidobacterium, around the age of 2 (Rodriguez et al. 2015). The adult ratio of anaerobic / aerobic bacteria is 1000/1. However, eubiosis, or the healthy balance of the microbiota function in the gastrointestinal tract, is a reference to their mutually cooperative effects on the maintainence of a healthy metabolism and not to defined relative counts of these microorganisms, which change between individuals (Shafquat et al. 2014). Our knowledge of other members of the microbiota, such as the archaea, viruses and eukaryotes, is still limited.

The human microbiota, in addition to preventing colonization by pathogenic microorganisms and supporting the immune system, also perform functions specific to their habitat such as the synthesis of vitamin K, stabilisation of the intestinal barrier and production of short-chain fatty acids (SCFA) (Backhed et al. 2005).

Fermentation of galactooligosaccharides and fructooligosaccharides from the mother's milk or adapted infant formulas by the gut microbiota results in the production of the SCFA acetic acid / acetate, propionic acid (PPA) / propionate and butyric acid / butyrate. After their fast absorption, only 5% of the SCFAs are found in feces. Lactic acid / lactate and succinic acid / succinate are the precursors of SCFA. While acetate is the major product, butyrate is the main energy source of the colonic epithelial cells and also prevents intestinal inflammation. SCFAs are capable of crossing the blood-brain barrier and are effective on histone acetylation in the human genome and as endogenous ligands of G protein-coupled receptors (Koh et al. 2016).

Expressions in the brain of neurotrophins and proteins such as PSD-95, synaptophysin and the brain-derived neurotrophic factor (BDNF) affecting brain development and plasticity have been found to be affected by gut microbiota (Diaz Heijtz et al. 2011, Bercik et al. 2011, Douglas Escobar et al. 2013). The central nervous system (CNS) can affect the gut microbiota by controlling food intake through peptides associated with appetite, by regulating gut motility by cortisol release through the hypothalamo-pituitary-adrenal axis (HPA), regulating mucin release by the intestinal epithelial cells and gut motility through neural pathways of the sympathetic and parasympathetic system (Wang and Kasoer 2014). Dysbiosis is the perturbation of the gut microbiota and has been subject of increasing investigations in neuropsychiatric disorders as a result of data accumulation on the bidirectional gut-CNS interaction, already called the microbiota-gut-brain axis (Averina and Danilenko 2017) although its significane in disease prevention and treatment has not yet been understood.

The microbiota-gut-brain axis activity is based on the neuroendocrine, neuroimmunity and the autonomic nervous system intermediations. The most important hypothesis about the disruption of this interaction is the leaky gut hypothesis. The gut microbiota is important in providing the integrity of the intestinal epithelium, the mucosal barrier and in determining which molecules pass into the blood. Dysbiosis results in the leaky gut by inflammattory disruption of the physical gut barrier, facilitating penetration of the mucosa and entry into the systemic circulation by bacteria, toxins and metabolites (Liu et al. 2005). Gut microbiota stimulates the secretion of proinflammatory cytokines such as IL-1, IL-6, IL-18, known to be increased in neuropsychiatric disorders (Petra et al. 2015, Maynard et al. 2012). Impairment of the innate functions of gut epithelial cells allow passage microorganisms such as Lactobacilli and microbiota products including lipopolysaccharides (LPS), serotonin and gammaaminobutyric acid (GABA) resulting in stimulation of the mucosal immune system and release of inflammatory cytokines IL-6, IL-1β, IFN-γ and TNF-α. Reduced immune response after vagotomy in animal model with GIS infection demonstrated the gut microbiota-CNS interaction conducted on the vagal pathway (Wang et al. 2002). Neuropeptides and other factors including dopamine, serotonin, GABA and SCFAs secreted by the stimulated enteric neurons are seen to affect brain functions through the vagal nerve or directly by passing the blood-brain barrier. Corticotropin release factor (CRF) release by vagal or cytokine activated hypothalamo-pituitary-adrenocortical (HPA) axis further increases gut permeability by intestinal cytokine release (Yarandi et al. 2016, Li et al. 2017). Microbiota may affect the brain by altering the plasma levels of kynurenic acid, quinolinic acid and serotonin, the end products of tryptophan metabolism, that can cross the blood-brain barrier (O'Mahony et al. 2015).

Autism spectrum disorder (ASD) is a neurodevelopmental psychiatric disorder of early childhood with symptoms beginning before the age of 36 months. It is characterized by deficits in social communication and interaction, stereotypical behaviors and restricted and/or circumscribed interests (American Psychiatric Association 2013). The cause being unknown, genetic and environmental factors are thought to have combined aetiological roles (Risch et al. 2014). In children with ASD, GIS complaints such as constipation / diarrhea, gastric reflux and food intolerance are common (Coury et al. 2012). Within the context of the brain-gut-microbiota axis, investigations have been started on the roles of gut microbiota on the basic symptoms of ASD and GIS. In this article, the results of the studies on microbiota in animal models of ASD (Table 1) and in children with ASD (Table 2) will be discussed.

METHOD

Pubmed, Google Academic and Web of Science databases were searched using the keywords "autism, microbiota, intestine, gut and animal model". The articles published between January 1975 and July 2018 were examined and the findings of the research and review articles of especially the recent years were given precedence.

The Relationship Between ASD and Gut Microbiota in Animal Models

Since the first use of mice without microorganisms or germ free (GF) mice in 1959, the microbiota has been understood to have an effect on every system in the body (Wostmann 1981, Yi and Li 2012). The effect of gut colonization on brain plasticity was noticed by normalization of the high stress response in the HPA axis in GF mice after colonization with a gut microbiota bacterium (Sudo et al. 2004). Microbiota may also alter blood-brain barrier permeability in mice by affecting endothelial binding molecules occludin and claudin-5 in tight junctions ((Braniste et al. 2014).

The effect of microbiota on social behavior was understood as experimental GF mice spent more time in an empty chamber while normal mice spent time in a chamber with another mouse and this preference was normalized after bacterial colonization. In addition, the normal mice spent longer time while the GF mice spent less time in the chamber with the 'familiar' mouse, and this was attributed to less willingness of the GF mice to socialize, thus giving rise to the idea of starting investigation on the microbiota in ASD (Desbonnet et al. 2014).

It is being thought that the socialising behaviour of GF mice is affected by a combination of factors including morphological changes in their brains especially involving the amygdala and the hippocampus, the involvement of gut microbiota in essential pathways such as the citric acid cycle, synaptic long-acting potential, cAMP-mediated signalling and also in neuronal communication by molecules such as serotonin, glutamate, dopamine (Needham et al. 2018).

One of the experimental animal models for ASD relies on maternal immune system activation (MIA) during gestation with the use of viral mimic polyinosinic: polycytidylic acid (poly I:C) to activate the immune system in pregnant rodents. The offspring born after MIA demonstrated ASD-like behaviors, increased levels of gut permeability and gut originated molecules such as 4-ethylphenylsulfate, indolepyruvate, serotonin and dysbiosis with increased Lachnospiraceae of the Clostridia class. These ASD-like symptoms were reversed by probiotic administration of Bacteroides fragilis (Hsiao et al., (2013). Also, inhibition of the proinflammatory cytokine IL-6 entry to the circulation in the leaky gut syndrome corrected the behavioural changes in the MIA model (Smith et al. 2007). Increased intestinal permeability was also found (Coretti et al. 2017) In the Black and Tan Brachyury (BTBR) mice, another animal model for ASD (Moy et al. 2007), with a thin hippocampal commissure and a congenitally missing corpus callosum (Wahlsten et al. 2003). In the animal model for ASD achieved by high-fat maternal diet, it has been demonstrated that probiotic use of Lactobacillus reuteri improved socialising and normalised the decreased oxytocin receptor concentration in the ventral tegmental area (Buffington et al. 2016), while Citrobacter rodentium reduced anxiety (Mackos et al. 2012) and Lactobacillus rhamnosus JB-1 improved the impaired socialisation after penicillin induced change in gut microbiota (Leclercg et al. 2017). Knock-out of the peptidoglycan-derived molecule Pglyrp2 from gut microbiota affects c-Met expression which is one of the risk genes for ASD and related socialization behaviours (Arentsen et al. 2017). In the Shank 3 knock-out mutant mice, another animal model for ASD, the concentration of GABA receptors in the brain changed due to the reduction of Lactobacillus reuteri and this and the ASD-like behaviours were reversed by probiotic replacement of L. reuteri, suggesting that probiotic treatment may reduce ASD symptoms caused not only environmentally but also genetically (Tabouy et al. 2018).

ASD-like symptoms have appeared by intracerebroventricular administration of PPA produced by Clostridia, Bacteroidetes and Desulfovibrio species in mice (Thomas et al. 2012) and rats (MacFabe et al. 2007). Immune activation with IgE in the intestine of whey-protein-sensitive allergic mice caused ASD-like behaviours by inducing various molecules and especially serotonin (De Theije et al. 2014a), while meta-tyrosine (m-tyrosine) lead to ASD-like behaviors in animals by catecholamine release in the brain (Dyck et al. 1982). The m-tyrosine analogue 3- (3-hydroxyphenyl) -3-hydroxypropionic acid (HPHPA) produced by the class Clostridia spp and found to be increased in the urine of ADS patients, was proposed to be used as an animal model for ASD (Shaw 2010).

In addition to the studies investigating gut microbiota in animal models for ASD, studies were also conducted on the effects of modified animal gut microbiota on social behavior by using GF rodents, probiotics, antibiotics and pathogens (Needham et al. 2018).

Ketogenic diet is a diet with low carbohydrate, high fat and sufficient protein content based on the use of ketone substances produced by the liver as energy source in the brain. The ketogenic diet acting on mitochondrial functions is thought to improve the excitation / inhibition balance of the

Table 1. Studies of Gut Microbiota in Animal Models of Autism

Study	ASD model	Results related with microbiota composition
Hsiao et al. (2013)	MIA	Porphyromonadaceae, Prevotellaceae, Bacteroidales Lachnospiraceae ↑
De Thieje et al. (2014b)	VPA	Bacteroidetes (Bacteroidales) ↑ Firmicutes (Clostridiales) ↑
Buffington et al. (2016)	MHFD	Fecal Lactobacillus reuteri ↓ Socialization increased after probiotic L. reuteri
Newell et al. (2016)	BTBR	Cecal and fecal Akkermansia muciniphila↑ Bifidobacterium spp. ↓
Coretti et al. (2017)	BTBR	Fecal Lactobacillus ↑ in males Fecal Coprobacillus ↑ in females
Golubeva et al. (2017)	BTBR	Cecal Akkermansia↑ Verrucomicrobiaceae↑ Porphyromonadaceae↓ Odirobacter↓
Tabouy et al. (2018)	Shank3 knock-out	Prevotella ↓ Veillonella↑ Lactobacillus (L. reuteri)↓

MIA: Maternal immune activation, VPA: Valproic acid, MHFD: Maternal high-fat diet, BTBR: Black and tan brachyury mice

brain. There are more studies investigating the effectiveness of the ketogenic diet in ADS animal models (Ruskin et al. 2013, Castro et al. 2017, Ruskin et al. 2017, Ahn et al. 2014 and Newell et al. 2016) and of probiotics (Hsiao et al. 2013, Gilbert et al. 2013, Tabouy et al. 2018), as compared to the studies on fecal transplantation and the use of prebiotics and antibiotics (Needham et al. 2018).

Studies on Gut Microbiotia in Children with Autistic Spectrum Disorder

While investigating the role of gut microbiota in GIS symptoms of children with ASD, it was hypothesized that neural developmental disorder in CNS may also be present in GIS neural network and impair GIS functions (Bernier et al. 2014). As previously mentioned, monoamines such as GABA and serotonin are synthesized by the gut microbiota (Cryan and Leonard 2000, Barrett et al. 2012). Serotonin is synthesized from tryptophan in the GIS mostly, not in the CNS (O'Mahony et al. 2015). Blood serotonin levels increase in ASD (Hanley et al. 1977) such that it could be hypothesised that serotonin synthesis is impaired due to dysbiosis and / or the action mechanisms of the synthesised serotonin is impaired due to neurodevelopmental disorder. Serotonin is responsible for secretion, motility and pain in the GIS. A relationship was not observed between hyperserotoninaemia and constipation in ASD, but the small sample size was suspected to be behind these results (Marler et al. 2016). In this section, the hypotheses explaining the relationship between ASD symptoms, GIS symptoms and the microbiota will be presented.

Hypothesis of the Species Change in Gut Microbiota

Although symptoms of ASD are frequently noted before 36 months; in some, the symptoms may start between 15 and 30 months after a normal developmental period, which is called autistic regression (Barger et al. 2013). It was hypothesised that in these children ASD symptoms may be due to Clostridium toxins released by the action of agents such as antibiotics on gut microbiota (Sandler et al. 2000). Clostridium proliferation was found to be 10-fold higher than the normal in the stool of autistic children with GIS symptoms (Finegold et al. 2002). Although the Clostridium species present in gut microbiota did not produce toxins in eubiosis, Clostridium bolteae toxin (Song et al. 2004) and Clostridium histolyticum toxin (Parracho et al. 2005) were detected in ASD. Exotoxin and PPA, produced by Clostridia spp, are increased in the presence of autistic symptoms (Frye et al. 2015).

The gram negative anaerobic bacterium genus Sutterella, from the Sutterellaceae family and the gram-positive genus Ruminicoccus torques of the Clostridia family were highly prevalent in the stools of autistic children with GIS symptoms (Williams et al. 2012, Wang et al. 2013) while the genus Prevotella was rare (Kang et al. 2013). Disruption of carbohydrate digestion in the absence of disaccharidase and hexose transporter was suggested to change the species in microbiota (Williams et al. 2011). High levels of IgA in the stool of autistic children was suggested to be due to altered intestinal immunity in dysbiosis (Zhou et al. 2017). Table 2 presents the findings of these studies that notably differ with respect to the analytical techniques used and, as mentioned earlier, the effects of microorganisms on each other and their ecosystem are more important than their types and numbers. Also, there are studies that do not report any relationship between the gut microbiota and ASD (Gondalia et al. 2012, Son et al. 2015).

The Leaky Gut Hypothesis

Leaky gut is the condition resulting with changes in microbiota and inflammation in the gut which by disrupting the gut epithelial integrity cause bacteria, toxins and metabolites to get into the systemic circulation (Liu et al. 2005).

Gut permeability was increased in 9 out of 21 children diagnosed with ASD as compared to the healthy control group (D'Eufemia et al. 1996). Zonulin, the haptoglobin 2 precursor protein that modulates the permeability of tight junctions between cells of the digestive tract was elevated in ASD patients (Esnafoğlu et al. 2017) and in autistic children with GIS symptoms (as compared to normal children with GIS symptoms) who responded to Toll-like receptor-4

Table 2. Studies of Gut Microbiota in Autistic Children

Study	Study group (number, gender, age)			Method/ Probiotic and diet in autistic group	Findings in autistic group
	Autism	Sibling	Control		
	GIS+/GIS-	GIS+/GIS-	GIS+/GiS-		
Finegold et al. (2002)	13/0 (autistic regression)		8	Fecal flora/ GFCF (+)	Some species of Clostridium ↑, non-spore-forming anaerobes and microaerophilic bacteria ↑
Song et al. (2004)	15		8	Real time PCR procedure with 16S rRNA sequencing in feces to detect Clostridia species/ Not mentioned	C.bolteae ↑ Clostridia cluster I and IX ↑
Parracho et al. (2005)	53/5 (48 M, 10 F) (3-16 y)	9/3 (7 M, 5 F) (2-10 y)	0/10 (6 M, 4 F) (3-12 y)	FISH analysis of feces/ Probiotic and/or GFCF	No significant difference between autistic and sibling group Clostridia species ↑ (C. histolyticum, cluster I and II)
Finegold et al. (2010)	33/0 (24 M, 9 F) (2-13 y)	0/7 (2 M, 5 F) (2-13 y)	0/8 (5 M, 3 F) (2-13 y)	bTEFAP in feces / Not mentioned	No significant difference between autistic and sibling group Bacteroides, Protebacteria ↑ Firmicutes, Actinobacteria ↓
Williams et al. (2012)	15/0 (3.5-5.9 y)		7/0 (3.9-5.5 y)	Ileal and cecal biopsy samples, qPCR; qRT-PCR and 16S rRNA/ Not mentioned	Bacteroides/Firmicutes ↓ Sutterella species ↑
Wang et al. (2011, 2013)	9/14 (21 M, 2 F) (123± 9 m)	6/16 (11 M, 11 F) (144±12 m)	1/8 (4 M, 5 F) (114±15 m)	Targeted qPCR in feces/ Diet and probiotic (+)	No significant difference between autistic and sibling group Bifidobacteria ↓ Akkermansia ↓(A. muciniphilia ↓) Sutterella spp ↑(C. difficile) Ruminococcus↑(R. torgues ↑)
Adams et al. (2011)	58/0 (50 M, 8 F) (6.91±3.4 y)		0/39 (18 M, 21 F) (7.7±4.4 y)	Bacterial stool culture/ Probiotics (+)	Bifidobacterium, Enterococcus ↓ Bacillus spp (Lactobacillus) ↑
Martirosian et al., (2011)	41 (32 M, 9 F) (3-18 y)		10 (5 M, 5 F)	Clostridium culture in stool/ Not mentioned	C. perfringens ↑
Gondalia et al. (2012)	28/23 (42 M, 9 F) (2-12 y)	4/49 (19 M, 34 F) (2-12 y)		bTEFAP of ile 16S rRNA pyrosequencing in feces / Probiotics (+)	No difference between groups
De Angelis et al. (2013)	0/10 (OSB) 0/10(YGB-BTA) (4-10 y)	0/10 (4-10 y)		Bacterial bTEPAP of 16S rRNA and rDNA in feces/ Not used	Firmicutes ↓ Fusobacterium ↓ Bacteroides ↑ Verrucumicrobia ↓
Kang et al. (2013)	20/0 (18 M, 2 F) (6.7±2.7 y)		7/13 (17 M, 3 F) (8.3±4.4 y)	16S rRNA pyrosequencing in feces/ GFCF, probiotics, nutrient supplements (+)	Prevotella, Coprococcus and Veillonellaceae ↓
Tomova et al. (2015)	9/1 (9 M, 1 F) (2-9 y)	7/2 (7 M, 2 F) (5-17 y)	6/4 (10 M) (2-11 y)	Real time PCR in feces (targeted qPCR)/ Probiotic supplement were given	No significant difference between autistic and sibling group Bacteroides/Firmicutes ↓ Lactobacillus spp ↑ Disulfovibrio spp ↑
Son et al. (2015)	25/34 (52 M, 7 F) (10.3±1.8 y)	13/31 (21 M, 23 F) (10.0 ±1.8 y)		16S rRNA pyrosequencing in feces / Not used	No difference in microbial composition Chloroplast ↓
Strati et al. (2017)	40 (5/35) (31 M, 9 F) (11.1±6.8y)		40 (11/29) (28 M, 12 F) (9.2±7.9y)	16S rRNA pyrosequencing in feces / Not used	Firmicutes/Bacteroides ↑
Kang et al. (2018)	23/0 (22 M, 1 F) (10.1 ±4.1 y)		0/21 (15 M, 6 F) (8.4±3.4 y)	16S rRNA pyrosequencing in feces / Not used	Prevotella ↓ Coprococcus ↓ Feacalibacterium prausnitzii ↓ Haemophilus parainfluenzae ↓
Rose et al. (2018)	21 (17M)/ 29 (25 M) 3-17 y		7(4M)/34(32 M) 3-17 y	16S rRNA pyrosequencing in feces / Not used	In GIS+ autistic group compared with GIS+ control group Bacteriodaceae, Lachnospiraceae, Prevotellaceae, Ruminococcaceae ↑

+/-GIS: with/without GIS symptoms, M: Male, F: Female, y: year, m: month, bTEFAP: Bacterial tag-encoded FLX-titanium amplicon pyrosequencing, FISH: Fluorecence in situ hybridization, SCFA: Short chain fatty acids, GFCF: Gluten-free, casein-free diet

stimulation with increased levels of the cytokines IL-5, IL-15, IL-17 and lowered level of the regulatory TGFβ1, suggesting imbalanced immune response and altered gut permeability in ASD (Rose et al. 2018). Calprotectin, a granulocyte-derived protein in feces, was observed to be increased in ASD patients independently of GIS symptoms (de Magistris et al. 2015). These results support the proposition of increased gut permeability. However, studies conducted with 103 autistic children (Dalton et al. 2014) and 61 autistic children with GIS symptoms (Kushak et al. 2016), gut permeability was found normal when compared to the healthy control groups.

Investigations have been made to identify the mechanisms underlying the correlation between ASD symptoms and the substances entering the circulation with increased gut permeability. Cytokines, especially the proinflammatory cytokines IL-6 and IL-1 were increased in children with autistic regression (Ashwood et al. 2011, Onore et al. 2012). The leaky gut hypothesis proposes that the bacterial lipopolysacchharides (LPSs) entering the blood stream activate the immune system and cause proinflammatory cytokine secretion (Qin et al. 2007). Neurotoxic effects were observed with the entry of phenol and paracresol (p-cresol) to the bloodstream through the leaky gut without being metabolised which is normally carried out by the gut microbiota species Clostridium difficile and Pseudomonas stutzeri before elimination from the body (Gabriele et al. 2014, Persico and Napolioni 2013). ASD symptom severity was found to be related to elevated urinary p-cresol levels especially in girls younger than 8 years of age and it was proposed that hepatic drug pharmacokinetics was altered by the competitive inhibiton between p-cresol and drug inactivating sulfatases which could increase the risk of drug side effects and toxicity in ASD (Altieri et al. 2011). Raised urinary p-cresol level was correlated with slow bowel transit time and chronic constipation in autism (Gabriele et al. 2016).

It was concluded that increased PPA produced by gut microbiota induced alterations of gene expression of the neurotransmitter systems, neuronal cell adhesion molecules, inflammation, oxidative stress, lipid metabolism and mitochondrial function all of which have been implicated in ASD (Nankova et al. 2014). Especially PPA among the SCFAs and ammonia were observed to be elevated in the stool samples of autistic children, but blood levels were not reported (Wang et al. 2012). It has been hypothesised that dysbiosis, impaired sulfur metabolism and oxidative stress are involved in a vicious cycle by augmenting the effects of each other (Heberling et al. 2013).

Other Results

Metabolism of free amino acids such as glutamate, glycine, serine, alanine in the gut is impaired in ASD. Glutamate is significant in neuropsychiatric disorders since excess levels may cause neural death (De Angelis et al. 2013). Plasma levels of p-hydroxyphenylacetate, an antioxidant metabolite of Lactobacillus and Bifidobacteria (West et al. 2014), and other antioxidants taurine and carnosine (Ming et al. 2012) were lowered in ASD. These results could be associated with dysfunction of bacterial metabolomics and mitochondrial dysfunction due to dysbiosis. Autoantibodies resulting from immune reaction against bacteria may also affect the CNS (Sandler et al. 2000, Finegold et al. 2010). Urinary excretion of HPHPA produced by Clostridia was higher in autistic children (Shaw 2010, Keşli et al. 2014, Noto et al. 2014). It has been argued that an impaired inhibition/excitation balance is reflected by the altered GABA/Glutamate ratio demonstrated by increased isopropanol and p-cresol, lowered GABA and unaltered propionate, butyrate and glutamate contents of stool samples of ASD patients as compared to healthy controls (Kang et al. 2018).

Clinical Reflections of The Gut Microbiota-ASD Relationship

ASD is diagnosed clinically following the same approach with all psychiatric disorders. Given the heterogeneity of the clinical process, objective measures are needed to estimate prognosis, plan treatment and evaluate the response to treatment. Patients would benefit from research on microbiota, metabolites and metabolomics, the disorder/patient-specific markers for diagnosis, treatment and prognosis, as well as from its contribution to the causes and treatment of the frequently seen GIS symptoms, which negatively affect the functionalityof autistic patients. The necessity of investigating the relationship between the microbiota and the higher prevalence of ASD in the male genders was proposed in the literature (Kopec et al. 2018, Kushak et al. 2018).

GIS symptoms in children with and without ASD are treated with the same approach (Coury et al. 2012). Treatment options for ASD using antibiotics and/or probiotics and diet regulation have been considered after the reported improvement in the neurobehavioral symptoms of 11 children with autistic regression by a 6-week oral vancomycin therapy (Sandler et al. 2000). However, antibiotic use for ASD symptoms is not adequately proven to be included in the standard treatment protocols (Levy and Hyman 2015). Antibiotics may also cause problems of hepatotoxicity, allergic reactions, diarrhea and antibiotic resistance. Probiotics are live bacteria and yeasts beneficial for the gut and usually produce lactic acid. Probiotics were shown to correct GIS symptoms and metabolic variables in autistic children and their improvements on ASD symptoms were considered to be secondary effects (Kaluzna-Czaplinska et al. 2012, Santocchi

et al. 2016, Parracho et al. 2010). Prebiotics are unabsorbable carbohydrate fibre types which allow the growth of beneficial bacteria in the intestine. In a currently ongoing study, probiotics and prebiotics together decreased GIS symptoms in ASD (Sanctuary et al. 2015). Although they are generally well tolerated and have few side effects, routine use is not yet recommended in autistic children without GIS symptoms (Li et al. 2017).

Gluten and casein-free diets, used to prevent the gluten and casein activation of the immune response in the gut that leads to GIS inflammation and adverse effects on the brain of proinflammatory cytokines by entering the blood stream from the leaky gut, have been shown not to have any effect on ASD symptoms (Mari-Bauset et al. 2014, Millward et al. 2008, Elder et al. 2015, Li et al. 2017). The ketogenic diet, suitable for the treatment of resistant epilepsy, and as previously mentioned, with positive effects on the microbiota and ASD-like behaviours in animal models, was also shown to affect the mocirobiota in humans (Zhang et al. 2018). However, the ketogenic diet is not routinely used in ASD treatment in humans, with only one case (Herbert and Buckley 2013) and a few studies on the subject having been reported in the literature (Frye et al. 2011, Evangeliou et al. 2003).

Fecal Microbiota Transplantation is mostly used in the treatment of Clostridium difficile infection (Bagdasarian et al. 2015). The only open-label study in the literature on 18 children aged 7-17 years with ASD and GIS symptoms, used the Microbiota Transfer Therapy by increasing the numbers of Bifidobacterium, Desulfovibrio and Prevotella species in the gut and reported that GIS symptoms of pain, indigestion, diarrhea /constipation and ASD symptom severity were decreased without occurrence and side effects (Kang et al. 2017). It is, however, too early to decide the effectiveness of this treatment.

When determining the diet of a physically and cognitively developing child, as long as there is not disease or gluten sensitivity or any other nutritional sensitivity, it should be taken into consideration that the required nutrients are different from those needed by adults. Also, the effect of nutrition taken out of and/or added to the diet on the development should not be ignored.

DISCUSSION

In this review, the relationship between gut microbiota and ASD is discussed. In studies on both the ASD symptoms and GIS symptoms in humans, conflicting results have been reported on gut microbiota with and without differences from the normal. Gondalia et al. (2012) argued that GIS symptoms may be due to psychiatric comorbidities in ASD and that gut may have been affected by the brain. Son et al. (2015), on the other hand, stated that although not severe enough to be diagnosed with ASD, there can be neurocognitive problems in the siblings of autistic children suggesting that the microbiota of the siblings may not be different. They determined that emotional and problems were more prevalent in autistic children with GIS symptoms although their microbiota did not vary from that of the autistic children without GIS symptoms, but stated that nutrition could affect the gut glora. They also drew attention to their large sample size and to have used standard scales in assessing GIS symptoms when arriving at these conclusions. Other studies reported that the microbiota of siblings of autistic children had characteristics comparable to those of healthy and autistic children which was attributed to the common environment and genetic predisposition (Parracho et al. 2005, Finegold et al. 2010, Tomova et al. 2015).

Following the study which proposed that toxin produced by Clostridium spp played a role especially in autistic regression (Sandler et al. 2000), other studies have also reported increased levels of Clostridia in ASD, but the results on the involved species are not consistent (Finegold et al. 2002, Song et al. 2004, Paracho et al. 2005). In ASD, numbers of normal intestinal flora such as Prevotella were decreased (Kang et al. 2013), while microorganisms such as Sutterella that are normally found in less numbers were increased (Williams et al. 2012, Wang et al. 2013). In studies with noticable methodological differences, the Firmicutes / Bacteroidetes ratio was reported to be increased (Williams et al. 2012), decreased (Finegold et al. 2010, De angelis et al. 2013) or remained unaltered in ASD (Kang et al. 2013, Gondalia et al. 2012). The Clostridium hypothesis states that antibiotics cause dysbiosis (Sandler et al. 2000). The possibility of carbohydrate metabolism disorder being a cause of dysbiosis was also proposed (Williams et al. 2011). Complexities of the relationship between microorganisms, absence of a numerical threshold value for microorganisms, and the influence of environmental conditions such as nutrition and antibiotic use prevent the interpretation and generalization of the results.

Within the context of the leaky gut hypothesis, the effects of bacteria, toxins and metabolites on the brain and the immune system are being investigated in order to explain the aetiology of ASD and to identify the disease biomarkers. As a result of the impairment of phenylalanine metabolism in ASD, increased concentrations of 3-hydroxyphenylacetic acid, HPHPA, and 3-hydroxyhippuric acid were determined in the urine of children with ASD (Xiong et al. 2016). Taking into consideration the experiments on animal models for ASD (MacFabe et al. 2007, Shaw 2010, Thomas et al. 2012), PPA and HPHPA may be more related to ASD.

Oxidant-antioxidant imbalance has also been associated with gut permeability (Ming et al. 2012, West et al. 2014). Phenol derivatives, as one type of bacterial toxins, were increased in the stool of childred with ASD (De Angelis et al. 2013) and p-cresol was increased in urine (Persico and Napolioni 2013). Leaky gut hypothesis has been associated with many diseases (Liu et al. 2005). Genetic/metabolic disorders and psychiatric comorbidities such as intellectual disability and anxiety are common in ASD, which makes its clinic heterogeneous (American Psychiatric Association 2013). Considering that the symptoms start in early childhood, it is important to investigate the effect of microbiota on the brain during infancy.

CONCLUSION

The brain develops through the interaction of molecules and transmission pathways within itself and external factors. Gut microbiota is one of the external factors affecting the development. The heterogeneity of the patients in terms of age, diet, and probiotic use prevents the generalization of the results of microbiota studies in ASD. It should also be noted that most of the studies are cross-sectional and predominantly investigating bacteria. The dose and duration in the long term of the treatments designed to change the microbiota are not yet clear. It is not always possible to determine correlations between the results of studies on humans and the animal models. The bidirectional working of the microbiota-gut-brain axis makes it difficult to determine the starting point of the problem and to establish the cause-effect relationship.

For these reasons, it is thought that using the current data, microbiota will assist the planning of patient-specific treatment in ASD diagnosed patients with GIS symptoms; and double-blind, placebo-controlled, prospective studies with homogenously distributed participants are needed.

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