Chapter

Interplay between Human Intestinal Microbiota and Gut-to-Brain Axis: Relationship with Autism Spectrum Disorders

Francisco Javier Díaz-García, Saúl Flores-Medina and Diana Mercedes Soriano-Becerril

Abstract

A growing body of scientific reports suggests a relevant key role of human intestinal microbiota (HIM) in maintaining the host's physiological and mental balance; thus any disturbance in the microbiota diversity and/or concentrations may result in impaired stimulation of the gastrointestinal (GI) system-central nervous system (CNS) bidirectional pathway, termed gut-to-brain axis. Recent data show that HIM composition is significantly unbalanced among a subset of autism spectrum disorder (ASD) subjects, as compared with non-ASD siblings or age-matched control subjects. Several authors claim that specific changes in HIM (diet-based alteration of Bacteroidetes/Firmicutes ratio and death of predominant microbiota after antibiotic treatments, among others) could either trigger or be highly associated events with persistent ASD signs and behaviors. Whether HIM plays a causative or a circumstantial role in ASD severity, then HIM manipulation might be applied as a therapeutic alternative to improve ASD clinical manifestations and behaviors.

Keywords: human intestinal microbiota, dysbiosis, gut-to-brain axis, autism spectrum disorders, short-chain fatty acids

1. Introduction

Communication between the gastrointestinal (GI) system and the central nervous system (CNS) occurs constantly and plays a critical role in maintaining the healthy status. That communication engages a bidirectional stimulation system, involving not only the brain and gut cells but endocrine-, immune-, and microbiota-derived components as well, the so-called gut-to-brain axis (GBA) [1, 2]. Consequently, impaired communication between both ends of the GBA, associated with or as consequence of disturbance of the GI microbial diversity, has been associated with a negative health outcome later in life [1, 3].

Increasing evidence points out that there is a link between alterations of gut microbiota and several disorders of the central nervous system including autism spectrum disorders (ASD) depression, anxiety, irritable bowel syndrome, attention deficit and hyperactivity disorder (ADHD), Parkinson's disease, disorders of mood and affect, and chronic pain [3–5].

The abovementioned psychiatric disorders frequently co-occur with each other, but interestingly they also occur in comorbidity with metabolic disorders, such as diabetes, cardiovascular disease, and metabolic syndrome [6–8], and are associated with adverse outcomes including higher mortality [6]. The insights of how those disorders are linked remain unclear. One likely explanation is that gut microbiota can trigger and guide the communication network of GBA and subsequently alter metabolic and psychological equilibrium [3, 5, 7, 8].

ASD are a heterogeneous set of lifelong neurodevelopmental diseases, whose incidence increased significantly over the past decades [9]. No unique etiology of ASD has been identified, though both genetic and environmental factors have been suggested [9, 10]. However, findings of candidate genes do not conclusively explain the etiopathology of ASD; thus, scientific research has been redirected to GI comorbidities of ASD, under the premise that the high frequency of gut microbiota alterations seen in these patients may be associated with autism symptoms severity [10]. Indeed, the independent observations of Rodakis [10] and Sandler et al. [11] about improvements in autism clinical manifestations after antibiotic treatments prompted intense research around the issue, including therapeutic interventions such as diet modification, supplementation with biotics (prebiotics, probiotics, synbiotics, and/or postbiotics), alternative antibiotic treatments, and fecal microbiota transplantation, among others, with variable outcomes [12].

2. Human intestinal microbiota

Colonization of the human body occurs after birth, and possibly before birth, with a diverse microbial community of archaea, bacteria, fungi, viruses, and protozoa. This diverse community is referred to as the HIM. The prokaryote organisms colonizing the human body encompass nearly 90% of all HIM [13, 14]. Resident microbiota of the human GI tract, the one that colonizes permanently, is one of the most densely populated communities, even more so than the soil, the subsoil, and the oceans [15].

Colonization of GI tract is influenced by many factors like mode of birth delivery, infant feeding method, and the environment (stress, frequency of exercise, hygiene habits, infections, pharmaceuticals use, and type of feeding) [16, 17]. Within the human intestinal microbiota, there are both types of microorganisms: those who are essential, and even indispensable, for the survival of the host, and those who are potentially pathogenic. The vast majority have beneficial rather than detrimental effects on the host's health [15].

The importance of the GI microbiota was overlooked for a long time, and efforts to determine its composition and functions were unsuccessful; on the one hand, cultures from stool samples are unproductive, and on the other hand, according to estimations, 80% of the GI microbiota are anaerobe uncultivable organisms [10]. Anaerobic bacteria outnumber aerobic and facultative anaerobic bacteria by 100- to 1000-fold [16, 17]. Calculations of microbial counts in the colon of adult humans reach a mean of 10^{11} organisms/gram of wet stool, a quantity updated that is similar to the total number of human cells [18]. Estimated HIM composition comprise up to 1800 genera representing 7000–40,000 bacterial strains belonging to 500–1000 resident species [17, 18].

Taking into account the presence of gene content and metabolic products, along with the microbiota organisms contained within a particular body site, we must refer to it as a microbiome [15]. Studies on composition and function of uncultured microbial communities, more specifically by sequencing-based assays, are referred to as metagenomics. First, community DNA is extracted from a sample containing

multiple microbial members. Second, bacterial taxa present in the community are then defined by amplification of the 16S rRNA gene followed by sequencing. Highly similar sequences are grouped into operational taxonomic units (OTUs) or phylotypes, which can be compared to 16S rRNA databases to identify them as accurately as possible. An alternate method identifies community taxa after the total DNA is metagenomically sequenced and compared to reference genomes or gene catalogs. The OTUs can be described in terms of their relative abundance and/or their phylogenetic relationships, while sequenced genomes can be described as relative abundances of its genes and pathways [19].

The human intestinal microbiome is mainly defined by the high abundance of two bacterial phylotypes: Bacteroidetes and Firmicutes. Other phylotypes present at lesser amounts are Proteobacteria, *Actinomyces*, Fusobacterium, and Verrucomicrobia [14]. The gut microbiome is conformed with nearly 470 phylotypes, more than 1000 bacterial species representing more than 5000 strains, which in turn encode between 5 and 10 million of nonredundant genes (150-fold the number of genes identified in the human genome) [16, 17]. Studies on intestinal microbiome in health and disease revealed two microbiome subpopulations, one with high-gene counts and the other with low-gene counts; the first one seems to be associated with a healthy digestive status [20, 21].

Every person has a unique microbiome profile; still there is a reduced number of species shared between persons. The aforesaid feature allowed to classify individuals into one of three enterotypes, each one based on the proportions of the three predominant intestinal genera, based on their abundance, *Bacteroides*, *Prevotella*, and *Ruminococcus*. The first two genera represent the Bacteroidetes, and the last one represents the Firmicutes. Enterotype 1 shows predominance of *Bacteroides*, while enterotypes 2 and 3 were defined by predominance of *Prevotella* and *Ruminococcus*, respectively [22].

Alterations of the typical GI microbiota, in number and abundance distribution of distinctive types of microorganisms, and the host's adverse response to such changes have been called as dysbiosis. Thus, dysbiosis with low diversity has been linked particularly with obesity, inflammatory bowel disease, and ASD [16].

There are two ongoing multi-group projects on human microbiome, the Europe-based Metagenomics of the Human Intestinal Tract (MetaHIT) and the US-based Human Microbiome Project (HMP). Both of them will allow to define to its finest details the microbiome diversity, at least to species level, their genetic load, and how microbiota interacts with the host [23, 24].

2.1 Biological role of GI microbiota

The intestinal microbiota maintains a symbiotic relationship with the host. Studies in both humans and mammals have implicated the intestinal microbiome in several physiological processes that are pivotal to the host health, from food digestion and energy homeostasis to immune and neurobehavioral development [25].

The single layer of intestinal epithelial cells, connected by tight junctions, constitutes itself a physical and biochemical barrier that segregates the commensal microbiota organisms to maintain intestinal homeostasis. This occurs through regulation of nutrients, electrolytes, and water absorption, as well as through release of mucins, antimicrobial peptides, and IgA for the prevention of the entry of pathogenic microorganisms [26–28]. The interaction between the microbiota and intestinal epithelial cells also promotes tissue restoration in the setting of injury or acute inflammation, thus supporting epithelium integrity. Besides the above statement, the microbiota provides protection against exogenous pathogenic organisms, either through competition for common nutrients and niches or by prompting

development and functional maturation of the gut immune system, including gut-associated lymphoid tissue, T-helper 17 cells, inducible regulatory T cells, IgA-producing B cells, and innate lymphoid cells [13].

The HIM microbiota has a considerable input on the metabolomic profile, the complete set of intestinal metabolites, of the host [29]. Specifically, the microbiota is a major source of both circulating organic acids and tryptophan metabolites, which have beneficial effects on the host health (**Table 1**) [30–53].

Fermentative processes of nondigestible complex carbohydrates, from dietary fiber, by Firmicutes and Bacteroidetes, result in the production of various short-chain fatty acids (SCFAs), such as acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate. These bacteria-derived SCFAs, in a physiological context, may serve as an energy source for enterocytes, stimulate water and sodium absorption, decrease colonic pH, etc. [30].

Hyperproduction or deficiency of SCFAs may also affect the pathogenesis of a diverse range of diseases, from allergies and asthma to neurological diseases [17, 29, 30]. For example, a diet high in fat and digestible saccharides provokes that majority of nutrients be absorbed in the duodenum, leaving very few substrates for the colonic bacteria, leading to dysbiosis. Higher levels of SCFAs can also alter the intercellular spaces between the cells, resulting in a leaky gut that allows for more metabolites and bacteria to pass through the epithelial barrier, where bacterial endotoxins and other microbial-derived metabolites can gain entry into the bloodstream [17] Furthermore, dysbiosis can affect host immunity and neurobehavioral responses [17, 29]. Among SCFAs, butyrate is a promoter of colonic functionality and physical integrity, via cholesterol-rich membrane microdomain, as well as the preferred metabolic substrate for the colonocytes' energy requirements [54].

Essential vitamins such as folate, vitamin K, and vitamin B12, for the host's growth, are synthesized by gut microbiota, which in turn may affect DNA and histone protein methylation [55–57]. Certain hormones and vitamins also participate in drug and poison removal [58].

Apart from carbohydrates, GI bacteria also metabolize complex lipids and proteins that are indigestible by the host [59–61]. Expression of colipase, a critical protein factor for lipid metabolism, and subsequent stimulation of the release of pancreatic lipases appear to be regulated by *Bacteroides thetaiotaomicron* [59].

The metabolism of tryptophan by the HIM and/or gut and immune cells follows three alternative pathways: (a) the transformation to ligands of the aryl hydrocarbon receptor (AhR), (b) the kynurenine pathway (via indoleamine 2,3-dioxygenase 1), and (c) the serotonin (5-HT) production pathway. These pathways are performed by HIM, enterocytes/immune cells, and enterochromaffin cells, respectively. The HIM pathway yields several molecules, indole-3-aldehyde, indole-3-acid-acetic, indole-3-propionic acid, indole-3-acetaldehyde, and indoleacrylic acid. AhR signaling is crucial for gut epithelium renewal and barrier integrity and acts over many immune cell types for responsiveness [62].

Microbial metabolism of tryptophan is very important for intestinal AhR activity, since the absence or imbalance of tryptophan-metabolizing organisms generate deficiency of AhR agonists [46]. The production of AhR ligands have been determined among few HIM species, *Peptostreptococcus russellii* and *Lactobacillus* ssp. Many GI and neuropsychiatric diseases have been related to dysbiotic impairment of tryptophan metabolism or to accumulation of the end products [62].

Several HIM species not only can synthesize but respond as well to hormones and neurotransmitters of bacterial and human origin, which impact their growth and virulence. Beneficial *Lactobacillus* spp. are able to synthesize acetylcholine and gamma-aminobutyric acid (GABA), while *Bifidobacterium* spp. produce GABA. *Escherichia* spp. produce norepinephrine, serotonin, and dopamine; other Firmicutes species

Pathway	Metabolite	Microbial agent	Health benefits	Refs.
Carbohydrate metabolism	Butyrate	Clostridia (clusters IV and IVa)	Increased intestinal barrier function	[30, 31]
		Faecalibacterium. Prausnitzii	Modulate intestinal macrophage function	[32]
		Eubacterium spp.	Regulation of colonic regulatory T cell homeostasis	[33, 34]
		Roseburia spp.	Induction of tolerogenic dendritic cells that polarize naive CD4+ T cells toward IL-10–producing regulatory T cells	[35]
		Coprococcus catus	Suppression of colonic inflammation	[36, 37]
		Anaerostipes hadrus	Improvements in insulin sensitivity	[38]
	Propionate	*Bacteroides* spp.	Regulation of colonic regulatory T cell homeostasis	[33, 34]
		Blautia obeum	Suppression of colonic inflammation	[39]
		C. catus	Decreased innate immune responses to microbial stimulation	[40]
		Roseburia inulinivorans	Protection from allergic airway inflammation	[41]
		P. copri	Improvements in insulin sensitivity and weight control in obese mice	[42]
Tryptophan metabolism	Indole	Various tryptophanase-producing bacteria such as *Lactobacillus* spp.	Maintenance of host–microbe homeostasis at mucosal surfaces via IL-22	[43]
		B. longum	Increased barrier function	[44]
		B. fragilis	Modulation of host metabolism	[45]
	I3A	*Lactobacillus* spp.	Maintenance of mucosal homeostasis and intestinal barrier function Protection against mouse intestinal inflammation.	[43, 46]
	IPA	*Clostridium sporogenes*	• Maintenance of intestinal barrier function and mucosal homeostasis • Increased production of antioxidant and neuroprotectant molecules	[47, 48]
Llipid metabolism	HYA	*Lactobacillus* spp.	• Maintenance of intestinal barrier function • Decreased inflammation • Increased intestinal IgA production	[49, 50]
	CLA	*Lactobacillus* spp.	Decreased inflammation	[51]
		Bifidobacterium spp.	Reduced adiposity	[52]
		F. prausnitzii	Improved insulin sensitivity	[53]

I3A, indole-3-aldehyde; IPA, indole-3-propionate; HYA, 10-hydroxy-cis-12-octadecoate (linoleic acid derivative); CLA, conjugated linoleic acid.
Modified from [29].

Table 1.
Examples of intestinal microbiota-derived metabolites and their beneficial effects on human health.

belonging to *Streptococcus* and *Enterococcus* produce serotonin, and *Bacillus* produce norepinephrine and dopamine [17]. Those bacteria-derived neurotransmitters released directly to the intestinal lumen may either induce epithelial cells to in turn release molecules that modulate neural signaling within the enteric nervous system (ENS) or, after passing through the gut wall, gain entry into the portal circulation to exert direct effects on afferent axons [63, 64]. Indeed, several reports documented elevated levels of noradrenaline and adrenaline in the plasma of subjects coursing with systemic infections by gram-negative Proteobacteria, like *Escherichia coli*. (Reviewed in [64]).

2.2 Stability of HIM

Bacterial colonization of the human gut likely occurs at the time of birth, when infants born via vaginal delivery are inoculated with a complex mixture of maternal vaginal microorganisms. According to Dominguez-Bello et al. [65], those infants had colonizing *Lactobacillus*, *Prevotella*, or *Sneathia* species in their skin and mucosae, which resembled their own mother's vaginal microbiota. In contrast, infants delivered by cesarean section had predominantly *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* species, akin to their mothers' skin microbiota. Thus, there is concern that babies delivered via cesarean section may receive an insufficient maternal bacterial load [17].

After birth, breastfeeding is the main factor defining the composition of newborn's GI microbiota, since breast milk provides a variety of specific antibodies and immediate immunity molecules that neutralize pathogenic bacteria. Breast milk also contains more than 200 oligosaccharides (prebiotics) that favor the growth of bifidobacteria [66, 67], which have been reported to prevent gastrointestinal infections by competitive exclusion of pathogens based on common binding sites on epithelial cells [67]. Therefore, in breastfed children, bifidobacteria reaches up to 90% of GI microbiota, followed by lactobacilli, *Bacteroides*, coliforms, and clostridia. In contrast, infants fed with infant formula have predominance of *Bacteroides*, enterococci, coliforms, and clostridia, with much lesser bifidobacteria, resembling the more diverse GI microbiota of adults [66, 67].

The initial breastfeeding-driven colonization is essential for induction of adaptive immunity and for early metabolic programming. After the introduction of complementary feeding, the microbiota differences between breastfed children and those fed with formula tend to disappear. It is assumed that the predominant bacteria in the intestinal microbiome of 3-year-olds are similar to those of adults and remain relatively stable lifelong [66, 67].

Daily variability of the HIM composition has been assessed in controlled feeding studies, specifically short-term administration of extreme amount of fat and fiber intake, which revealed disturbance of the intestinal microbiome, but this effect was of low-scale and transient that not changed the individual's enterotype designation [66, 67].

3. Gut-to-brain axis

The basis of the GBA cross-communication includes an array of multichannel sensing and trafficking pathways (neural, endocrine, immune, and metabolic) to transfer the enteric signals to the brain (**Figure 1**), which ultimate results in keeping proper maintenance of GI homeostasis, although its multiple effects likely impacts on brain performance and higher cognitive functions [1–3, 68].

The GBA comprises highly interconnected body systems. Those systems are the CNS, the autonomic nervous system (vagal and spinal nerves), and the ENS

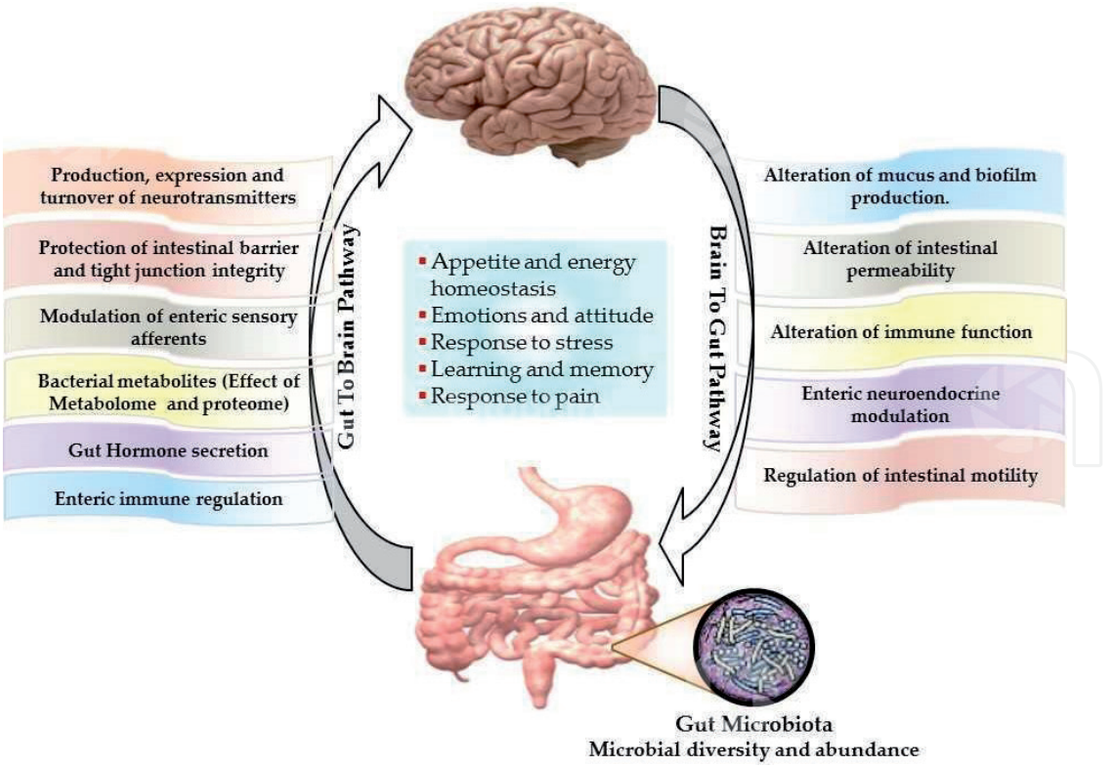

Figure 1.
The bidirectional pathways of the gut-to-brain axis and their effects. Modulation of the CNS by the gut microbiome (through microbial-derived molecules such as SCFAs, neurotransmitters, hormones and tryptophan metabolites) occurs primarily via neuro-immune and neuroendocrine mechanisms. Those microbial molecules reach brain sites directly or only induce central responses through long-distance neural signaling by vagal and/or spinal afferents. The autonomic nervous system regulates gut functions (motility, secretion, intestinal permeability, and mucosal immune response), which ultimately affect the microbial habitat, thereby modulating microbiota composition and activity.

(the arrangement of neurons and supporting cells throughout and embedded within GI tract, from the esophagus to the anus). Other critical components of GBA include the hypothalamic pituitary adrenal axis (HPA; release of gut hormones), the immune system (release of multiple cytokines), and bacteria-derived metabolites (SCFAs and free amino acids). In fact, gut microbes have evolved alongside their host, through complex relationships, so influencing their own genotypic and phenotypic features [1–3]. However, failures in the GBA cross talk may lead to a number of health disorders, from inflammatory to metabolic and neurodevelopmental conditions, including ASD [1].

The following pathways may explain the influence of the gut microbiota on neurologic disorders through GBA: (a) production of neurotransmitters, (b) triggering release of gut hormones from entero-endocrine cells, (c) stimulation of the ENS and signaling to the brain via ascending neural pathways, and (d) activation of the immune system via cytokine release by the mucosa-associated immune cells.

At physiological conditions, GBA modulates the digestive processes like motility and secretion, immune function, and perception and emotional response to visceral stimuli [17]. The high comorbidity of stress-related neurologic disorders with GI disorders proves the impact of altered function of GBA [3].

4. Autism spectrum disorders

ASD is a group of neurodevelopmental abnormalities whose clinical manifestations begin in early childhood (although their diagnosis may delay months to years later in life). Clinically ASDs show complex and heterogeneous features but

generally are defined by a core symptomatology including impaired social communication (oral and nonverbal languages, eye contact), behavioral problems (fixated interests in the daily routine, engagement in repetitive manners, exacerbated responses to external stimuli), and self-isolation, with or without impairment of cognitive abilities and competences [9, 69].

According to the latest American Psychiatric Association's diagnostic criteria [69], ASDs include conditions known as autism disorder (AD), Asperger's syndrome, childhood disintegrative disorder, and pervasive developmental disorder not otherwise specified (PDD-NOS).

Noteworthy ASD clinical features show extensive heterogeneity among affected subjects, according to the developmental stage, to chronological age, and to specific disorder within the spectrum (and even within the same disorder) [9, 69].

Until of April 2018, ASD were estimated to affect, in average, 1 in every 160 children worldwide, with a yearly rising incidence, and an estimated boy to girl ratio of 5:1 [70]. Data from the USA reveal that prevalence of ASDs has dramatically increased from 4.5 in 10,000 children in 1966 to 1 in 68 in 2010 and finally to 1 in 59 children in 2014 [71].

This recent outburst in frequency may be partly attributed to increased public awareness and or to better diagnosis; however, the occurrence of other factors, such as exposure to environmental chemicals, diet alterations, metabolic status, and changes in microbiota composition, cannot be excluded [17].

Despite the alarming rise trend in frequency of diagnosed cases in developed countries, the etiopathogenesis of ASD is still unknown; thus, there are no consensus in medical, neurologic, or psychiatric treatments [10]. Moreover, a diversity of comorbidities also affect ASD individuals, including one or more of the following: anxiety, intellectual disability, epilepsy/seizures, attention deficit and hyperactivity disorder, GI disorders, sleep disorders, obesity, depression, bipolar disorder, and Tourette's syndrome, among others (**Figure 2**) [6, 7, 9].

Among the most frequent GI comorbidities in ASD subjects are exacerbated flatulence (60%), bloating (38%), abdominal pain (37%), diarrhea (28%), burping/belching (25%), gastroesophageal reflux symptoms (16%), and constipation (10%) [8].

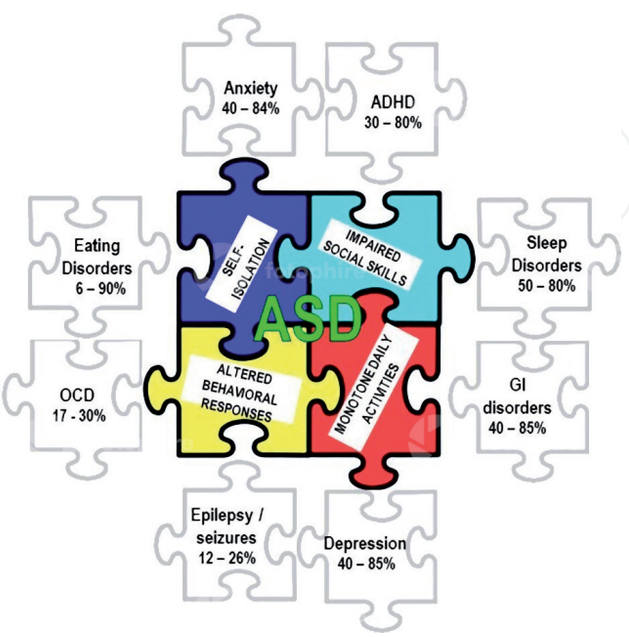

Figure 2.
Relevant features of ASD and their most frequent comorbidities. The colored figures represent typical features defining ASD, while colorless figures represent the most prevalent of its comorbidities. ADHD, attention deficit and hyperactivity disorder; GI, gastrointestinal, OCD, obsesive –compulsive disorder.

Research on ASD was primarily focused on genetic associations, but recent evidence has suggested that other environmental factors, including pre- or postnatal exposure to chemicals and drugs, air pollution, stress, maternal infection, the HIM, and dietary factors, may play a role in the clinical manifestations of the ASD [17].

5. Interplay between HIM and GBA in the context of ASD

About 40–60% of ASD children suffer from gastrointestinal comorbidities [8], although due to their social and communicative impairments, the real prevalence of gastrointestinal issues among ASD patients may be higher. Such intestinal dysfunction in this group of patients may be caused by disturbances in the pathways underlying the GBA, with a central role of the HIM and including an immune component.

Several studies have demonstrated HIM dysbiosis in ASD subjects; however, little or null correlation between studies has been obtained, mainly due to variations in study groups, control groups, and the use of diverse methods for microbiota/microbiome determinations and analysis (**Table 2**) [67, 72]. In short, 13 of the 15 studies showed some degree of dysbiosis among ASD patients as compared with controls (total combined sample of 585 individuals, 339 ASD, 61 control siblings, and 185 unrelated neurotypical controls), whereas 2 of the 15 studies found no significant differences among ASD subjects as compared with siblings controls (no neurotypical controls were included).

Altogether the microbiome data from the studies showed in **Table 2** suggests some important features among stool samples of ASD subjects: (a) levels of clostridia, *Desulfovibrio*, and *Sutterella* seem consistently elevated; (b) on the opposite, levels of *Prevotella* and bifidobacteria appears to be reduced; (c) the Bacteroidetes/Firmicutes ratio showed inconsistent results over different cohorts. There are significant, but not consistent, distinctive different microbiome compositions in ASD patients, regardless of gastrointestinal problems, compared to controls [73–90]. Moreover, the presence of HIM dysbiosis may correlate with ASD phenotype [91].

Dysbiosis in ASD is also associated with increased permeability of the GI tract, the leaky gut, which leads to the entry of endotoxins, and other bacterial products into the bloodstream [92]. Bacterial lipopolysaccharide (LPS) can alter neuronal as well as microglial activity in brain regions involved in emotional control [93–95]. In fact, serum levels of LPS were significantly higher among ASD subjects compared to healthy individuals and correlated with impaired social behavioral scores [96].

Serotonin synthesis in the gut and the brain depends on the availability of dietary tryptophan. High levels of blood serotonin were found in children with ASD [97–99], which contrasts with finding of decreased brain serotonin synthesis in ASD subjects [100]. A significant correlation between whole-blood serotonin levels and low-grade intestinal inflammation in ASD was demonstrated [101]. Regarding these findings, a likely explanation was proposed by de Theije et al. (2011) [91]: After GI inflammation, the intestinal serotonin release provokes changes in motility, secretion, vasodilation, and permeability, leading to functional intestinal dysmotility, stool inconsistency, and abdominal pain. Since the majority of dietary tryptophan is transformed in serotonin by HIM during inflammation, less tryptophan (and serotonin) will be available for the brain resulting in mood and cognitive dysfunction in ASD and increased autistic behavior [102].

Propionic acid, a major SCFA produced by clostridia, *Bacteroides*, and *Desulfovibrio,* has been associated with ASD, since it can induce ASD-like behavioral deficits in rats [103, 104]. Detrimental effects of propionic acid are suggested to be through mitochondrial and epigenetic modulation of ASD-associated genes. In fact, elevated levels of SCFAs are described in the stool of ASD children [82, 105].

Country (Year)	Study Group			Specimen type	Analytical method	Changes in fecal microbiome in ASD	Refs.
	ASD (GI+/GI−)	SIB (GI+/GI−)	NTC (GI+GI−)				
USA (2002)	13	–	8	Stool	Bacterial cultures	↑ Nine species of *Clostridium*	[73]
USA (2004)	15	–	8	Stool	16S rRNA gene sequencing	↑ *C. bolteae* and cluster I/IX	[74]
United Kingdom (2005)	58	12	10	Stool	FISH analysis	↑ *C. histolyticum* and cluster I/II. Siblings show intermediate levels.	[75]
USA (2010)	33 (33/0)	7 (0/7)	8 (0/8)	Stool	16S rRNA gene sequencing	↑ Bacteroidetes and Proteobacteria: *Desulfovibrio, B. Alkaliflexus, Acetanaerobacterium, Parabacteroides* ↓ Firmicutes and Actinobacteria: *Clostridium*, *Weissella*, *Turicibacter*, *Anaerofilum*, *Ruminococcus*, *Streptococcus, Pseudoramibacter*,	[76]
USA (2011)	58 (58/0)	–	39 (0/39)	Stool	Bacterial cultures	↓ *Bifidobacterium* and *Enterococcus* ↑ *Bacillus* spp. *(Lactobacillus)*	[77]
Poland (2011)	41	–	10	Stool	Bacterial cultures	↑ *Clostridium perfringens*	[78]
USA (2011, 2012)	23 (23/0)	–	9 (9/0)	Intestinal biopsies	16S rRNA gene sequencing	↓ Bacteroidetes ↑ Firmicutes, Proteobacteria, *Sutterella*	[79, 80]
Australia (2011, 2012 2013)	23 (9/14)	22 (6/16)	9 (1/8)	Stool	Targeted qPCR GC HPLC	↓*Bifidobacterium* spp., *Akkermansia muciniphilia* ↑ *Sutterella* spp. (↑ Relative abundance of *Clostridium difficile* in ASD, NS) ↑ *Ruminococcus torques* (only in ASD-GI+). ↑Ammonia and SCFA (acetic, butyric, isobutyric, valeric, isovaleric acids), likely microbial-derived. No differences in phenol and p-cresol levels	[81–83]

Country (Year)	Study Group			Specimen type	Analytical method	Changes in fecal microbiome in ASD	Refs.
	ASD (GI+/GI−)	SIB (GI+/GI−)	NTC (GI+GI−)				
Australia (2012)	51 (28/23)	53 (4/49)	–	Stool	16S rRNA gene sequencing	No differences	[84]
USA (2013)	20 (20/0)	–	20 (0/20)	Stool	16S rRNA gene sequencing	↓ *Prevotella, Coprocuccus, Veillonellaceae*	[85]
Italy (2013)	10	10	10	Stool	16S rRNA gene sequencing GC-MS/SPME	↓ *Caloramator, Sarcina, Clostridium, Sutterellaceae* ↓ *Eubacterium, Bifidobacterium* ↓SCFA (except PPA) ↑ Phenol, 4-(1,1-dimethylethyl)-phenol, and p-cresol. ↑ Free amino acids (Proteolytic bacteria)	[86]
USA (2015)	59 (25/34)	44 (13/31)	-	Stool	16S rRNA gene sequencing	No differences	[87]
Slovakia (2015)	10	10	10	Stool	Targeted qPCR	↓Bacteroidetes/Firmicutes ↑ *Lactobacillus* spp. (↑Clostridia cluster I and *Desulfovibrio*, NS.) *Desulfovibrio* spp.: strong positive association with ASD severity	[88]
USA (2017)	14 (14/0)	-	21 (15/6)	Rectal biopsies	16S rDNA PCR and sequencing HPLC of mucosal supernatant.	↑ Clostridiales (*C lituseburense, Lachnoclostridum bolteae, L hathewayi, C aldenense*, and *Flavonifractor plautii*) ↓ *Dorea formicigenerans, Blautia luti, Sutterella* spp. ↓Tryptophan (correlation with ↑*Erysipelotrichaceae*, *C. lituseburense*, and *Terrisporobacter*) ↑ Serotonergic metabolites, including 5-HIAA (associated with abdominal pain and with the following: ↓ *Akkermansia muciniphila*, *Coprococcus catus*, *Odoribacter splanchnicus*, *C. lactatifermentans*, and *Ruminococcus lactaris;* ↑ *L. bolteae, L. hathewayi*, and *F. plautii)*.	[89]

Country (Year)	Study Group			Specimen type	Analytical method	Changes in fecal microbiome in ASD	Refs.
	ASD (GI+/GI−)	SIB (GI+/GI−)	NTC (GI+GI−)				
USA (2018)	21	-	23	Stool	^{1}H NMR spectroscopy 16S rRNA gene sequencing	↑ Isopropanol, p-cresol. ↓ GABA (associated to ↓ *Streptococcus thermophiles)* ↓Phylotypes closely related to *Prevotella copri* , ↓ *Feacalibacterium prausnitzii and Haemophilus parainfluenzae*.	[90]

Data presented here include microbial phylotypes or species and/or relevant metabolites pertaining ASD-associated alterations, compared to non-ASD siblings or unrelated healthy controls.
ASD, children with autism spectrum disorder; SIB, siblings without ASD; NTC, neuro typical controls; GI+, with gastrointestinal comorbidities; GI−, without gastrointestinal comorbidities, ↑, increased level(s); ↓, decreased level(s); NS, non statistically significant; FISH, fluorescent in situ hybridization; GC, gas chromatography; HPLC, high performance liquid chromtography; SCFA, short-chain fatty acids; PPA, propionic acid MS, mass spectroscopy; SPME, solid phase microextraction; 5-HIAA: 5-hydoxy-indoleacetic acid; H-NMR, proton nuclear magnetic resonance; GABA, gamma-amino butyric acid.

Table 2.
Studies on gut microbiome in ASD.

Scientific literature supports the notion that the HIM plays a crucial role in the pathogenesis of ASD, so scientists are now targeting gut microbiome as a therapeutic approach for such disorder (reviewed in [106]). First, modification of high lipid and sugar diet for a fiber- and protein-containing one showed improved skills while ameliorated ASD behavioral deficits. Second, supplementation with prebiotics (inulin, fructo-oligosaccharides, galacto-oligosaccharides, and lactulose) allows specific changes, both in the composition and/or activity of the gut microflora, mainly inducing the growth of indigenous lactobacilli and bifidobacteria. Third, probiotics administration, either *Bacteroides fragilis* or *Lactobacillus reuteri*, there were improvements in ASD-associated behaviors, counteract effect of harmful infections and stimulation of the host's immune system. Fourth, fecal microbiota transplant, usually applied for treating recurrent *Clostridium difficile* infection and other GI disorders, consists of a sample containing about a thousand indigenous bacterial species of the GI from a neurotypical donor, treatment showed sustained improvement of both GI- and ASD related symptoms (up to 8 weeks posttreatment).

6. Conclusion

After the complete sequencing of the human genome was achieved, the scientific community began, in the second half of the past decade, the task of mapping the human microbiota, mainly the intestinal microbiota. In parallel, the notion that the ENS interplay with the intestinal microbiota, generating responses in the CNS, through the GBA and HPA axis, has opened an avenue for the study of gastrointestinal, metabolic, and/or neuropsychiatric disorders.

In this landscape, an increasing body of evidence suggests that HIM has a key role in gut and brain development and functionality but also in pathogenesis of mental disorders, including ASD. Studies on ASD have showed that HIM dysbiosis, with altered Bacteroidetes/Firmicutes ratio, presence of detrimental key species, and dysregulation of bacterial metabolite release, appears to correlate with severity of ASD symptoms. In this regard, intervention measures to restore HIM homeostasis are likely promising.

However, the part concerning the microbiota is only one more piece of the puzzle that are ASDs, mainly because the etiology of such disorders remains elusive.

Acknowledgements

The following Mexican institutions supported this paper: National Autonomous University of Mexico, the National Institute of Perinatology and the National Polytechnic Institute.

Conflict of interest

The authors have declared that no competing interests exist.

Notes/thanks/other declarations

FJDG. Thanks to my beloved son, Manuel, a youngster with ASD who encourages me to understand how the world is seen through their eyes.

Author details

Francisco Javier Díaz-García[1*], Saúl Flores-Medina[2,3] and Diana Mercedes Soriano-Becerril[2]

1 Department of Biology, Faculty of Chemistry, National Autonomous University of Mexico, Mexico City, Mexico

2 Department of Infectology, National Institute of Perinatology "Isidro Espinosa de los Reyes", Health Ministry of Mexico, México City, México

3 Center of Scientific and Technological Studies No. 15 "DAE". National Polytechnic Institute, México City, México

*Address all correspondence to: jdiazgr@hotmail.com

References

[1] Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: Interactions between enteric microbiota, central and enteric nervous systems. Annals of Gastroenterology. 2015;**28**:203-209

[2] Cani PD, Knauf C. How gut microbes talk to organs: The role of endocrine and nervous routes. Molecular Metabolism. 2016;**5**(9):743-752. DOI: 10.1016/j.molmet.2016.05.011

[3] Martin CR, Osadchiy V, Kalani A, Mayer EA. The brain-gut-microbiome axis. Cellular and Molecular Gastroenterology and Hepatology. 2018;**6**(2):133-148. DOI: 10.1016/j.jcmgh.2018.04.003

[4] Dinan TG, Cryan JF. Brain-gut-microbiota axis and mental health. Psychosomatic Medicine. 2017;**79**:920-926. DOI: 10.1097/PSY.0000000000000519

[5] Cryan JF, Dinan TG. Mind-altering microorganisms: The impact of the gut microbiota on brain and behavior. Nature Reviews. Neuroscience. 2012;**13**:701-712. DOI: 10.1038/nrn3346

[6] Groen RN, de Clercq NC, Nieuwdorp M, Hoenders HJR, Groen AK. Gut microbiota, metabolism and psychopathology: A critical review and novel perspectives. Critical Reviews in Clinical Laboratory Sciences. 2018;**55**(4):283-293. DOI: 10.1080/10408363.2018.1463507

[7] Mannion A, Leader G. Gastrointestinal symptoms in autism spectrum disorder: A literature review. Review Journal of Autism and Developmental Disorders. 2014;**1**:11-17. DOI: 10.1007/s40489-013-0007-0

[8] Hsiao EY. Gastrointestinal issues in autism spectrum disorder. Harvard Review of Psychiatry. 2014;**22**(2):104-111. DOI: 10.1097/HRP.0000000000000029

[9] Hahler EM, Elsabbagh M. Autism: A global perspective. Current Developmental Disorders Reports. 2015;**2**:58-64. DOI: 10.1007/s40474-014-0033-3

[10] Rodakis J. An n=1 case report of a child with autism improving on antibiotics and a father's quest to understand what it may mean. Microbial Ecology in Health and Disease. 2015;**26**:26382. DOI: 10.3402/mehd.v26.26382

[11] Sandler RH, Finegold SM, Bolte ER, Buchanan CP, Maxwell AP, Väisänen ML, et al. Short-term benefit from oral vancomycin treatment of regressive-onset autism. Journal of Child Neurology. 2000;**15**(7):429-435. DOI: 10.1177/088307380001500701

[12] Frye RE, Slattery J, MacFabe DF, Allen-Vercoe E, Parker W, Rodakis J, et al. Approaches to studying and manipulating the enteric microbiome to improve autism symptoms. Microbial Ecology in Health and Disease. 2015;**26**:26878. DOI: 10.3402/mehd.v26.26878

[13] Sommer F, Bäckhed F. The gut microbiota--masters of host development and physiology. Nature Reviews. Microbiology. 2013;**11**(4):227-238. DOI: 10.1038/nrmicro2974

[14] Wang HX, Wang YP. Gut microbiota-brain Axis. Chinese Medical Journal. 2016;**129**:2373-2380. DOI: 10.4103/0366-6999.190667

[15] Icaza-Chávez ME. Gut microbiota in health and disease. Revista de Gastroenterología de México. 2013;**78**(4):240-248. DOI: 10.1016/j.rgmx.2013.04.004

[16] Mayer EA, Tillisch K, Gupta A. Gut/brain axis and the microbiota. The Journal of Clinical Investigation. 2015;**125**(3):926-938. DOI: 10.1172/JCI76304

[17] Rosenfeld CS. Microbiome disturbances and autism Spectrum disorders. Drug Metabolism and Disposition. 2015;**43**:1557-1571. DOI: 10.1124/dmd.115.063826

[18] Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. PLoS Biology. 2016;**14**(8):e1002533. DOI: 10.1371/journal.pbio.1002533

[19] Morgan XC, Huttenhower C. Chapter 12: Human microbiome analysis. PLoS Computational Biology. 2012;**8**(12):e1002808. DOI: 10.1371/journal.pcbi.1002808

[20] Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, et al. Richness of human gut microbiome correlates with metabolic markers. Nature. 2013;**500**(7464):541-546. DOI: 10.1038/nature12506

[21] Cotillard A, Kennedy SP, Kong LC, Prifti E, Pons N, Le Chatelier E, et al. Dietary intervention impact on gut microbial gene richness. Nature. 2013;**500**(7464):585-588. DOI: 10.1038/nature12480

[22] Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. Nature. 2011;**473**(7346):174-180. DOI: 10.1038/nature09944

[23] Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature. 2012;**486**(7402):207-214. DOI: 10.1038/nature11234

[24] Integrative HMP (iHMP) Research network consortium. The integrative human microbiome project. Nature. 2019;**569**(7758):641-648. DOI: 10.1038/s41586-019-1238-8

[25] Felice VD, O'Mahony SM. The microbiome and disorders of the central nervous system. Pharmacology, Biochemistry, and Behavior. 2017;**160**:1-13. DOI: 10.1016/j.pbb.2017.06.016

[26] Farhadi A, Banan A, Fields J, Keshavarzian A. Intestinal barrier: An interface between health and disease. Journal of Gastroenterology and Hepatology. 2003;**18**:479-497

[27] Groschwitz KR, Hogan SP. Intestinal barrier function: Molecular regulation and disease pathogenesis. The Journal of Allergy and Clinical Immunology. 2009;**124**:3-20. DOI: 10.1016/j.jaci.2009.05.038

[28] Johansson ME, Larsson JM, Hansson GC. The two mucus layers of colon are organized by the MUC2 mucin, whereas the outer layer is a legislator of host-microbial interactions. Proceedings of the National Academy of Sciences of the United States of America. 2011;**108**(Suppl 1):4659-4665. DOI: 10.1073/pnas.1006451107

[29] Durack J, Lynch SV. The gut microbiome: Relationships with disease and opportunities for therapy. The Journal of Experimental Medicine. 2018;**216**(1):20-40. DOI: 10.1084/jem.20180448

[30] Kelly CJ, Zheng L, Campbell EL, Saeedi B, Scholz CC, Bayless AJ, et al. Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. Cell Host & Microbe. 2015, 2015;**17**:662-671. DOI: 10.1016/j.chom.2015.03.005

[31] Zheng L, Kelly CJ, Battista KD, Schaefer R, Lanis JM, Alexeev EE, et al. Microbial-derived butyrate promotes epithelial barrier function through

IL-10 receptor-dependent repression of Claudin-2. Journal of Immunology. 2017;**199**:2976-2984. DOI: 10.4049/jimmunol.1700105

[32] Chang PV, Hao L, Offermanns S, Medzhitov R. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. Proceedings of the National Academy of Sciences of the United States of America. 2014;**111**:2247-2252. DOI: 10.1073/pnas.1322269111

[33] Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013;**504**:446-450. DOI: 10.1038/nature12721

[34] Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science. 2013;**341**:569-573. DOI: 10.1126/science.1241165

[35] Kaisar MMM, Pelgrom LR, van der Ham AJ, Yazdanbakhsh M, Everts B. Butyrate conditions human dendritic cells to prime type 1 regulatory T cells via both histone deacetylase inhibition and G protein-coupled receptor 109A signaling. Frontiers in Immunology. 2017;**8**:1429. DOI: 10.3389/fimmu.2017.01429

[36] Singh N, Gurav A, Sivaprakasam S, Brady E, Padia R, Shi H, et al. Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. Immunity. 2014;**40**:128-139. DOI: 10.1016/j.immuni.2013.12.007

[37] Simeoli R, Mattace-Raso G, Pirozzi C, Lama A, Santoro A, Russo R, et al. An orally administered butyrate-releasing derivative reduces neutrophil recruitment and inflammation in dextran sulphate sodium-induced murine colitis. British Journal of Pharmacology. 2017;**174**:1484-1496. DOI: 10.1111/bph.13637

[38] Khan S, Jena G. Sodium butyrate reduces insulin-resistance, fat accumulation and dyslipidemia in type-2 diabetic rat: A comparative study with metformin. Chemico-Biological Interactions. 2016;**254**:124-134. DOI: 10.1016/j.cbi.2016.06.007

[39] Tong LC, Wang Y, Wang ZB, Liu WY, Sun S, Li L, et al. Propionate ameliorates dextran sodium sulfate-induced colitis by improving intestinal barrier function and reducing inflammation and oxidative stress. Frontiers in Pharmacology. 2016;**7**:253. DOI: 10.3389/fphar.2016.00253

[40] Ciarlo E, Heinonen T, Herderschee J, Fenwick C, Mombelli M, Le Roy D, et al. Impact of the microbial derived short chain fatty acid propionate on host susceptibility to bacterial and fungal infections in vivo. Scientific Reports. 2016;**6**:37944. DOI: 10.1038/srep37944

[41] Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. Nature Medicine. 2014;**20**:159-166. DOI: 10.1038/nm.3444

[42] den Besten G, Bleeker A, Gerding A, van Eunen K, Havinga R, van Dijk TH, et al. Short-chain fatty acids protect against high-fat diet–induced obesity via a PPARγ-dependent switch from lipogenesis to fat oxidation. Diabetes. 2015;**64**:2398-2408. DOI: 10.2337/db14-1213

[43] Zelante T, Iannitti RG, Cunha C, De Luca A, Giovannini G, Pieraccini G, et al. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. Immunity.

2013;**39**:372-385. DOI: 10.1016/j.immuni.2013.08.003

[44] Bansal T, Alaniz RC, Wood TK, Jayaraman A. The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**:228-233. DOI: 10.1073/pnas.0906112107

[45] Chimerel C, Emery E, Summers DK, Keyser U, Gribble FM, Reimann F. Bacterial metabolite indole modulates incretin secretion from intestinal enteroendocrine L cells. Cell Reports. 2014;**9**:1202-1208. DOI: 10.1016/j.celrep .2014.10.032

[46] Lamas B, Richard ML, Leducq V, Pham HP, Michel ML, Da Costa G, et al. CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands. Nature Medicine. 2016;**22**:598-605. DOI: 10.1038/nm.4102

[47] Hwang IK, Yoo KY, Li H, Park OK, Lee CH, Choi JH, et al. Indole-3-propionic acid attenuates neuronal damage and oxidative stress in the ischemic hippocampus. Journal of Neuroscience Research. 2009;**87**:2126-2137. DOI: 10.1002/jnr.22030

[48] Venkatesh M, Mukherjee S, Wang H, Li H, Sun K, Benechet AP, et al. Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and toll-like receptor 4. Immunity. 2014;**41**:296-310. DOI: 10.1016/j.immuni.2014.06.014

[49] Miyamoto J, Mizukure T, Park SB, Kishino S, Kimura I, Hirano K, et al. A gut microbial metabolite of linoleic acid, 10-hydroxy-cis-12-octadecenoic acid, ameliorates intestinal epithelial barrier impairment partially via GPR40-MEK-ERK pathway. The Journal of Biological Chemistry. 2015;**290**:2902-2918. DOI: 10.1074/jbc.M114.610733

[50] Kaikiri H, Miyamoto J, Kawakami T, Park SB, Kitamura N, Kishino S, et al. Supplemental feeding of a gut microbial metabolite of linoleic acid, 10-hydroxy-cis-12-octadecenoic acid, alleviates spontaneous atopic dermatitis and modulates intestinal microbiota in NC/nga mice. International Journal of Food Sciences and Nutrition. 2017;**68**:941-951. DOI: 10.1080/09637486.2017.1318116

[51] Viladomiu M, Hontecillas R, Bassaganya-Riera J. Modulation of inflammation and immunity by dietary conjugated linoleic acid. European Journal of Pharmacology. 2016;**785**:87-95. DOI: 10.1016/j.ejphar.2015.03.095

[52] SA1 S, MH1 V, C1 G, XD1 Z, Reynolds CM. Conjugated linoleic acid supplementation improves maternal high fat diet-induced programming of metabolic dysfunction in adult male rat offspring. Scientific Reports. 2017;**7**:6663. DOI: 10.1038/s41598-017-07108-9

[53] Garibay-Nieto N, Queipo-García G, Alvarez F, Bustos M, Villanueva E, Ramírez F, et al. Effects of conjugated linoleic acid and metformin on insulin sensitivity in obese children: Randomized clinical trial. The Journal of Clinical Endocrinology and Metabolism. 2017;**102**:132-140

[54] Suzuki T, Yoshida S, Hara H. Physiological concentrations of short-chain fatty acids immediately suppress colonic epithelial permeability. The British Journal of Nutrition. 2008;**100**(2):297-305. DOI: 10.1017/S0007114508888733

[55] Macfarlane S, Macfarlane GT. Regulation of short-chain fatty acid production. The Proceedings of the Nutrition Society. 2003;**62**:67-72. DOI: 10.1079/PNS2002207

[56] Le Galliard JF, Cote J, Fitze PS. Lifetime and intergenerational fitness

consequences of harmful male interactions for female lizards. Ecology. 2008;**89**:56-64. DOI: 10.1890/06-2076.1

[57] LeBlanc JG, Milani C, de Giori GS, Sesma F, van Sinderen D, Ventura M. Bacteria as vitamin suppliers to their host: A gut microbiota perspective. Current Opinion in Biotechnology. 2013;**24**:160-168. DOI: 10.1016/j.copbio.2012.08.005

[58] Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. The role of short-chain fatty acids in health and disease. Advances in Immunology. 2014;**121**:91-119. DOI: 10.1016/B978-0-12-800100-4.00003-9

[59] Hooper LV, Midtvedt T, Gordon JI. How host-microbial interactions shape the nutrient environment of the mammalian intestine. Annual Review of Nutrition. 2002;**22**:283-307. DOI: 10.1146/annurev.nutr.22.011602.092259

[60] Dai ZL, Li XL, Xi PB, Zhang J, Wu G, Zhu WY. Metabolism of select amino acids in bacteria from the pig small intestine. Amino Acids. 2012;**42**(5):1597-1608. DOI: 10.1007/s00726-011-0846-x

[61] Saulnier DM, Gibson GR, Kolida S. In vitro effects of selected synbiotics on the human faecal microbiota composition. FEMS Microbiology Ecology. 2008;**66**:516-527

[62] Agus A, Planchais J, Sokol H. Gut microbiota regulation of tryptophan. Cell Host & Microbe. 2018;**23**(6):716-724. DOI: 10.1016/j.chom.2018.05.003

[63] Wall R, Cryan JF, Ross RP, Fitzgerald GF, Dinan TG, Stanton C. Bacterial neuroactive compounds produced by psychobiotics. Advances in Experimental Medicine and Biology. 2014;**817**:221-239. DOI: 10.1007/978-1-4939-0897-4_10

[64] Lyte M. Microbial endocrinology in the microbiome-gut-brain axis: How bacterial production and utilization of neurochemicals influence behavior. PLoS Pathogens. 2013;**9**(11):e1003726. DOI: 10.1371/journal.ppat.1003726

[65] Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**(26):11971-11975. DOI: 10.1073/pnas.1002601107

[66] O'Callaghan A, van Sinderen D. Bifidobacteria and their role as members of the human gut microbiota. Frontiers in Microbiology. 2016;**7**:925. DOI: 10.3389/fmicb.2016.00925

[67] Hughes HK, Rose D, Ashwood P. The gut microbiota and dysbiosis in autism spectrum disorders. Current Neurology and Neuroscience Reports. 2018;**18**(11):81. DOI: 10.1007/s11910-018-0887-6

[68] Galland L. The gut microbiome and the brain. Journal of Medicinal Food. 2014;**17**(12):1261-1272. DOI: 10.1089/jmf.2014.7000

[69] American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Arlington, VA: American Psychiatric Association; 2013. 947 p. DOI: 10.1176/appi.books.9780890425596

[70] World Health Organisation. Autism spectrum disorders. [Internet]. 2018. Available from: https://www.who.int/en/news-room/fact-sheets/detail/autism-spectrum-disorders [Accessed: 03 August 2019]

[71] Centers for Disease Control and Prevention. Autism Spectrum Disorder

(ASD) [Internet]. 2019. Available from: https://www.cdc.gov/ncbddd/autism/data.html [Accessed: 04 August 2019]

[72] Kraneveld AD, Szklany K, de Theije CG, Garssen J. Gut-to-brain axis in autism spectrum disorders: Central role for the microbiome. International Review of Neurobiology. 2016;**131**:263-287. DOI: 10.1016/bs.irn.2016.09.001

[73] Finegold SM, Molitoris D, Song Y, Liu C, Vaisanen ML, Bolte E, et al. Gastrointestinal microflora studies in late-onset autism. Clinical Infectious Diseases. 2002;**35**(Suppl 1):s6-s16. DOI: 10.1086/341914

[74] Song Y, Liu C, Finegold SM. Real-time PCR quantitation of clostridia in feces of autistic children. Applied and Environmental Microbiology. 2004;**70**(11):6459-6465. DOI: 10.1128/AEM.70.11.6459-6465.2004

[75] Parracho HM, Bingham MO, Gibson GR, McCartney AL. Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. Journal of Medical Microbiology. 2005;**54**(Pt 10):987-991. DOI: 10.1099/jmm.0.46101-0

[76] Finegold SM, Dowd SE, Gontcharova V, Liu C, Henley KE, Wolcott RD, Youn E, Summanen PH, Granpeesheh D, Dixon D, Liu M, MolitorisDR,GreenJA3rd.Pyrosequencing study of fecal microflora of autistic and control children. Anaerobe 2010;**16**(4):444-453. DOI: 10.1016/j.anaerobe.2010.06.008

[77] Adams JB, Johansen LJ, Powell LD, Quig D, Rubin RA. Gastrointestinal flora and gastrointestinal status in children with autism–comparisons to typical children and correlation with autism severity. BMC Gastroenterology. 2011;**11**:22. DOI: 10.1186/1471-230X-11-22

[78] Martirosian G, Ekiel A, Aptekorz M, Wiechula B, Kazek B, Jankowska-Steifer E, et al. Fecal lactoferrin and *Clostridium* spp. in stools of autistic children. Anaerobe. 2011;**17**(1):43-45. DOI: 10.1016/j.anaerobe.2010.12.003

[79] Williams BL, Hornig M, Buie T, Bauman ML, Cho Paik M, Wick I, et al. Impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children with autism and gastrointestinal disturbances. PLoS One. 2011;**6**(9):e24585

[80] Williams BL, Hornig M, Parekh T, Lipkin WI. Application of novel PCR-based methods for detection, quantitation, and phylogenetic characterization of *Sutterella* species in intestinal biopsy samples from children with autism and gastrointestinal disturbances. MBio. 2012;**3**(1):e00261-11. DOI: 10.1128/mBio.00261-11

[81] Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, Conlon MA. Low relative abundances of the mucolytic bacterium *Akkermansia muciniphila* and *Bifidobacterium* spp. in feces of children with autism. Applied and Environmental Microbiology. 2011;**77**(18):6718-6721. DOI: 10.1128/AEM.05212-11

[82] Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, Conlon MA. Elevated fecal short chain fatty acid and ammonia concentrations in children with autism spectrum disorder. Digestive Diseases and Sciences. 2012;**57**(8):2096-2102. DOI: 10.1007/s10620-012-2167-7

[83] Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, Conlon MA. Increased abundance of *Sutterella* spp. and *Ruminococcus torques* in feces of children with autism spectrum disorder.

Molecular Autism. 2013;**4**:42. DOI: 10.1186/2040-2392-4-42

[84] Gondalia SV, Palombo EA, Knowles SR, Cox SB, Meyer D, Austin DW. Molecular characterisation of gastrointestinal microbiota of children with autism (with and without gastrointestinal dysfunction) and their neurotypical siblings. Autism Research. 2012;**5**(6):419-427. DOI: 10.1002/aur.1253

[85] Kang DW, Park JG, Ilhan ZE, Wallstrom G, Labaer J, Adams JB, et al. Reduced incidence of Prevotella and other fermenters in intestinal microflora of autistic children. PLoS One. 2013;**8**(7):e68322. DOI: 10.1371/journal.pone.0068322

[86] De Angelis M, Piccolo M, Vannini L, Siragusa S, De Giacomo A, Serrazzanetti DI, et al. Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. PLoS One. 2013;**8**(10):e76993. DOI: 10.1371/journal.pone.0076993

[87] Son JS, Zheng LJ, Rowehl LM, Tian X, Zhang Y, Zhu W, et al. Comparison of fecal microbiota in children with autism Spectrum disorders and Neurotypical siblings in the Simons simplex collection. PLoS One. 2015;**10**(10):e0137725. DOI: 10.1371/journal.pone.0137725

[88] Tomova A, Husarova V, Lakatosova S, Bakos J, Vlkova B, Babinska K, et al. Gastrointestinal microbiota in children with autism in Slovakia. Physiology & Behavior. 2015;**138**:179-187. DOI: 10.1016/j.physbeh.2014.10.033

[89] Luna RA, Oezguen N, Balderas M, Venkatachalam A, Runge JK, Versalovic J, et al. Distinct microbiome-neuroimmune signatures correlate with functional abdominal pain in children with autism spectrum disorder. Cellular and Molecular Gastroenterology and Hepatology. 2016;**3**(2):218-230. DOI: 10.1016/j.jcmgh.2016.11.008

[90] Kang DW, Ilhan ZE, Isern NG, Hoyt DW, Howsmon DP, Shaffer M, et al. Differences in fecal microbial metabolites and microbiota of children with autism spectrum disorders. Anaerobe. 2018;**49**:121-131. DOI: 10.1016/j.anaerobe.2017.12.007

[91] de Theije CG, Wu J, da Silva SL, Kamphuis PJ, Garssen J, Korte SM, et al. Pathways underlying the gut-to-brain connection in autism spectrum disorders as future targets for disease management. European Journal of Pharmacology. 2011;**668**(Suppl 1):S70-S80. DOI: 10.1016/j.ejphar.2011.07.013

[92] de Magistris L, Familiari V, Pascotto A, Sapone A, Frolli A, Iardino P, et al. Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. Journal of Pediatric Gastroenterology and Nutrition. 2010;**51**(4):418-424. DOI: 10.1097/MPG.0b013e3181dcc4a5

[93] Audet MC, Jacobson-Pick S, Wann BP, Anisman H. Social defeat promotes specific cytokine variations within the prefrontal cortex upon subsequent aggressive or endotoxin challenges. Brain, Behavior, and Immunity. 2011;**25**(6):1197-1205. DOI: 10.1016/j.bbi.2011.03.010

[94] Haba R, Shintani N, Onaka Y, Wang H, Takenaga R, Hayata A, et al. Lipopolysaccharide affects exploratory behaviors toward novel objects by impairing cognition and/or motivation in mice: Possible role of activation of the central amygdala. Behavioural Brain Research. 2012;**228**(2):423-431. DOI: 10.1016/j.bbr.2011.12.027

[95] van Heesch F, Prins J, Konsman JP, Westphal KG, Olivier B, Kraneveld AD, et al. Lipopolysaccharide-induced

anhedonia is abolished in male serotonin transporter knockout rats: An intracranial self-stimulation study. Brain, Behavior, and Immunity. 2013;**29**:98-103. DOI: 10.1016/j.bbi.2012.12.013

[96] Emanuele E, Orsi P, Boso M, Broglia D, Brondino N, Barale F, et al. Low-grade endotoxemia in patients with severe autism. Neuroscience Letters. 2010;**471**(3):162-165. DOI: 10.1016/j.neulet.2010.01.033

[97] Anderson GM, Freedman DX, Cohen DJ, Volkmar FR, Hoder EL, McPhedran P, et al. Whole blood serotonin in autistic and normal subjects. Journal of Child Psychology and Psychiatry. 1987;**28**(6):885-900

[98] Hanley HG, Stahl SM, Freedman DX. Hyperserotonemia and amine metabolites in autistic and retarded children. Archives of General Psychiatry. 1977;**34**(5):521-531. DOI: 10.1001/archpsyc.1977.01770170031002

[99] Schain RJ, Freedman DX. Studies on 5-hydroxyindole metabolism in autistic and other mentally retarded children. The Journal of Pediatrics. 1961;**58**:315-320. DOI: 10.1016/s0022-3476(61)80261-8

[100] Chugani DC1, Muzik O, Behen M, Rothermel R, Janisse JJ, Lee J, Chugani HT. Developmental changes in brain serotonin synthesis capacity in autistic and nonautistic children. Annals of Neurology. 1999;**45**(3):287-295

[101] Marler S, Ferguson BJ, Lee EB, Peters B, Williams KC, McDonnell E, et al. Brief report: Whole blood serotonin levels and gastrointestinal symptoms in autism spectrum disorder. Journal of Autism and Developmental Disorders. 2016;**46**(3):1124-1130. DOI: 10.1007/s10803-015-2646-8

[102] McDougle CJ, Naylor ST, Cohen DJ, Aghajanian GK, Heninger GR, Price LH. Effects of tryptophan depletion in drug-free adults with autistic disorder. Archives of General Psychiatry. 1996;**53**(11):993-1000. DOI: 10.1001/archpsyc.1996.01830110029004

[103] Foley KA, MacFabe DF, Vaz A, Ossenkopp KP, Kavaliers M. Sexually dimorphic effects of prenatal exposure to propionic acid and lipopolysaccharide on social behavior in neonatal, adolescent, and adult rats: Implications for autism spectrum disorders. International Journal of Developmental Neuroscience. 2014;**39**:68-78. DOI: 10.1016/j.ijdevneu.2014.04.001

[104] Foley KA, MacFabe DF, Kavaliers M, Ossenkopp KP. Sexually dimorphic effects of prenatal exposure to lipopolysaccharide, and prenatal and postnatal exposure to propionic acid, on acoustic startle response and prepulse inhibition in adolescent rats: Relevance to autism spectrum disorders. Behavioural Brain Research. 2015;**278**:244-256. DOI: 10.1016/j.bbr.2014.09.032

[105] Wang L, Conlon MA, Christophersen CT, Sorich MJ, Angley MT. Gastrointestinal microbiota and metabolite biomarkers in children with autism spectrum disorders. Biomarkers in Medicine. 2014;**8**(3):331-344. DOI: 10.2217/bmm.14.12

[106] Yang Y, Tian J, Yang B. Targeting gut microbiome: A novel and potential therapy for autism. Life Sciences. 2018;**194**:111-119. DOI: 10.1016/j.lfs.2017.12.027