Life Sciences 194 (2018) 111–119

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Review article

Targeting gut microbiome: A novel and potential therapy for autism

Yongshou Yang[a,*], Jinhu Tian[b], Bo Yang[a]

[a] Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima 739-8528, Japan
[b] Department of Food Science and Nutrition, Zhejiang University, 866 Yuhangtang Road, Hangzhou, Zhejiang Province 310058, PR China

ARTICLE INFO

Keywords:
Gut microbiome
Probiotics
Gut-brain axis
Autism spectrum disorders
Gastrointestinal problems
Therapy

ABSTRACT

Autism spectrum disorder (ASD) is a severely neurodevelopmental disorder that impairs a child's ability to communicate and interact with others. Children with neurodevelopmental disorder, including ASD, are regularly affected by gastrointestinal problems and dysbiosis of gut microbiota. On the other hand, humans live in a co-evolutionary association with plenty of microorganisms that resident on the exposed and internal surfaces of our bodies. The microbiome, refers to the collection of microbes and their genetic material, confers a variety of physiologic benefits to the host in many key aspects of life as well as being responsible for some diseases. A large body of preclinical literature indicates that gut microbiome plays an important role in the bidirectional gut-brain axis that communicates between the gut and central nervous system. Moreover, accumulating evidences suggest that the gut microbiome is involved in the pathogenesis of ASD. The present review introduces the increasing evidence suggesting the reciprocal interaction network among microbiome, gut and brain. It also discusses the possible mechanisms by which gut microbiome influences the etiology of ASD via altering gut-brain axis. Most importantly, it highlights the new findings of targeting gut microbiome, including probiotic treatment and fecal microbiota transplant, as novel and potential therapeutics for ASD diseases.

1. Introduction

Neurodevelopmental disorders are characterized by disturbances of the growth and development of the brain or central nervous system (CNS). Autism spectrum disorder (ASD) is a severely neurodevelopmental disorder that impairs a child's ability to communicate and interact with others [1]. The core symptoms also include restricted repetitive patterns of behaviors, interests and activities. In addition to the core symptoms of ASD, related conditions and behaviors have been reported recently, including abnormalities in sensory processing (hypo or hypersensitivity), gastrointestinal (GI) symptoms or even self-injurious behaviors [2]. The reported prevalence of ASD has increased dramatically in recent years. Based on the extensive surveys conducted by the Centers for Disease Control and Prevention, the incidence of ASD from 1 in 150 children in 2000 increased to 1 in 68 in 2012 in the United States (https://www.cdc.gov/ncbddd/autism/data.html). In spite of the extensive efforts, the exact mechanisms of ASD have not been clearly elucidated. Recently, emerging works suggested that various environmental factors (including chemical exposures, viral infections, and metabolic imbalances) have been implicated in the etiology and pathogenesis of ASD [3,4]. However, due to the lack of consensus on the causes of ASD, there have been no widely accepted and efficient therapies for such disorder.

A large body of recent studies elucidated the crucial roles of gut microbiome in the functions of CNS, neuroendocrine and neuroimmune systems [5,6]. The altered bidirectional neurohumoral communication system between gut and brain – known as the gut-brain axis – may cause a series of diseases, such as autoimmune and CNS disorders [6]. GI distress, which affects millions of people worldwide, is a most common syndrome among several comorbidities in ASD patients. Morover, a recent multi-case analysis of about 15,000 ASD patients indicated that almost 12% of ASD individuals had comorbidity with bowel disorders [7]. Based on a great many previous studies, dysbiosis of the gut microbiome was implicated in the etiology of several diseases, including inflammatory bowel disease (IBD), obesity, and cardiovascular disorders, in both human and animal models [8,9]. In addition, increasing evidences revealed that the compositions of gut microbiota in ASD patients were largely different compared with the healthy control individuals [10–13]. Taken together, we speculate that gut microbiome, acting singly or in interaction with other factors, may be associated with the pathogenesis of ASD.

This review hopes to provide a better understanding of the gut microbiome as a potential therapy for ASD and its role with respect to neurodevelopmental disorders (e.g., ASD). Then the possible and underlying mechanisms by which the gut microbiome interacts with ASD are fully analyzed. Most importantly, the very recent and compelling

* Corresponding author.
E-mail address: d156196@hiroshima-u.ac.jp (Y. Yang).

https://doi.org/10.1016/j.lfs.2017.12.027
Received 18 August 2017; Received in revised form 12 December 2017; Accepted 21 December 2017
Available online 23 December 2017

studies that suggested probiotic and fecal microbiota transplant treatments (FMT) can help to improve GI- and ASD-related behavioral symptoms are summarized.

2. Overview of gut microbiome

Human beings, including other mammals, live in a co-evolutionary association with huge numbers of microorganisms that resident on the exposed and internal surfaces of their bodies. The collection of microbes and their genetic material is termed microbiome. The human GI tract contains approximately 10^{14} bacteria belonging to approximately 1000 species [14]. It is estimated that commensal microbiome outnumbers at least 100-folds more than the human somatic genome [15]. The healthy adult GI tract is most dominated by *Bacteroidetes* and *Firmicutes* phyla (both account for up to 70–90% of total bacteria), followed by *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* [16]. These symbiotic microbiota dwelling in the gut have long been appreciated for the various beneficial effects they offered to the host including providing essential nutrients by metabolizing indigestible dietary compounds, defending against opportunistic pathogen colonization by nutrient competition and antimicrobial substance production, and contributing to intestinal epithelial barrier [17]. Moreover, the studies on the immune defects in germ-free (GF) mice have suggested that gut microbiome was essential to the host immune system [18,19]. A recent review also indicated that gut bacterial colonization could drive maturation and functionality of the host adaptive immune system [17].

The beneficial partnership between gut microbiome and the host immune system contributes greatly to the normal host homeostasis during life. Disruption of a balanced composition of gut microbiome can cause immunological dysregulation that may underlie several inflammatory disorders, such as irritable bowel syndrome (IBS) and IBD or even several kinds of cancer [17]. Moreover, altered gut microbiome also affects microbiota-derived products and metabolites, including pro- and anti-inflammatory materials, which in turn can influence development or composition of the gut microbiome [20]. However, some probiotics, such as *Bifidobacteria* and *Lactobacillus*, may re-establish the composition of the gut microbiome and exert benefits to gut microbial communities, leading to amelioration or prevention of gut inflammation and other intestinal or systemic diseases [21].

3. Communications among microbiome, gut and brain

Ever since the term of gut-brain axis was proposed and the first publication of preclinical studies that revealed the communication among gut microbiome, gut and brain, there are fast-growing studies focusing on this field and further deepening our understanding of those correlations [22]. The gut-brain axis refers to the bidirectional biochemical communications between the GI tract and the nervous system [6]. The reciprocal interaction network consists of CNS, the autonomic nervous system (ANS), the enteric nervous system (ENS), hypothalamic pituitary adrenal (HPA) axis, gut microbiome, as well as the vagus nerve that is the major pathway in the connection between the viscera and CNS [6,23].

In the past several years, many efforts exploring the roles of gut microbiome in modulating brain functions have been performed mainly on animals. Studies conducted on the GF animals demonstrated that bacterial colonization of the gut was vitally significant to the development and maturation of both CNS and ENS [24,25]. Based on the previous publications [6,23,26], we summarized the major categories of mechanisms by which the gut microbiome regulates the brain functions. First, the gut microbiome could influence the production, expression and turnover of neurotransmitters, such as serotonin and gamma-aminobutyric acid (GABA), and brain-derived neurotrophic factor (BDNF), which are an important factor to memory. Second, the gut microbiome contributed to the intestinal barrier and tight junction integrity, and thus attenuated HPA axis and ANS functions. Third, some species of probiotics modulated enteric sensory afferents and ENS activity. Fourth, the gut microbiome could exert effects on the brain through bacteria-derived and/or co-metabolites, including short-chain fatty acids (SCFAs), serotonin and kynurenine productions. Finally, immunological pathways (systemic and mucosal immune) also played a significant role in the regulation of the gut-brain axis by the gut microbiome.

Evidence from animal research have shown that several types of social stressors had an influence on mucus secretion, and the composition as well as total biomass of gut microbiota [27,28]. The fact suggests that brain functions play an important role in the regulation of brain-gut-microbiome signaling. The CNS directly exerts control over the gut microbiota composition via peptides which are sent upon satiation and thus influence nutrient availability. The release of signaling molecules by neurons, immune and enterocromaffin cells under controls of the brain may directly affect the gut microbiome [23]. Moreover, brain can modulate gut functions, including motility, secretion of acid and mucins, and handling of intestinal fluid, all crucial for the maintenance of the mucosal biofilms where bacteria grow in a multiplicity of microenvironment [29]. Furthermore, gut microbiota composition and function may be regulated by the brain via alteration of intestinal permeability, leaving some pathogens across the epithelium and activating the immune response in the mucosa [23]. There is even evidence from rat models that stress in mothers during prenatal development can induce long-term alterations in the gut microbiota, and impact upon other major physiological systems in their offspring [30]. This research implies that a propensity to heightened social stress may also be transmitted biologically via the microbiota through vaginal delivery. Last but not least, HPA regulates cortisol secretion, and cortisol can affect immune function. Cortisol also can influence gut permeability and barrier function, as well as change gut microbiota composition. For instance, a recent study on mice indicated that olfactory bulbectomy induced chronic depression elevated corticotropin-releasing hormone expression and serotonin levels, associated with alteration of motor activity and the microbial profile in the colon likely via activation of the HPA [31].

4. Underlying mechanisms by which gut microbiome is involved in ASD

Despite numerous studies focused on the possible pathogenesis of ASD, the exact causes of this disease have not been clearly stated and the current theory points that ASD should be a result of combinations including several genetic and environmental risk factors. Particularly, accumulating studies underlined the potential roles of environmental risk factors and associated comorbidities in the contribution to core neurobehavioral symptoms of ASD. The microbiome habituating in the body is well known for its functions of interaction between genes and environment. With the increasing knowledge of microbiome, it is believed that ASD-associated alterations of gut microbiome and its metabolites can directly and indirectly modulate corresponding immune and GI disorders, and may play a possible role in the etiology of ASD (see Fig. 1).

4.1. Microbiome-associated maternal risk factors

There are strong evidences from many epidemiological, clinical, and animal studies that showed that maternal infections as primarily influencing the development of ASD symptoms [32–35]. For decades, fetuses were thought to be born germ-free, but recent researches using meconium samples from healthy babies suggested that fetuses were already exposed to maternal bacteria in utero [36,37]. Subsequently, the microbial colonization performed in the infants during vaginal delivery was largely different from that of microbiome acquired in the newborns by cesarean section [38,39]. Therefore, microbial dysbiosis in the maternal microbiome in response to environmental risk exposure or

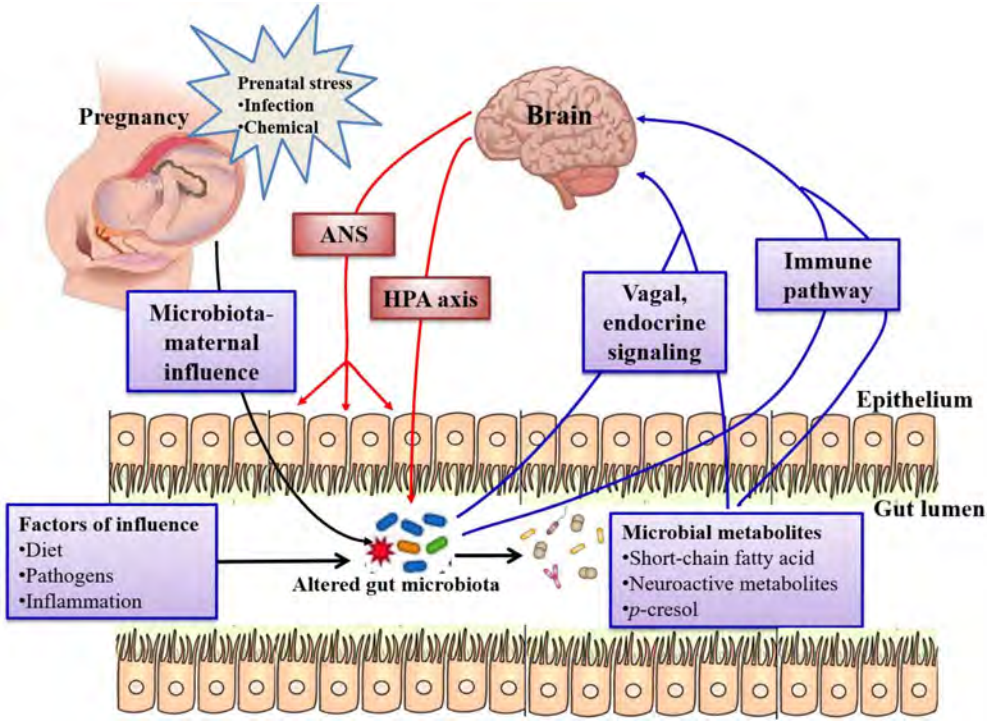

Fig. 1. The potential mechanisms by which the gut microbiome is involved in ASD. The maternal influence, like maternal bacterial dysbiosis, is believed the first reason to cause the compositional and functional changes of gut microbiota in the offspring. The altered gut microbiome and their subsequently aberrant metabolites are able to cause GI problems in the gut, as well as influence the brain function via endocrine and immune signaling pathways. The altered microbiome related signaling to the brain may play a role in the possible pathogenesis of ASD. Conversely, the brain mainly affects GI functions, including motility, intestinal barriers, and gut epithelial integrity, through ANS pathway. Additionally, the brain is likely to have a major impact on the composition and function of the gut microbiota by modulation of the HPA axis. ASD, autism spectrum disorder; ANS, autonomic nervous system; GI, gastrointestinal; HPA, hypothalamic pituitary adrenal; P-cresol, para-cresol.

genetic risk heritance may be passed onto the offspring at birth. Consistent with this concept, increasing evidences from human epidemiology and animal studies suggested that maternal high-fat diet and several metabolic conditions exposure could increase the risk of developing neurodevelopmental and behavioral disorders in offspring [40–42]. Moreover, animal data indicated that microbiome alterations induced by maternal stress were passed onto the offspring at birth, causing lasting microbial dysbiosis and other long-term consequences for the offspring [30,43].

Maternal immune activation (MIA) is a useful model to investigate the effects of maternal infection influence on the offspring in the pathogenesis of neurodevelopmental diseases, including ASD. The findings from animal models suggested prenatal exposure to poly (I:C)-induced MIA could cause offspring lifelong neuropathology and altered behaviors through changing the composition of gut microbiome and altering other transcriptional pathways in the offspring [44–46]. ASD-associated maternal risk factors increase the possibility of ASD-like behaviors in the offspring may through several other potential pathways, including altered modulation of placenta, epigenetic modification, as well as immune dysregulation [47–49]. Future studies will require cautious consideration of study subjects in order to address the specific roles of these prenatal risk factors.

4.2. ASD-associated gut microbiome alterations

ASD patients were noticed for alterations in the composition of gut microbiome as compared with healthy control subjects. By pyrosequencing gut microbiome from fecal DNA samples, Kang et al. [12] found that distinct and less diverse gut microbial compositions and decreased levels of several genera in the children with ASD, including *Prevotella*, *Corprococcus*, and *Veilonellaceae*, which were responsible for carbohydrate ingestion and fermentation. At the phylum level, analysis of fecal microflora from children with regressive autism reported an elevated abundance of Firmicutes, and relative lower abundances of other phyla, including Bacteroidetes, Actinobacteria and Proteobacteria [50]. ASD children also showed increased Firmicutes/Bactteroidetes ratio, as well as higher levels of *Lactobacillus* and *Desulfovibrio* species [13,51]. Of particular interest was that *Desulfovibrio* abundance positively related to the severity of ASD [13]. The genus *Sutterella*, associated with mucosal metabolism, was found at elevated abundance in biopsies taken from the GI tract of ASD patients with GI disturbance relative to control subjects with GI disturbance [52]. Additionally, a study performed by Wang et al. [53] revealed that increased numbers of *Sutterella*, as well as mucosa-associated *Ruminococcus torques*, in the stools from ASD patients compared with community controls, further confirmed and supplemented this conclusion.

Clostridium, a common bacterium in GI, is well-documented and the most possible cause for certain cases of ASD [54,55]. Several studies have demonstrated a significantly higher prevalence of genus *Clostridium* in ASD patients [50,56,57]. According to the extremely close association of ASD symptoms and chronic tetanus infection of GI, Bolte [58] has proposed the hypothesized role of *Clostridium* in the etiology of ASD. *Clostridium tetani*, an ubiquitous anaerobic bacillus, produces a potent neurotoxin which inhibited neurotransmitter release, and was thought to be related to behavioral disruptions of ASD [58]. *Clostridia* contained a heterogeneous group of spore-forming and gram-positive bacilli, with some species belonged to this genus were believed to have profitable effects on the host, such as *Faecalibacterium prausnitzii* and *Ruminococcus*. However, several *Clostridium* species, including *C. perfringens*, *C. difficile*, and *C. botulinum*, produced numerous toxins and subsequently causing GI disorders or other toxin-mediated diseases [59,60]. Increasing evidences from analyses of fecal specimens from both ASD and control healthy children, indicated greater abundances in *Clostridium* clusters I and XI (which contain toxin-producing *C. perfringens* and *C. difficile*), with reduced level of cluster XIVab (considered to be beneficial bacteria) [50,56]. Additionally, a similar study conducted by Parracho et al. [57] found a higher incidence of the *C. histolyticum* group (*Clostridium* clusters I and II) in ASD children than in healthy controls, and an intermediate level in their siblings. Some literatures suggested that *Clostridium* acted as a pivotal factor in the etiology of ASD because ASD children exhibited ameliorated behavior, communication, and social skills in the GI manifestations of their diseases after oral vancomycin treatment, an antibiotic well known mainly against clostridia [58,61]. However, the GI disorders and the core behavioral symptoms relapsed again after the discontinuation of vancomycin administration. This relapse might be due to the fact that spores

of *Clostridium*, which were difficult to be killed by antibiotics, could grow into infective forms later [58]. Consistent with this concept, Finegold et al. [50] reported larger numbers of non-spore-forming anaerobes and microaerophilic bacteria from children with ASD, but no such bacteria were found in healthy children.

Although many studies reported changes of the gut microbiome in ASD patients, there is still little consensus on specific bacterial species that were similarly changed among independent researches. To date, the physiological functions of numerous of bacterial species are not fully understood. Several factors, including dietary lifestyles, microbial infection, and xenobiotics, could influence the normal microflora profile and then cause functional alterations [62]. Therefore, not only the alterations of bacterial abundance can be estimated, but also the changes of bacterial functions interacting with other risk factors, may contribute to the pathogenesis of certain ASD.

4.3. ASD-associated microbial metabolites

With the emergence of the effective measurement technique, such as metabolomics, liquid chromatography-mass spectrometry (LC-MS) and gas chromatography–mass spectrometry (GS-MS), it is possible and efficient to analysis the alterations of various metabolites in ASD patients. Increasing evidence revealed that abnormalities of microbial metabolites, such as excessive production of SCFAs, *para*-cresol (*p*-cresol) and ammonia, were found inside the bodies responding to abnormal gut microbiome by analyses of urinary, serum and fecal samples from ASD children [63,64]. As some metabolites generated by gut microbiome play an important role in health and diseases, they are considered possible pathways to trigger the etiology of ASD.

The overproduction of SCFAs detected in feces from ASD patients was reported by several recent studies [64,65]. A recent review concluded that enteric SCFAs, existed in diet and also produced by gut microbiome following fermentation of dietary carbohydrates, might be environmental triggers of ASD [66]. Propionic acid is a major SCFAs produced by ASD-associated gut bacteria, such as *Clostridium*, *Bacteroides*, and *Desulfovibrio*, in GI and also used as a common preservative in traditional food industry. Several studies performed in rodent animals showed that rats treated with propionic acid displayed restricted behavioral interest, impaired social behaviors and cognition, as well as an induced innate neuroinflammatory response [67–69]. The underlying mechanisms of influences by SCFAs in the pathogenesis of ASD were considered through the altered mitochondrial functions via the citric acid cycle and carnitine metabolism, or the epigenetic modulation of ASD-associated genes [66].

Another microbial metabolite *p*-cresol and its conjugated derivative *p*-cresylsulfate, elevated in the urinary samples of ASD children below 8 years of age, were suggested as biomarkers of ASD in small children, especially females and more severely affected males [63,70]. It is common that the skin, GI and respiratory systems absorb *p*-cresol into bodies when exposed to some environment. However, the most widespread source of this compound produced by some gut bacteria which expresses *p*-cresol synthesizing enzymes have not been found in human cells [71]. The gut- or environmentally-derived *p*-cresol is potentially able to worsen ASD severity and gut function, but the precise mechanisms are still poorly understood [71].

There was a study using urinary metabolic phenotyping in ASD children that indicated abnormal levels of mammalian-microbial common metabolites, including dimethylamine, hippuric and phenylacetylglutamine [72]. Another urinary metabolomic research demonstrated that ASD children and healthy children displayed larger difference with the tryptophan and purine metabolic pathways associated with gut microbiome [73]. There was also a discovery with the metabolomic analysis of serum that identified 11 metabolites consistently different in 2 independent cohorts of patients with ASD and controls. Among these metabolites, sphingosine 1-phosphate and docosahexaenoic acid were consistently replicated in a multiple logistic regression model [74]. Different kinds of abnormal ASD-associated metabolites were reported by many studies which suggested that the intricate and mutable metabolism existed in ASD patients. Future research is needed to reduce the confounding factors in metabolic analyses, such as ethnics, sex, and lifestyles, as well as the widespread and representative biomarkers that should be established for ASD.

4.4. Gut microbiome-associated immune deregulation

Accumulating evidences indicated that immune dysregulation increased the risk, and contributed to the pathophysiology, of ASD in human and animal models. Extensive immune aberration have been described in ASD individuals, including ongoing inflammation in brain specimens, elevated pro-inflammatory cytokine profiles in the cerebrospinal fluid and blood, increased presence of brain-specific autoantibodies and raised immune cell dysfunction [75]. The ongoing neuroinflammation, observed in postmortem brain specimens from ASD individuals, which was marked by excess microglia activation and, increased inflammatory cytokine and chemokine production, including interferon (IFN)-γ, IL-1β, IL-6, IL-12p40, tumor necrosis factor (TNF)-α and chemokine C-C motif ligand (CCL)-2 [76,77]. Furthermore, elevated levels of these inflammatory cytokines in the circulating system were found to be associated with a regressive form of ASD and more impaired stereotypical behaviors, and increased levels of chemokines CCL-2 and CCL-5 in ASD patients were associated with higher aberrant behavioral scores and more impaired development [78]. Additionally, increasing evidence showed impaired immune cell function and responsiveness following immune challenge in ASD patients, including defective natural killer cells, and abnormal monocytes that exhibited impaired responses to challenge with ligands for Toll-like receptor 4 [79,80].

Commensal microbiome is considered to play important roles in regulation of nervous-immune-endocrine system. The initially microbial colonization, dominated by maternal microbiome during vaginal delivery, assists in priming the developing immune system and regulates immune homeostasis [81,82]. A variety of immune dysregulation observed in ASD patients may be associated with gut microbiome. For example, some species of gut bacteria modulate differentiation of T lymphocyte subtypes. Intestinal colonization by segmented filamentous bacteria could promote pro-inflammatory IL-17 production in the gut, and subsequently trigger symptoms of autoimmune disease in mouse models [83]. Inversely, *Bacteroides fragilis* colonization could improve symptoms of intestinal disease and multiple sclerosis in animal models by restraining T helper 17 cell responses and enhancing levels of IL-10-producing T regulatory cells [84,85]. Despite many studies that have reported that impaired immune system seen in ASD that may be associated with gut microbiome, the precise mechanisms of how and whether gut microbiome alterations in ASD can cause immune abnormalities observed in the disorder are not currently understood.

5. Novel therapeutic approaches targeting gut microbiome for ASD

Since a large number of publications have proved that gut microbiota played critical roles in the pathogenesis of ASD, we can naturally think targeting gut microbiome may be an effective therapeutic approach for such disorder. Diet and particular macronutrients are one of the main factors that affect the composition of gut microbiota. Western-style diet patterns could negatively influence anxiety-like behavior and memory, depending on inflammatory state [86]. In contrast, high levels of polyunsaturated fatty acid administration could alleviate depression in rat models [87]. Prebiotics, defined as selectively fermented ingredient that allows specific changes, both in the composition and/or activity of the gut microflora conferring benefits upon host well-being and health, are mainly inulin, fructo-oligosaccharides and galacto-oligosaccharides, and lactulose [88]. Savignac et al. [89] have

Table 1
Probiotics administration improves ASD-associated behavioral abnormalities in ASD patients and rodent models.

Subject	Behavior(s)	Probiotics	Key findings	Reference(s)
Mice	Stress response	*Bifidobacterium infantis*	GF mice displayed elevated response to restraint stress, and reduced BDNF in the cortex and hippocampus. The defects were reversed by reconstitution with *B.infantis*.	[92]
Mice (AKR)	Anxiety-like behavior	*Bifidobacterium longum* NCC3001	GI inflammation caused anxiety-like behavior, decreased hippocampal BDNF mRNA and increased TNFα, IFN γ as well as kynurenine and kynurenine/tryptophan. *B. longum* normalized behavior and BDNF mRNA.	[93]
Mice	Anxiety- and depression-related behavior	*Lactobacillus rhamnosus* (JB-1)	*L. rhamnosus* treatment induced cortical $GABA_{B1b}$ mRNA expression, and decreased $GABA_{A\alpha2}$ mRNA in the prefrontal cortex and amygdala, but increased in the hippocampus. *L. rhamnosus* reduced stress-induced corticosterone and anxiety- and depression-related behavior.	[94]
Mice	Anxiety-like behavior	*Bifidobacterium longum* NCC3001	*B. longum* normalized behavior that altered in chemical colitis mouse model, but had no effect on inflammatory activity and BDNF expression.	[95]
Mice	ASD-like behavior	*Bacteroides fragilis*	MIA offspring exhibited GI barrier defects, altered serum metabolites, microbial dysbiosis, and ASD-like behavior. *B. fragilis* treatment restored these abnormalities in MIA offspring.	[44]
Mice	Social behavior	*Lactobacillus reuteri*	Maternal high-fat diet induced deficient synaptic plasticity in the VTA, reduced oxytocin production as well as social deficits in offspring. *L.reuteri* restored oxytocin levels, VTA plasticity and social behavior.	[96]
Mice (Innately anxious BALB/c)	Anxiety-like behavior	*Bifidobacterium longum* 1714, *B. breve* 1205	Both *Bifidobacteria* reduced anxiety-like behavior and improved cognitive processes, but had no effect on visceral sensitivity.	[97,98]
Mice/rats	Visceral hypersensitivity	*Lactobacillus paracasei* NCC2461 or *Bifidobacterium infantis* 35,624	The two probiotics corrected visceral sensitivity and reduced visceral pain, respectively.	[99,100]
Rats	Depression-like behavior	*Bifidobacterium infantis* 35,624	*B.infantis* treatment normalize the immune response, recovered behavioral deficits in the forced swim test, and restored noradrenaline concentrations in the brainstem	[101]
Rats and humans	anxiety, depression, stress	*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175	Probiotics treatment reduced anxiety-like behavior in rats and alleviated psychological distress in healthy humans.	[102]
Humans (n = 20)	Negative thoughts associated with sad mood	Multispecies probiotic containing *B. bifidum W23*, *B. lactis W52*, *L. acidophilus W37*, *L. brevis W63*, *L. casei W56*, *L. salivarius W24*, and L. *lactis* (W19 and W58)	Administration of 4-week multispecies probiotics decreased cognitive reactivity to sad mood, which was largely accounted for by reduced rumination and aggressive thoughts.	[103]
Humans (n = 12)	Healthy woman without GI or psychiatric symptoms	*B. animalis* subsp. *lactis*, *Streptococcus thermophiles*, *L. bulgaricus*, and *Lactococcus lactis* subsp. *Lactis*	Four-week consumption of fermented milk product with probiotic modulated activity of brain regions that control central processing of emotion and sensation.	[104]
Humans (n = 18)	Children with ASD also suffering GI disorders	Standardized human gut microbiota	Administration of 8-week fecal microbiota transplant significantly improved both GI- and ASD-related symptoms in 16 out of 18 ASD children.	[105]

Abbreviation: GF, germ free; GI, gastrointestinal; BDNF, brain-derived neurotrophic factor; TNF-α, tumor necrosis factor alpha; IFN-γ, including interferon gamma; GABA, gamma-aminobutyric acid; mRNA, messenger RNA; VTA, ventral tegmental area; ASD, autism spectrum disorder; MIA, maternal immune activation.

demonstrated that oligosaccharides induced the growth of indigenous beneficial gut bacteria such as *Lactobacilli* and *Bifidobacteria*, and exhibited neurotropic effects in rat models. The same group also confirmed that Bimuno®-galactooligosaccharides supplement suppressed the neuroendocrine stress response and increased the processing of positive versus negative attentional vigilance in healthy human [90]. Together, these data suggested that prebiotics can maintain brain health, may be through modulating gut microbiome, and possibly useful as adjunctive treatment of neuropsychiatric disorders.

Another charming area of the therapeutics showing renewed interest in recent years is probiotics, which have the ability to confer beneficial effects upon the host. A special focus has been placed on their ability to affect neural and endocrine systems and behavioral phenotypes [91]. The emerging evidence demonstrated that probiotics administration is able to improve and ameliorate behavioral and neurophysiological conditions associated with ASD in animal models and human studies (see Table 1). Particularly, some most distinguished studies confirmed the beneficial roles of probiotics for ASD-related symptoms in mouse model, which will be discussed in the following section.

5.1. Probiotics administration

A recently notable study by Hsiao et al. [44] demonstrated that the vital roles of a probiotic, *Bacteroides. fragilis*, in the improvement of ASD-associated behaviors as well as modulation of several metabolites. In this study, the authors made a mouse model of MIA to evaluate the influence of gut microbiome on ASD-like behaviors [44]. MIA offspring exhibited disruption of GI barrier integrity and functional defects in intestinal permeability, as well as microbial alterations. Moreover, MIA offspring displayed stereotypic ASD-like behaviors, and differences in serum metabolomic profile. Interestingly, administration of the probiotic *B. fragilis* corrected the intestinal permeability in MIA offspring. Despite treatment with this probiotic the microbial composition was not restored completely, but recovered the relative abundance of specific species. Remarkably, *B. fragilis* treatment dramatically ameliorated some behavioral defects, including communicative deficits, stereotypic, anxiety-like and sensorimotor behaviors [44].

B. fragilis has been shown to modulate the development and function of the host immune system by producing purified capsular polysaccharide A (PSA) in the intestine [106,107]. However, the authors likely have not checked whether PSA was associated in the mediation of the probiotic effect on ASD-behavioral alterations [44]. Instead, several kinds of serum metabolites, involved in ASD-like behaviors, altered in MIA offspring were corrected to normal levels. Intriguingly, a specific serum metabolite, 4-ethyphenyl sulfate (4EPS) which was elevated in mice with behavioral defects, was sufficient to induce ASD-like behaviors [44]. As 4EPS was considered to be a microbial product, the result further demonstrated that gut microbiome played vital roles in the pathogenesis of ASD.

Increasing epidemiological evidences suggested that maternal obesity and diabetes have been shown to be associated with ASD in the offspring [108,109]. In order to investigate the neurobiological mechanisms by which maternal obesity affects offspring behaviors and brain functions, a recent study performed by Buffington et al. [96] used a maternal high-fat diet (MHFD) in mice to induce impaired social behavior in the offspring. In this study, MHFD caused dysbiosis of gut microbiome and social behavioral deficits in the offspring. In addition, MHFD-induced alterations in gut microbiome of the offspring blocked neural adaption in the mesolimbic dopamine reward system [96]. Notably, co-housing control mice with MHFD offspring restored both social dysfunction and microbial profile of MHFD offspring, indicating a key role for gut microbiome in mediating mouse social behaviors [96]. Furthermore, administration of gut bacterium *Lactobacillus. reuteri* alone, but not other species, could restore oxytocin levels, mesolimbic dopamine reward system, and social behaviors in MHFD offspring, demonstrating specificity of social behavioral regulation to a particular bacterial species [96]. However, repetitive behaviors were not reversed by the gut microbiome manipulation, and L. *reuteri* treatment had no effect on anxiety, suggesting the limitation of the specific-gut microbiota effects on behavioral modulation [96].

L. reuteri is a gram-positive bacterium that naturally inhabits GI of mammals. The probiotics L. *reuteri* benefits its host in a variety of ways, particularly by fighting against harmful infections and mediating the host's immune system [110,111]. One of the most well-documented effects of L. *reuteri* is in the treatment of diarrheal diseases in children, where it has been shown to markedly reduce the duration of symptoms [112]. *L. reuteri* has been indicated to increase oxytocin levels, a key hormone that plays a role in regulation of social behaviors [113]. Interestingly, direct treatment of oxytocin in MHFD offspring also reversed the behavioral and electrophysiological deficits, which was similar with that of L. *reuteri* treatment [96].

5.2. Fecal microbiota transplant

Comparing with probiotics which contain several bacterial species, FMT contains about a thousand bacterial species native to the GI and is well studied for treating recurrent *Clostridium difficile* infection and other GI disorders [114,115]. Since ASD patients often have unusual bacterial profiles and GI problems, it is not difficult to consider FMT has a promising potential for treatment of such disorder through rebalancing gut microbiota. To confirm this hypothesis, a most recent open-label study by Kang et al. [105] investigated the safety, tolerability, and efficacy of FMT for GI and behavior symptoms in ASD children. In this study, 8-week FMT treatment significantly improved both GI- and ASD-related symptoms in 16 out of 18 ASD children, even if the improvements were sustained at least 8 weeks after the treatment [105]. The engraftment of both microbiota and phage in the recipients from the donors, toward that of neurotypical children, was considered responsible for these clinical improvements [105]. This study is the first clinical trial to apply FMT treatment in ASD disorder in humans and sheds light on the potential of targeting gut microbiome for ASD treatment [105]. However, the adverse effects were observed in FMT treatment in this study and previous study [105,116], therefore, a large number size will be essential to clarify long-term safety and tolerability of FMT treatment in ASD patients. Furthermore, the composition of standardized human gut microbiota used in the study was largely dependent on the donor conditions. It is vital to determinate which bacterial specie(s) is/are responsible for the improvement of both GI- and ASD-related symptoms in the future study.

6. Conclusion

Significant advances have been achieved recently in recognizing the importance of gut microbiome in the regulation of brain function. Key findings introduced in this review showed that alterations of the gut microbiome might promote the symptoms of ASD through microbial metabolites, immune regulation and direct interaction with the brain. Accumulating information of animal and human research have demonstrated the beneficial effects of probiotics treatment in the improvement of comorbid symptoms seen in ASD, providing an insight that targeting gut microbiome may be useful as a potential treatment for patients suffering such disorder.

However, the detailed roles of gut microbiome in the pathology of ASD are still unclear, and thus studies investigating mechanisms, such as the contributions of immune, neural, and endocrine pathways in microbiome-brain communications, are significant. Moreover, the disputed microbial alterations seen in ASD patients need to be examined deeply. Additionally, caution should be taken in using the probiotics applied in animal models into human clinics. Finally, the effects of a large number of symbiotic species and their metabolites in the body of hosts remain unknown, and they may act as carriers for therapeutic

substances. It is therefore crucial to elucidate their functions in the future.

Author contribution

Y Yang conceived the original idea. Y Yang wrote the manuscript with the help of J Tian. B Yang collected and organized some part of data and made the figure. All authors read and approved the final manuscript.

Acknowledgements

We are grateful to Professor Lawrence M. Liao for the English language review and Junjun Zheng for helping to make the illustration (both of them in Hiroshima University). This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] H.E. Vuong, E.Y. Hsiao, Review emerging roles for the gut microbiome in autism spectrum disorder, Biol. Psychiatry (2016) 1–13, http://dx.doi.org/10.1016/j.biopsych.2016.08.024.
[2] G.N. Soke, B.A. Steven Rosenberg, B.F. Richard Hamman, B. Tasha Fingerlin, C. Robinson, B. Laura Carpenter, et al., Lisa Wiggins, Brief report: prevalence of self-injurious behaviors among children with autism spectrum disorder—a population-based study, J. Autism Dev. Disord. 46 (2016) 3607–3614, http://dx.doi.org/10.1007/s10803-016-2879-1.
[3] H. Won, W. Mah, E. Kim, Autism spectrum disorder causes, mechanisms, and treatments: focus on neuronal synapses, Front. Mol. Neurosci. 6 (2013) 19, , http://dx.doi.org/10.3389/fnmol.2013.00019.
[4] T.L. Arndt, C.J. Stodgell, P.M. Rodier, The teratology of autism, Int. J. Dev. Neurosci. 23 (2005) 189–199, http://dx.doi.org/10.1016/j.ijdevneu.2004.11.001.
[5] E.A. Mayer, D. Padua, K. Tillisch, Altered brain-gut axis in autism: comorbidity or causative mechanisms? BioEssays 36 (2014) 933–939, http://dx.doi.org/10.1002/bies.201400075.
[6] Y. Wang, L.H. Kasper, The role of microbiome in central nervous system disorders, Brain Behav. Immun. 38 (2014) 1–12, http://dx.doi.org/10.1016/j.bbi.2013.12.015.
[7] I.S. Kohane, A. McMurry, G. Weber, D. MacFadden, L. Rappaport, L. Kunkel, J. Bickel, et al., The co-morbidity burden of children and young adults with autism spectrum disorders, PLoS One 7 (2012) e33224, , http://dx.doi.org/10.1371/journal.pone.0033224.
[8] R. Blumberg, F. Powrie, Microbiota, disease, and back to health: a metastable journey, Sci. Transl. Med. 4 (2012) 137rv7, , http://dx.doi.org/10.1126/scitranslmed.3004184.
[9] A.K. DeGruttola, D. Low, A. Mizoguchi, E. Mizoguchi, Current understanding of dysbiosis in disease in human and animal models, Inflamm. Bowel Dis. 22 (2016) 1137–1150, http://dx.doi.org/10.1097/mib.0000000000000750.
[10] J.B. Adams, L.J. Johansen, L.D. Powell, D. Quig, R. a Rubin, Gastrointestinal flora and gastrointestinal status in children with autism-comparisons to typical children and correlation with autism severity, BMC Gastroenterol. 11 (2011) 22, , http://dx.doi.org/10.1186/1471-230X-11-22.
[11] S.M. Finegold, J. Downes, P.H. Summanen, Microbiology of regressive autism, Anaerobe 18 (2012) 260–262, http://dx.doi.org/10.1016/j.anaerobe.2011.12.018.
[12] D.W. Kang, J.G. Park, Z.E. Ilhan, G. Wallstrom, J. LaBaer, J.B. Adams, et al., Reduced incidence of prevotella and other fermenters in intestinal microflora of autistic children, PLoS One 8 (2013), http://dx.doi.org/10.1371/journal.pone.0068322.
[13] A. Tomova, V. Husarova, S. Lakatosova, J. Bakos, B. Vlkova, K. Babinska, et al., Gastrointestinal microbiota in children with autism in Slovakia, Physiol. Behav. 138 (2015) 179–187, http://dx.doi.org/10.1016/j.physbeh.2014.10.033.
[14] A.S. Neish, Microbes in gastrointestinal health and disease, Gastroenterology 136 (2009) 65–80, http://dx.doi.org/10.1053/j.gastro.2008.10.080.
[15] Y. Belkaid, S. Naik, Compartmentalized and systemic control of tissue immunity by commensals, Nat. Immunol. 14 (2013) 646–653, http://dx.doi.org/10.1038/ni.2604.
[16] G.P. Donaldson, S.M. Lee, S.K. Mazmanian, Gut biogeography of the bacterial microbiota, Nat. Rev. Microbiol. 14 (2015) 20–32, http://dx.doi.org/10.1038/nrmicro3552.
[17] J.L. Round, S.K. Mazmanian, The gut microbiota shapes intestinal immune responses during health and disease, Nat. Rev. Immunol. 9 (2009) 313–324, http://dx.doi.org/10.1038/nri2515.
[18] P.G. Falk, L.V. Hooper, T. Midtvedt, J.I. Gordon, Creating and maintaining the gastrointestinal ecosystem: what we know and need to know from gnotobiology, Microbiol. Mol. Biol. Rev. 62 (1998) 1157–1170 http://www.ncbi.nlm.nih.gov/pubmed/9841668.
[19] A.J. Macpherson, N.L. Harris, Interactions between commensal intestinal bacteria and the immune system, Nat. Rev. Immunol. 4 (2004) 478–485, http://dx.doi.org/10.1038/nri1373.
[20] C.A. Lopez, D.D. Kingsbury, E.M. Velazquez, A.J. Bäumler, Collateral damage: microbiota-derived metabolites and immune function in the antibiotic era, Cell Host Microbe 16 (2014) 156–163, http://dx.doi.org/10.1016/j.chom.2014.07.009.
[21] P. Hemarajata, J. Versalovic, Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation, Ther. Adv. Gastroenterol. 6 (2013) 39–51, http://dx.doi.org/10.1177/1756283X12459294.
[22] S.M. Collins, P. Bercik, The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease, Gastroenterology 136 (2009) 2003–2014, http://dx.doi.org/10.1053/j.gastro.2009.01.075.
[23] M. Carabotti, A. Scirocco, M.A. Maselli, C. Severi, The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems, Ann. Gastroenterol. 28 (2015) 203–209, http://dx.doi.org/10.1038/ajgsup.2012.3.
[24] G. Barbara, V. Stanghellini, G. Brandi, C. Cremon, G. Di Nardo, R. De Giorgio, et al., Interactions between commensal bacteria and gut sensorimotor function in health and disease, Am. J. Gastroenterol. 100 (2005) 2560–2568, http://dx.doi.org/10.1111/j.1572-0241.2005.00230.x.
[25] R.M. Stilling, T.G. Dinan, J.F. Cryan, Microbial genes, brain & behaviour-epigenetic regulation of the gut-brain axis, Genes Brain Behav. 13 (2014) 69–86, http://dx.doi.org/10.1111/gbb.12109.
[26] S.S. Yarandi, D.A. Peterson, G.J. Treisman, T.H. Moran, P.J. Pasricha, Modulatory effects of gut microbiota on the central nervous system: how gut could play a role in neuropsychiatric health and diseases, J. Neurogastroenterol. Motil. 22 (2016) 201–212, http://dx.doi.org/10.5056/jnm15146.
[27] M.T. Bailey, S.E. Dowd, J.D. Galley, A.R. Hufnagle, R.G. Allen, M. Lyte, Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation, Brain Behav. Immun. 25 (2011) 397–407, http://dx.doi.org/10.1016/j.bbi.2010.10.023.
[28] C.A. Rubio, C.B. Huang, Quantification of the sulphomucin-producing cell population of the colonic mucosa during protracted stress in rats, In Vivo (Brooklyn), Vol. 6 1992, pp. 81–84.
[29] S. Macfarlane, J.F. Dillon, Microbial biofilms in the human gastrointestinal tract, J. Appl. Microbiol. 102 (2007) 1187–1196, http://dx.doi.org/10.1111/j.1365-2672.2007.03287.x.
[30] A.V. Golubeva, S. Crampton, L. Desbonnet, D. Edge, O. O'Sullivan, K.W. Lomasney, et al., Prenatal stress-induced alterations in major physiological systems correlate with gut microbiota composition in adulthood, Psychoneuroendocrinology 60 (2015) 58–74, http://dx.doi.org/10.1016/j.psyneuen.2015.06.002.
[31] A.J. Park, J. Collins, P.A. Blennerhassett, J.E. Ghia, E.F. Verdu, P. Bercik, et al., Altered colonic function and microbiota profile in a mouse model of chronic depression, Neurogastroenterol. Motil. 25 (2013), http://dx.doi.org/10.1111/nmo.12153.
[32] O. Zerbo, Y. Qian, C. Yoshida, J.K. Grether, J. Van de Water, L.A. Croen, Maternal infection during pregnancy and autism spectrum disorders, J. Autism Dev. Disord. 45 (2015) 4015–4025, http://dx.doi.org/10.1007/s10803-013-2016-3.
[33] O. Zerbo, A.M. Iosif, C. Walker, S. Ozonoff, R.L. Hansen, I. Hertz-Picciotto, Is maternal influenza or fever during pregnancy associated with autism or developmental delays? Results from the CHARGE (childhood autism risks from genetics and environment) study, J. Autism Dev. Disord. 43 (2013) 25–33, http://dx.doi.org/10.1007/s10803-012-1540-x.
[34] N.V. Malkova, C.Z. Yu, E.Y. Hsiao, M.J. Moore, P.H. Patterson, Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism, Brain Behav. Immun. 26 (2012) 607–616, http://dx.doi.org/10.1016/j.bbi.2012.01.011.
[35] B.K. Lee, C. Magnusson, R.M. Gardner, Å. Blomström, C.J. Newschaffer, I. Burstyn, et al., Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders, Brain Behav. Immun. 44 (2015) 100–105, http://dx.doi.org/10.1016/j.bbi.2014.09.001.
[36] M.C. Collado, S. Rautava, J. Aakko, E. Isolauri, S. Salminen, Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid, Sci. Rep. 6 (2016) 23129, , http://dx.doi.org/10.1038/srep23129.
[37] R. Hansen, K.P. Scott, S. Khan, J.C. Martin, S.H. Berry, M. Stevenson, et al., First-pass meconium samples from healthy term vaginally-delivered neonates: an analysis of the microbiota, PLoS One 10 (2015) e0133320, , http://dx.doi.org/10.1371/journal.pone.0133320.
[38] M.G. Dominguez-Bello, E.K. Costello, M. Contreras, M. Magris, G. Hidalgo, N. Fierer, et al., Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 11971–11975, http://dx.doi.org/10.1073/pnas.1002601107.
[39] G. Biasucci, M. Rubini, S. Riboni, L. Morelli, E. Bessi, C. Retetangos, Mode of delivery affects the bacterial community in the newborn gut, Early Hum, Dev. Suppl. 186 (2010) 13–15, http://dx.doi.org/10.1016/j.earlhumdev.2010.01.004.
[40] E.L. Sullivan, E.K. Nousen, K.A. Chamlou, K.L. Grove, The impact of maternal high-fat diet consumption on neural development and behavior of offspring, Int. J. Obes. Suppl. 2 (2012) S7–S13, http://dx.doi.org/10.1038/ijosup.2012.15.
[41] A.G. Edlow, Maternal obesity and neurodevelopmental and psychiatric disorders in offspring, Prenat. Diagn. 37 (2016) 95–110, http://dx.doi.org/10.1002/pd.

4932.
[42] P. Krakowiak, C.K. Walker, A.A. Bremer, A.S. Baker, S. Ozonoff, R.L. Hansen, et al., Maternal metabolic conditions and risk for autism and other neurodevelopmental disorders, Pediatrics 129 (2012) e1121–1128, http://dx.doi.org/10.1542/peds.2011-2583.
[43] E. Jašarević, C.L. Howerton, C.D. Howard, T.L. Bale, Alterations in the vaginal microbiome by maternal stress are associated with metabolic reprogramming of the offspring gut and brain, Endocrinology 156 (2015) 3265–3276, http://dx.doi.org/10.1210/en.2015-1177.
[44] E.Y. Hsiao, S.W. McBride, S. Hsien, G. Sharon, E.R. Hyde, T. McCue, et al., Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders, Cell 155 (2013) 1451–1463, http://dx.doi.org/10.1016/j.cell.2013.11.024.
[45] M.L. Estes, A.K. McAllister, Maternal immune activation: implications for neuropsychiatric disorders, Science 353 (2016) 772–777, http://dx.doi.org/10.1126/science.aag3194.
[46] M. Mandal, R. Donnelly, S. Elkabes, P. Zhang, D. Davini, B.T. David, et al., Maternal immune stimulation during pregnancy shapes the immunological phenotype of offspring, Brain Behav. Immun. 33 (2013) 33–45, http://dx.doi.org/10.1016/j.bbi.2013.04.012.
[47] P.H. Patterson, Maternal infection and immune involvement in autism, Trends Mol. Med. 17 (2011) 389–394, http://dx.doi.org/10.1016/j.molmed.2011.03.001.
[48] S. Nardone, E. Elliott, The interaction between the immune system and epigenetics in the etiology of autism spectrum disorders, Front. Neurosci. 10 (2016) 1–9, http://dx.doi.org/10.3389/fnins.2016.00329.
[49] E.Y. Hsiao, P.H. Patterson, Activation of the maternal immune system induces endocrine changes in the placenta via IL-6, Brain Behav. Immun. 25 (2011) 604–615, http://dx.doi.org/10.1016/j.bbi.2010.12.017.
[50] S.M. Finegold, D. Molitoris, Y. Song, C. Liu, M. Vaisanen, E. Bolte, et al., Gastrointestinal microflora studies in late-onset autism, Clin. Infect. Dis. 35 (2002) S6–S16, http://dx.doi.org/10.1086/341914.
[51] S.M. Finegold, S.E. Dowd, V. Gontcharova, C. Liu, K.E. Henley, R.D. Wolcott, et al., Pyrosequencing study of fecal microflora of autistic and control children, Anaerobe 16 (2010) 444–453, http://dx.doi.org/10.1016/j.anaerobe.2010.06.008.
[52] B.L. Williams, M. Hornig, T. Parekh, W. Ian Lipkin, 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 3 (2012) 1–11, http://dx.doi.org/10.1128/mBio.00261-11.
[53] L. Wang, C.T. Christophersen, M.J. Sorich, J.P. Gerber, M.T. Angley, M.A. Conlon, Increased abundance of *Sutterella* spp. and *Ruminococcus torques* in feces of children with autism spectrum disorder, Mol. Autism 4 (2013) 42, http://dx.doi.org/10.1186/2040-2392-4-42.
[54] S.M. Finegold, Therapy and epidemiology of autism-clostridial spores as key elements, Med. Hypotheses 70 (2008) 508–511, http://dx.doi.org/10.1016/j.mehy.2007.07.019.
[55] H.T. Ding, Y. Taur, J.T. Walkup, Gut microbiota and autism: key concepts and findings, J. Autism Dev. Disord. 0 (2016) 1–10, http://dx.doi.org/10.1007/s10803-016-2960-9.
[56] Y. Song, C. Liu, S.M. Finegold, Real-time PCR quantitation of clostridia in feces of autistic children, Appl. Environ. Microbiol. 70 (2004) 6459–6465, http://dx.doi.org/10.1128/AEM.70.11.6459-6465.2004.
[57] H.M.R.T. Parracho, M.O. Bingham, G.R. Gibson, A.L. McCartney, Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children, J. Med. Microbiol. 54 (2005) 987–991, http://dx.doi.org/10.1099/jmm.0.46101-0.
[58] E.R. Bolte, Autism and clostridium tetani, Med. Hypotheses 51 (1998) 133–144, http://dx.doi.org/10.1016/S0306-9877(98)90107-4.
[59] P.A. Efron, J.E. Mazuski, Clostridium difficile colitis, Surg. Clin. North Am. 89 (2009) 483–500, http://dx.doi.org/10.1016/j.suc.2008.09.014.
[60] J. McLauchlin, K.A. Grant, *Clostridium botulinum* and *Clostridium perfringens*, (2007), pp. 41–78, http://dx.doi.org/10.1007/978-1-59745-501-5_3.
[61] R.H. Sandler, S.M. Finegold, E.R. Bolte, C.P. Buchanan, A.P. Maxwell, M.-L. Vaisanen, et al., Short-term benefit from oral vancomycin treatment of regressive-onset autism, J. Child Neurol. 15 (2000) 429–435, http://dx.doi.org/10.1177/088307380001500701.
[62] K. Lu, R. Mahbub, J.G. Fox, Xenobiotics: interaction with the intestinal microflora, ILAR J. 56 (2015) 218–227, http://dx.doi.org/10.1093/ilar/ilv018.
[63] L. Altieri, C. Neri, R. Sacco, P. Curatolo, A. Benvenuto, F. Muratori, et al., Urinary p-cresol is elevated in small children with severe autism spectrum disorder, Biomarkers 16 (2011) 252–260, http://dx.doi.org/10.3109/1354750X.2010.548010.
[64] L. Wang, C.T. Christophersen, M.J. Sorich, J.P. Gerber, M.T. Angley, M.A. Conlon, Elevated fecal short chain fatty acid and ammonia concentrations in children with autism spectrum disorder, Dig. Dis. Sci. 57 (2012) 2096–2102, http://dx.doi.org/10.1007/s10620-012-2167-7.
[65] D.F. Macfabe, Short-chain fatty acid fermentation products of the gut microbiome: implications in autism spectrum disorders, Microb. Ecol. Health Dis. 23 (2012) 1–24, http://dx.doi.org/10.3402/mehd.v23i0.19260.
[66] D.F. MacFabe, Enteric short-chain fatty acids: microbial messengers of metabolism, mitochondria, and mind: implications in autism spectrum disorders, Microb. Ecol. Health Dis. 26 (2015) 28177, , http://dx.doi.org/10.3402/mehd.v26.28177.
[67] D.F. MacFabe, N.E. Cain, F. Boon, K.P. Ossenkopp, D.P. Cain, Effects of the enteric bacterial metabolic product propionic acid on object-directed behavior, social behavior, cognition, and neuroinflammation in adolescent rats: relevance to autism spectrum disorder, Behav. Brain Res. 217 (2011) 47–54, http://dx.doi.org/10.1016/j.bbr.2010.10.005.
[68] S.R. Shultz, D.F. MacFabe, K.P. Ossenkopp, S. Scratch, J. Whelan, R. Taylor, et al., Intracerebroventricular injection of propionic acid, an enteric bacterial metabolic end-product, impairs social behavior in the rat: implications for an animal model of autism, Neuropharmacology 54 (2008) 901–911, http://dx.doi.org/10.1016/j.neuropharm.2008.01.013.
[69] S.R. Shultz, D.F. MacFabe, S. Martin, J. Jackson, R. Taylor, F. Boon, et al., Intracerebroventricular injections of the enteric bacterial metabolic product propionic acid impair cognition and sensorimotor ability in the Long-Evans rat: further development of a rodent model of autism, Behav. Brain Res. 200 (2009) 33–41, http://dx.doi.org/10.1016/j.bbr.2008.12.023.
[70] S. Gabriele, R. Sacco, S. Cerullo, C. Neri, A. Urbani, G. Tripi, et al., Urinary p-cresol is elevated in young French children with autism spectrum disorder: a replication study, Biomarkers 19 (2014) 463–470, http://dx.doi.org/10.3109/1354750X.2014.936911.
[71] A.M. Persico, V. Napolioni, Urinary p-cresol in autism spectrum disorder, Neurotoxicol. Teratol. 36 (2013) 82–90, http://dx.doi.org/10.1016/j.ntt.2012.09.002.
[72] I.K.S. Yap, M. Angley, K.A. Veselkov, E. Holmes, J.C. Lindon, J.K. Nicholson, Urinary metabolic phenotyping differentiates children with autism from their unaffected siblings and age-matched controls, J. Proteome Res. 9 (2010) 2996–3004, http://dx.doi.org/10.1021/pr901188e.
[73] F. Gevi, L. Zolla, S. Gabriele, A.M. Persico, Urinary metabolomics of young Italian autistic children supports abnormal tryptophan and purine metabolism, Mol. Autism 7 (2016) 47, , http://dx.doi.org/10.1186/s13229-016-0109-5.
[74] H. Wang, S. Liang, M. Wang, J. Gao, C. Sun, J. Wang, et al., Potential serum biomarkers from a metabolomics study of autism, J. Psychiatry Neurosci. 41 (2016) 27–37, http://dx.doi.org/10.1503/jpn.140009.
[75] C. Onore, M. Careaga, P. Ashwood, The role of immune dysfunction in the pathophysiology of autism, Brain Behav. Immun. 26 (2012) 383–392, http://dx.doi.org/10.1016/j.bbi.2011.08.007.
[76] X. Li, A. Chauhan, A.M. Sheikh, S. Patil, V. Chauhan, X.M. Li, et al., Elevated immune response in the brain of autistic patients, J. Neuroimmunol. 207 (2009) 111–116, http://dx.doi.org/10.1016/j.jneuroim.2008.12.002.
[77] J.T. Morgan, G. Chana, C.A. Pardo, C. Achim, K. Semendeferi, J. Buckwalter, et al., Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism, Biol. Psychiatry 68 (2010) 368–376, http://dx.doi.org/10.1016/j.biopsych.2010.05.024.
[78] P. Ashwood, P. Krakowiak, I. Hertz-Picciotto, R. Hansen, I.N. Pessah, J. Van de Water, Associations of impaired behaviors with elevated plasma chemokines in autism spectrum disorders, J. Neuroimmunol. 232 (2011) 196–199, http://dx.doi.org/10.1016/j.jneuroim.2010.10.025.
[79] R.P. Warren, A. Foster, N.C. Margaretten, Reduced natural killer cell activity in autism, J. Am. Acad. Child Adolesc. Psychiatry 26 (1987) 333–335, http://dx.doi.org/10.1097/00004583-198705000-00008.
[80] A.M. Enstrom, C.E. Onore, J.A. Van de Water, P. Ashwood, Differential monocyte responses to TLR ligands in children with autism spectrum disorders, Brain Behav. Immun. 24 (2010) 64–71, http://dx.doi.org/10.1016/j.bbi.2009.08.001.
[81] N.H. Salzman, Microbiota-immune system interaction: an uneasy alliance, Curr. Opin. Microbiol. 14 (2011) 99–105, http://dx.doi.org/10.1016/j.mib.2010.09.018.
[82] H.J. Wu, E. Wu, The role of gut microbiota in immune homeostasis and autoimmunity, Gut Microbes 3 (2012) 4–14, http://dx.doi.org/10.4161/gmic.19320.
[83] H.J. Wu, I.I. Ivanov, J. Darce, K. Hattori, T. Shima, Y. Umesaki, et al., Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells, Immunity 32 (2010) 815–827, http://dx.doi.org/10.1016/j.immuni.2010.06.001.
[84] J.L. Round, S.M. Lee, J. Li, G. Tran, B. Jabri, T.A. Chatila, et al., The Toll-Like receptor 2 pathway establishes colonization by a commensal of the human microbiota, Science 332 (2011) 974–977, http://dx.doi.org/10.1126/science.1206095.
[85] J. Ochoa-Repáraz, D.W. Mielcarz, L.E. Ditrio, A.R. Burroughs, S. Begum-Haque, S. Dasgupta, et al., Central nervous system demyelinating disease protection by the human commensal *Bacteroides fragilis* depends on polysaccharide A expression, J. Immunol. 185 (2010) 4101–4108, http://dx.doi.org/10.4049/jimmunol.1001443.
[86] C.L. Ohland, L. Kish, H. Bell, A. Thiesen, N. Hotte, E. Pankiv, et al., Effects of *Lactobacillus helveticus* on murine behavior are dependent on diet and genotype and correlate with alterations in the gut microbiome, Psychoneuroendocrinology 38 (2013) 1738–1747, http://dx.doi.org/10.1016/j.psyneuen.2013.02.008.
[87] K. Gilbert, J. Arseneault-Breard, F. Flores Monaco, A. Beaudoin, T.M. Bah, T.A. Tompkins, et al., Attenuation of post-myocardial infarction depression in rats by n-3 fatty acids or probiotics starting after the onset of reperfusion, Br. J. Nutr. 109 (2013) 50–56, http://dx.doi.org/10.1017/S0007114512003807.
[88] M. Pineiro, N.-G. Asp, G. Reid, S. Macfarlane, L. Morelli, O. Brunser, et al., FAO technical meeting on prebiotics, J. Clin. Gastroenterol. 42 (Suppl. 3) (2008) S156–159, http://dx.doi.org/10.1097/MCG.0b013e31817f184e.
[89] H.M. Savignac, G. Corona, H. Mills, L. Chen, J.P.E. Spencer, G. Tzortzis, et al., Prebiotic feeding elevates central brain derived neurotrophic factor, N-methyl-d-aspartate receptor subunits and d-serine, Neurochem. Int. 63 (2013) 756–764, http://dx.doi.org/10.1016/j.neuint.2013.10.006.
[90] K. Schmidt, P.J. Cowen, C.J. Harmer, G. Tzortzis, S. Errington, P.W.J. Burnet, Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers, Psychopharmacology 232 (2015) 1793–1801, http://dx.doi.org/10.1007/s00213-014-3810-0.
[91] T.G. Dinan, J.F. Cryan, G.D. Abrams, H. Bauer, H. Sprinz, I. Adlerberth, et al.,

Regulation of the stress response by the gut microbiota: implications for psychoneuroendocrinology, Psychoneuroendocrinology 37 (2012) 1369–1378, http://dx.doi.org/10.1016/j.psyneuen.2012.03.007.
[92] N. Sudo, Y. Chida, Y. Aiba, J. Sonoda, N. Oyama, X.-N.X. Yu, et al., Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice, J. Physiol. 558 (2004) 263–275, http://dx.doi.org/10.1113/jphysiol.2004.063388.
[93] P. Bercik, E.F. Verdu, J.A. Foster, J. MacRi, M. Potter, X. Huang, et al., Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice, Gastroenterology 139 (2010) 2102–2112, http://dx.doi.org/10.1053/j.gastro.2010.06.063.
[94] J.A. Bravo, P. Forsythe, M.V. Chew, E. Escaravage, H.M. Savignac, T.G. Dinan, et al., Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 16050–16055, http://dx.doi.org/10.1073/pnas.1102999108.
[95] P. Bercik, A.J. Park, D. Sinclair, A. Khoshdel, J. Lu, X. Huang, et al., The anxiolytic effect of Bifidobacterium longum NCC3001 involves vagal pathways for gut-brain communication, Neurogastroenterol. Motil. 23 (2011) 1132–1139, http://dx.doi.org/10.1111/j.1365-2982.2011.01796.x.
[96] S.A. Buffington, G.V. Di Prisco, T.A. Auchtung, N.J. Ajami, J.F. Petrosino, M. Costa-Mattioli, Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring, Cell 165 (2016) 1762–1775, http://dx.doi.org/10.1016/j.cell.2016.06.001.
[97] H.M. Savignac, B. Kiely, T.G. Dinan, J.F. Cryan, Bifidobacteria exert strain-specific effects on stress-related behavior and physiology in BALB/c mice, Neurogastroenterol. Motil. 26 (2014) 1615–1627, http://dx.doi.org/10.1111/nmo.12427.
[98] H.M. Savignac, M. Tramullas, B. Kiely, T.G. Dinan, J.F. Cryan, Bifidobacteria modulate cognitive processes in an anxious mouse strain, Behav. Brain Res. 287 (2015) 59–72, http://dx.doi.org/10.1016/j.bbr.2015.02.044.
[99] E.F. Verdú, P. Bercik, M. Verma-Gandhu, X.-X. Huang, P. Blennerhassett, W. Jackson, et al., Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice, Gut 55 (2006) 182–190, http://dx.doi.org/10.1136/gut.2005.066100.
[100] D.P. McKernan, P. Fitzgerald, T.G. Dinan, J.F. Cryan, The probiotic Bifidobacterium infantis 35624 displays visceral antinociceptive effects in the rat, Neurogastroenterol. Motil. 22 (2010) 1029–1035, http://dx.doi.org/10.1111/j.1365-2982.2010.01520.x.
[101] L. Desbonnet, L. Garrett, G. Clarke, B. Kiely, J.F. Cryan, T.G. Dinan, Effects of the probiotic *Bifidobacterium infantis* in the maternal separation model of depression, Neuroscience 170 (2010) 1179–1188, http://dx.doi.org/10.1016/j.neuroscience.2010.08.005.
[102] M. Messaoudi, R. Lalonde, N. Violle, H. Javelot, D. Desor, A. Nejdi, et al., Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects, Br. J. Nutr. 105 (2011) 755–764, http://dx.doi.org/10.1017/S0007114510004319.
[103] L. Steenbergen, R. Sellaro, S. van Hemert, J.A. Bosch, L.S. Colzato, A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood, Brain Behav. Immun. 48 (2015) 258–264, http://dx.doi.org/10.1016/j.bbi.2015.04.003.
[104] K. Tillisch, J. Labus, L. Kilpatrick, Z. Jiang, J. Stains, B. Ebrat, et al., Consumption of fermented milk product with probiotic modulates brain activity, Gastroenterology 144 (2013) 1394–1401, http://dx.doi.org/10.1053/j.gastro.2013.02.043.
[105] D.-W. Kang, J.B. Adams, A.C. Gregory, T. Borody, L. Chittick, A. Fasano, et al., Microbiota transfer therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study, Microbiome 5 (2017) 10, , http://dx.doi.org/10.1186/s40168-016-0225-7.
[106] E.B. Troy, D.L. Kasper, Beneficial effects of *Bacteroides fragilis* polysaccharides on the immune system, Front. Biosci. 15 (2010) 25–34, http://dx.doi.org/10.2741/3603 (Landmark Ed.).
[107] S.K. Mazmanian, J.L. Round, D.L. Kasper, A microbial symbiosis factor prevents intestinal inflammatory disease, Nature 453 (2008) 620–625, http://dx.doi.org/10.1038/nature07008.
[108] M. Li, M.D. Fallin, A. Riley, R. Landa, S.O. Walker, M. Silverstein, et al., The association of maternal obesity and diabetes with autism and other developmental disabilities, Pediatrics 137 (2016) e20152206, , http://dx.doi.org/10.1542/peds.2015-2206.
[109] K.D.N. Sacks, M. Friger, E. Spiegel, H. Abokaf, R. Sergienko, D. Landau, et al., Prenatal exposure to gestational diabetes mellitus as an independent risk factor for long-term neurologic morbidity of the offspring, Am. J. Obstet. Gynecol. 214 (2016) S48–S49, http://dx.doi.org/10.1016/j.ajog.2016.03.030.
[110] R. Francavilla, E. Lionetti, S.P. Castellaneta, A.M. Magistà, G. Maurogiovanni, N. Bucci, et al., Inhibition of *Helicobacter pylori* infection in humans by *Lactobacillus reuteri* ATCC 55730 and effect on eradication therapy: a pilot study, Helicobacter 13 (2008) 127–134, http://dx.doi.org/10.1111/j.1523-5378.2008.00593.x.
[111] C.M. Thomas, T. Hong, J.P. van Pijkeren, P. Hemarajata, D.V. Trinh, W. Hu, et al., Histamine derived from probiotic *Lactobacillus reuteri* suppresses TNF via modulation of PKA and ERK signaling, PLoS One 7 (2012) e31951, , http://dx.doi.org/10.1371/journal.pone.0031951.
[112] M. Urbańska, D. Gieruszczak-Białek, H. Szajewska, Systematic review with meta-analysis: *Lactobacillus reuteri* DSM 17938 for diarrhoeal diseases in children, Aliment. Pharmacol. Ther. 43 (2016) 1025–1034, http://dx.doi.org/10.1111/apt.13590.
[113] Z.R. Donaldson, L.J. Young, Oxytocin, vasopressin and the neurogenics of sociality, Science 322 (2008) 900–904, http://dx.doi.org/10.1126/science.1158668.
[114] H. Sbahi, J.A. Di Palma, Faecal microbiota transplantation: applications and limitations in treating gastrointestinal disorders, BMJ Open Gastroenterol. 3 (2016) e000087, , http://dx.doi.org/10.1136/bmjgast-2016-000087.
[115] N. Bagdasarian, K. Rao, P. Malani, Diagnosis and treatment of *Clostridium difficile* in adults, JAMA 313 (2015) 398, http://dx.doi.org/10.1001/jama.2014.17103.
[116] J. Aas, C.E. Gessert, J.S. Bakken, Recurrent *Clostridium difficile* colitis: case series involving 18 patients treated with donor stool administered via a nasogastric tube, Clin. Infect. Dis. 36 (2003) 580–585, http://dx.doi.org/10.1086/367657.