DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

DMD # 63826

Microbiome Disturbances and Autism Spectrum Disorders

Cheryl S. Rosenfeld

Departments of: Bond Life Sciences Center (CSR), Thompson Center for Autism and Neurobehavioral Disorders (CSR), Genetics Area Program (CSR), Biomedical Sciences (CSR), University of Missouri, Columbia, MO 65211 USA

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

Short Title: Microbiomes and ASD

Correspondence: Cheryl S. Rosenfeld, DVM, PhD, Associate Professor, University of Missouri, 440F Bond Life Sciences Center, 1201 E. Rollins Rd., Columbia, MO 65211, USA. E-mail: rosenfeldc@missouri.edu, phone: (573) 882-5132, and fax: (573) 884-9395.

Document Statistics

Number of text pages: 32
Tables: 2
Figures: 1
References: 222
Number of words in Abstract: 249
Number of words in Introduction: 980
Number of words in Discussion: 283

Non-standard Abbreviations:

4-EPS	4-Ethylphenylsulfate
ADI-R	Autism Diagnostic Interview-Revised
ADOS	Autism Diagnostic Observation Schedule
ASD	Autism Spectrum Disorders
BA	Butyric Acid
BBB	Blood Brain Barrier
CNS	Central Nervous System
DA	D-arabinitol
DOHaD	Developmental Origins of Health and Disease
EPM	Elevated Plus Maze
FDA	Food and Drug Administration
FMT	Fecal Microbiota Transplantation
GF	Germ Free
GI	Gastrointestinal
HAT	histone acetyl transferases
HDAC	Histone Deacetylases
HE	Hepatic Encephalopathy
HPA	Hypothalamic-Pituitary-Axis
LA	L-arabinitol
LPS	Lipopolysaccharide
MD	Mitochondrial Disease
MEND	Microbiota Epimutations Neuro Development
MIA	Maternal Immune Activation
NAS	Non-caloric Artificial Sweeteners
NE	Norepinephrine
OTUs	Operational Taxonomic Units
PPA	Propionic Acid
RCDI	Recurrent Clostridium difficile infection
SCFAs	Short Chain Fatty Acids
SPF	Specific Pathogen Free
VPA	Valproic Acid

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

Autism spectrum disorders (ASD) are considered a heterogenous set of neurobehavioral diseases with the rates of diagnosis dramatically increasing in the past few decades. As genetics alone does not explain the underlying cause in many cases, attention has turned to other environmental factors as potential etiological agents. Gastrointestinal disorders are a common comorbidity in ASD patients. It was thus hypothesized that a gut-brain link may account for some autistic cases. With the characterization of the human microbiome, this concept has been expanded to include the microbiota-gut-brain axis. There are mounting reports in animal models and human epidemiological studies linking disruptive alterations in the gut microbiota or dysbiosis and ASD symptomology. In this review, we will explore the current evidence that gut dysbiosis in animal models and ASD patients correlates with disease risk and severity. The studies to date have surveyed how gut microbiome changes may affect these neurobehavioral disorders. However, we harbor other microbiomes reside in the body that might impact brain function. We will consider these other microbiomes in the oral cavity, vagina, and the most recently discovered one in the placenta. Based on the premise that gut microbiota alterations may be causative agents in ASD, several therapeutic options have been tested, such as diet modulations, prebiotics, probiotics, synbiotics, postbiotics, antibiotics, fecal transplantation, and activated charcoal. The potential benefits of these therapies will be considered. Lastly, the possible mechanisms by which changes in the gut bacterial communities may result in ASD and related neurobehavioral disorders will be examined.

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

Autism Spectrum Disorders (ASD) are currently estimated to affect about 1 in every 68 children (http://www.cdc.gov/ncbddd/dd/addmprevalence.htm), with the number of diagnosed boys outnumbering girls by 4:1 (Bespalova and Buxbaum, 2003; Fombonne, 2005). The recent surge in frequency may be partly attributed to increased awareness/diagnosis, but intrisic and extrisinc factors (including enviromental chemicals, diet alterations, metabolic status, and microbiota changes) cannot be excluded (Rizzo et al., 1997; Bello, 2007; Newschaffer et al., 2007; Deth et al., 2008; Rogers, 2008; Leeming and Lucock, 2009; Currenti, 2010; Landrigan, 2010; Beard et al., 2011; LaSalle, 2011; Chaste and Leboyer, 2012; Krakowiak et al., 2012; Sullivan et al., 2012; Jones et al., 2013; Lyall et al., 2013; Gore et al., 2014). While extensive heterogeneity exists in ASD patients, this class of disorders is typified by a range of symptoms including decreased verbal communication, social skills to outright withdrawal, insistence on sameness in the daily routine, engagement in repetive behaviors, and heightened response to external stimuli. ASD core symptoms are classified based on the Autism Diagnostic Interview-Revised (ADI-R;(Rutter, 2003)), Autism Diagnostic Observation Schedule (ADOS;(Lord, 2002)), and Social Responsiveness Scale (SRS; (Constantino, 2000)).

Comorbidity with gastrointestinal disturbances is often observed in ASD patients with estimates ranging from 9 to 70% (Buie et al., 2010). This diverse range likely reflects variation in sample size, self-reporting, and speciality area of the reporting clinic. Even so, it is recognized that there is a linkage between the gastrointestinal system and and brain function; thereby leading to the coinnage of the term "*gut-brain axis*". With the mounting evidence that gut microbiota contained may underpin some neurobehavioral disorders, this term has been since broadened to

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

"*microbiome (microbiota)-gut-brain axis*" (Collins and Bercik, 2009; Rhee et al., 2009; Cryan and Dinan, 2012). In fact, 90% of the cells contained in most mammalian organisms are of prokaryotic orgin. The gut microbiota population is comprised of 500 to 1,000 denizen species representing 7,000 to 40,000 bacterial strains spanning 1800 genera (Luckey, 1972; Ley et al., 2006; Frank and Pace, 2008; Qin et al., 2010; Clemente et al., 2012; Douglas-Escobar et al., 2013; Forsythe and Kunze, 2013; Gilbert et al., 2013). The 1 X 10^3 to 1 X 10^4 microorganism gut inhabitants possess a divese and complex genome encompassing approximately 150 times more genes than the human genome (Gill et al., 2006; Qin et al., 2010). The gut microbiota are thus considered a forgotten "organ" in of itself with the 100 trillion prokaryotic cells weighing about 1 to 2 kg (O'Hara and Shanahan, 2006; Forsythe and Kunze, 2013). The Human Microbiome Project was initiated with the overarching goal to understand the comprehensive effects microbiota populations exert on host health and disease, including neurological disorders (2012a; 2012b). To understand this interaction, we need to examine when microbes initially inhabit the gut.

Bacterial colonization of the gut likely occurs at the time of birth with infants born via natural delivery innoculated with a complex mixture of maternal vaginal microorganisms. Thus, concern has risen that babies delivered via C-section may receive insufficient maternal bacterial transmission (Murgas Torrazza and Neu, 2011; Walker, 2013; Mueller et al., 2014). Notwithstanding, the intestinal microbiome of most infants resembles that of their mother, but after 1 year of age, a complex and distinct microbiome profile develops (Mackie et al., 1999; Palmer et al., 2007). The perinatal data suggests that there are critical windows for gut

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

microbiome establishment and later health and disease effects. This temporal requirement may have important ramifications for potential preventative and therapeutic remediation strategies.

Animals are dependent upon intestinal microbes for several functions. Gut microbiota synthesize various essential vitamins and co-factors, such as folate and vitamin B12, that may affect DNA and histone protein methylation (Le Galliard et al., 2008; LeBlanc et al., 2013). Bacteria also metabolize complex lipids, proteins, and carbohydrates, including those that are indigestible by the host (Hooper et al., 2002; Saulnier et al., 2008; Dai et al., 2012). Bacterial fermentative processes result in the production of various short chain fatty acids (SCFAs), such as acetic, propionic, and butyric acid (Hooper et al., 2002). The bacterial-derived SCFAs may serve as a fuel source for enterocytes lining the intestinal system, but these chemicals 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, which as discussed below can lead to detrimental neurological effects. Further, disturbances in the gut and other microbiomes (dysbiosis) can affect host immunity and neurobehavioral responses (Cryan and Dinan, 2012; Douglas-Escobar et al., 2013; Ding and Schloss, 2014; Galland, 2014; Stilling et al., 2014; Sherman et al., 2015). In this review, we will primarily focus on how alterations in microbiomes, especially in the gut, and their products affect the risk for ASD and related neurobehavioral disorders.

First, the evidence in animal models that gut dysbiosis can lead to ASD-like behavioral disturbances will addressed. Next, we will review the handful of human epidemiological studies correlating microbiota and bacterial metabolite changes in ASD patients. While the majority if

not all of the studies to date have focused on the potential role of gut microbiome alterations and ASD, it is now clear that other organs and systems, including the oral cavity (Ding and Schloss, 2014), lung (Dickson et al., 2014), placenta (Aagaard et al., 2014; Amarasekara et al., 2014; Antony et al., 2014; Doyle et al., 2014), and vagina (Stumpf et al., 2013; Ding and Schloss, 2014), possess unique microbiomes, that may influence distal target systems. Therefore, we will consider whether disruptions in these other microbiomes may be contributing etiologies to ASD risk. Prebiotics, probiotics, antibiotics, and related treatments have been proposed to be useful adjuvant treatments for ASD. Thus, the evidence that these of these factors may be useful adjuvant treatments forASD will be explored. Lastly, a sampling of the potential mechanisms by which gut dysbiosis may contribute to ASD will be considered.

ASD and Gut Microbiome Animal Model Studies

Table 1 summarizes animal model studies to date linking alterations in the gut microbiota and neurobehavioral changes. The first evidence associating gut microbiota disturbances and neurobehavioral disorders originated from germ-free (GF, axenic) mice. They are delivered via C-section and then maintained in a sterile gnobiotic environment. These mice are likely devoid of any microorganisms. Direct inferences can then be made on how the absence or presence of a gut microbiota may influence behavioral patterns. The initial study demonstrated that restraint stress of adult GF mice resulted in hypersecretion of adrenocorticotropic hormone (ACTH) and corticosterone (two commonly associated stress hormones) relative to specific pathogen free (SPF) controls (Sudo et al., 2004). Reconstitution of GF mice with *Bifidobacterium infantis*, however, ameliorated the exaggerated HPA responses. Transplantation of feces from SPF to GF animals partially reversed the hormonal abnormalities but only if such intervention was

performed early in life, suggestive microbes must colonize the gut at a critical post-natal period for normal neural programming to occur. This study also showed the essential neurotropin, BDNF, and NR2A proteins were suppressed in the cerebral cortex and hippocampus of GF mice.

While this pioneering study did not test behaviors in the GF animals, the hormonal findings suggest GF animals may be more anxious. This prediction was not borne out. Instead, GF animals were more anxious and less exploratory (Diaz Heijtz et al., 2011; Neufeld et al., 2011b; Clarke et al., 2013). Further, the anxiolytic behavior was resistant to SPF fecal transplantation (Neufeld et al., 2011a).

GF mice did show cognitive deficits in non-spatial and working memory tasks (Gareau et al., 2011). A subsequent study supported these original findings, especially in males, and further revealed these animals avoided social situations (Desbonnet et al., 2014). Post-weaning bacterial colonization abolished the latter but not the former disturbance, in line with the critical window for gut microbiota colonization.

Two approaches have been employed to discern how gut dysbiosis may lead to behavioral abnormalities in animal models that recapitulate the clinical signs observed in ASD patients. The first approach entails administration of bacterial metabolites or virulence factors to such rodent models. Other studies instead compared the gut microbiome in affected models to control counterparts and in some cases, whether fecal transplantation/monospecies transfer improves the symptomology.

Daily injection of pregnant rats with the bacterial-metabolite proprionic acid (PPA, 500 mg/kg SC) or the virulence factor, lipopolysaccharide (LPS, 50 mg/kg) led to ASD-like behavioral impairments in male and female offspring, including olfactory-mediated social recognition abnormalities, persistence in examining a novel object, hyperlocomotion, and social deficits (Foley et al., 2014a). Two additional studies by this same group with a similar approach showed pre- and post-natal exposure to these substances resulted in other sex-dependent behavioral disruptions (Foley et al., 2014b; Foley et al., 2014c). Males but not females subjected to prenatal LPS treatment were hypersensitive in acoustic startle testing. In contrast, females exposed to pre and post-natal PPA (double hit) became sensitized in this test. In the pre-pulse inhibition test, animals are first exposed to a weak acoustic stimulus (pre-pulse), which should decrease the reflexive flinching startle response when they are then exposed to a more intense stimulus (pulse). Normal animals should be able to filter out irrelevant auditory information; whereas, those with neurobehavioral deficits are not be able to do so. Females exposed to PPA during the pre-natal period exhibited a lower pre-pulse inhibition threshold, but similar effects were not observed in males. Males and females exposed to prenatal PPA treatment spent less time in the center of the open field maze, suggestive of increased anxiety-like behaviors. Anxiogenic behaviors were also exhibited by females exposed to PPA pre- and post-natally when tested in the elevated plus maze (EPM), where more time spent in the closed arms suggests increased anxiety. Increased amount of time in the open arms is indicative of anxiolytic or increased exploratory behaviors. The “double-hit” female group engaged in more repetitive behaviors in the open-field tests. Intraventricular injection of PPA for an acute period (8 days) to rats resulted in hyperlocomotion and architectural changes in the brain reminiscent of the pathobiology observed in select ASD cases (Thomas et al., 2012).

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

Intracerebroventricular injection of PPA (4 ml of 0.26 M solution) to Long-Evans, seizure-resistant (SLOW), and seizure prone (FAST) rats led to social behavioral impairments, exemplified by increased mean distance apart and less time spent in proximity to other animals, reduced playful bouts, and altered response to playful intentions from companions. Neuroinflammation (astrogliosis) was evident in these animals (Shultz et al., 2008). This same treatment also impaired cognition and sensorimotor ability in Long-Evans rats (Shultz et al., 2009; Shultz et al., 2014).

Valproic acid (VPA) is a commonly used drug to treat epilepsy and other neuropsychological disorders. A linkage exists between maternal usage of this drug while pregnant and later risk of ASD in her offspring (Christensen et al., 2013). Mice exposed *in utero* to VPA demonstrated later autistic-like social behavioral deficiencies, altered intestinal SCFAs, and gut dysbiosis (de Theije et al., 2014). VPA exposed animals displayed changes in the operational taxonomic units (OTUs) for genera classified within the main phyla of Bacteroidetes and Firmicutes and the order of Desulfovibrionales, which is similar to bacterial shifts observed in human ASD patients (described below). Male offspring developmentally exposed to VPA demonstrated alterations in OTUs in the Alistipes, Enterohaabdus, Mollicutes, and Erysipelotrichalis genera. In general, microbiome differences were more pronounced in VPA exposed males than females. The gut microbiome of these males positively correlated with increasing levels of cecal butyrate and neutrophil inflammation but was negatively linked to increasing intestinal concentrations of serotonin and social behavior scores.

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

The most convincing study linking gut microbiota alterations and ASD-like behaviors in an animal model employed the maternal immune activation (MIA) mouse model for ASD (Hsiao et al., 2013). Gestational administration to the mother of the immunostimulant polyinosinic:polycytidylic acid (Poly I:C) recapitulates the offspring neurodisruptive effects of a viral infection. The data revealed that this treatment significantly disturbed the offspring gut-microbiome-brain axis, including compromising the intestinal barrier, which was likely due to gut dysbiosis with ~8% of bacterial metabolites diverging in MIA offspring with a "leaky gut". Notably, administration of *Bacteroides fragilis*, a human commensal bacteria, restored the gut permeability and microbiota population, corrected the metabolome changes, and mitigated the defects in communicative, stereotypic, anxiety-like, and sensorimotor behaviors. One metabolite reduced to normal levels in the treated MIA offspring was 4-ethylphenylsulfate (4-EPS), which has been implicated as an autistic biomarker (Persico and Napolioni, 2013). Administration of 4-EPS sulfate to wild-type mice replicated the anxiety-like behaviors observed in ASD animal models (Hsiao et al., 2013). Collectively, this single study provides strong causative evidence that gut dysbiosis results in a cascade of effects culminating in ASD-like behavioral disturbances. However, probiotic treatment of this animal model may reverse pathological changes in the gut, bacterial metabolic disruptions, and mitigate the neurobehavioral abnormalities.

Human Epidemiological Studies Linking Gut Dysbiosis and ASD

GI system disorders are a common comorbidity in ASD patients (Buie et al., 2010; Mayer et al., 2014). Disruptions in the gut microbiota may be one common thread linking these two disparate systems. For instance, intestinal colonization by the anerobic bacteria, *Clostridium tetani*, was postulated to increase the risk and severity of ASD, but this paper did not test for a direct linkage

(Bolte, 1998). This bacteria produces a neurotoxin that may reach the brain via the vagus nerve. Once there, the chemical impacts neurotransmitter release with the possibility of a wide-range of ensuing behavioral deficits. Antimicrobial treatment against this disease partially alleviates the stereotypic behaviors observed in these patients.

PCR-based approaches have paved the way to isolate the microbe changes in ASD children with and without concurrent GI symptoms, as detailed in Table 2. Only one study to date has suggsted no association between the gut microbiota composition and ASD symptoms (Gondalia et al., 2012). Other follow-up studies showed other Clostridial groups to be signficantly elevated in ASD children, such as *C. bolteae* and clusters I and XI (Song et al., 2004). Another study by this group revealed 9 species of Clostridium present in ASD but not control children, where 3 unique species were identified. Non-spore-forming anaerobes and microaerophilic bacteria were abudant in the stool of ASD but absent in control children (Finegold et al., 2002). *C. histolyticum* (Clostrium clusters I and II) is another toxin-producing species abundant in the fecal flora of ASD children. Non-autistic siblings possess intermediate levels of this microbe, suggestive that it can be transmitted in the home environment (Parracho et al., 2005).

Newer technologies, such as 16S rDNA sequencing, have revealed other gut microbiota imbalances in ASD children. *Desulfovibrio*, another anaerobic bacillus possessing several virulence factors and generating various metabolic byproducts, was plentiful in ASD children. As with *C. histolyticum*, the fecal contents of non-ASD siblings contained intermediate amounts of *Delsulfovibrio* (Finegold, 2011). An earlier report suggested that *Delsufovibrio spp* was elevated in the stool of severely autistic children. These children also showed high amounts of

fecal *Bacteroides vulgatus*. Dybiosis was evident at the phylum level with autistic showing greater amounts of Bacteroidetes in the stool than controls. In contrast, Firmicutes predominated in the stool of control children. The two groups also had significant differences in the phyla Actinobacterium and Proteobacterium (Finegold et al., 2010).

Slovakian children with ASD dislayed significant reduction in the Bacteroidetes/Firmcutes ratio but increased amount of *Lactobacillus* spp. *Delsulfovibrio* spp showed a trend to be increased, especially with increasing autistic severity (as determined by the ADI, restricted/repetitive behavior subscale score). The clinical severity of GI symptoms was positively correlated with autism severity. Supplementation of these patients with a probiotic diet corrected the imbalanced Bacteroidetes/Firmicutes ratio, suppressed *Delsufovibrio* spp, and increased the amount of *Bifidobacterium* spp present in the stool (Tomova et al., 2015).

Another study suggested ASD children exhibit suppression of transcripts encoding disaccharidases, hexose transporters, and the transcription factor CDX2. The host transcriptomic changes correlated with the degree of gut dysbiosis observed in this ASD child cohort, as revealed by a decrease in *Bacteroidetes* and ratio of *Bacteroidetes* to Firmcutes, and greater preponderance of *Betaproteobacteria* in the intestinal biopsy samples (Williams et al., 2011).

ASD children with and without GI disorders possessed greater amounts of fecal *Sutterella* spp.; whereas, *Ruminococcus torques* was elevated in the stool of children with ASD and GI symptoms compared to those without such disorders (Wang et al., 2013). Another study indicated that in addition to phylum changes in Bacteroidetes, Firmicutes, Fusbobacteria, and

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

Verrucomicrobia in ASD compared to health children, other microbial disruptions were evident in these two groups. Caloramator, *Sarcina,* and Clostridium genera were greater in ASD children. Variations within the Lachnospracease family were also observed. ASD children showed high amounts of fecal Bacteroidetes genera, select *Alistipes* and *Akkermansia* species, and Sutterellaceae, but Enterobacteriaceae, Eubacteriaceae and *Bifidobacterium* species were reduced in this group. Correspondingly, levels of free amino acids and volatile organic compounds within the stool were affected in the autistic group with some increasing and others decreasing relative to controls (De Angelis et al., 2013). A separate study found similar findings with lower levels of Bifidobacteria species but greater abundance of the mucolytic bacterium, *Akkermansia muciniphilia* in ASD children (Wang et al., 2011). *Sutterella* spp. (*wadsworthensis* and *stercoicanis*) predominated in the gut microbiota of ASD children with concurrent GI dysfunction, but these species were absent in children with GI symptoms only (Williams et al., 2012), further highlighting that such species might play an important link between the microbiota-gut-brain axis.

Presence of ASD rather than GI symptoms may be a better predictor of a less diverse gut microbiota composition. This same study disclosed the genera *Prevotella*, *Coprococcus*, and unclassified *Veillonellaceae* to be reduced in the stool of ASD children. It is not clear though if the microbial changes were due to variation in dietary habits or directly correlated with ASD symptomology (Kang et al., 2013).

Associations exist between bacterial metabolites in the feces or urine and behavioral impairments in ASD, including those receiving antibiotic or probiotic treatment.. Adams et al (Adams et al.,

2011) reported a strong positive correlation with GI symptoms and ASD clinical severity. Further, decreased levels of fecal SCFAs, specifically acetate, propionate, and valerate, especially in those individuals on a probiotic treatment were noted. The stool of these children contained less *Bifidobacter* but greater amounts of *Lactobacillus*. Lysozyme was suppressed in children with autism, which may partly be attributed to the probiotic treatment. A separate study treated eleven children who had regressive-onset autism with a poorly absorbed oral antibiotic, vancomycin, and their disease symptomology was monitored. The treatment improved the behavioral performance of these children, but the beneficial effects were transient with the clinical signs recurring upon discontinuation of the antibiotic (Sandler et al., 2000).

In contrast to (Adams et al., 2011), another study found fecal SCFAs were significantly higher in children with ASD. Acetic, butyric, isobutryic, valeric, and isovaleric acids were elevated in the stool of ASD children; whereas, caproic acid was reduced. ASD patients had greater concentration of ammonia in the stool than controls (Wang et al., 2012). In the urine, the free amino acids glutamate and taurine were increased in ASD children, possibly suggestive of disruptions in sulfur and amino acid metabolism. Disturbances in the patterns of bacterial metabolites dimethylamine, hippurate, and phenylacetylglutamine were also identified in this study (Yap et al., 2010). The concentration of D-arabinitol (DA) in the urine was higher in ASD children compared to controls before and after probiotic supplementation. However, the probiotic therapy appeared to partially mitigate the elevated urinary concentrations of DA and ratio of DA/LA as there was a noticeable improvement in the behaviors of the cohort ASD children, particularly in their concentration ability and following orders (Kaluzna-Czaplinska and Blaszczyk, 2012).

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

The conclusions that can be drawn from the collective studies to date suggest that *Clostridia*, *Desulfovibrio, Sutterella,* and *Bacteroidetes* are elevated in the stool of ASD children. In contrast, *Firmicutes*, *Prevotella*, and *Bifidobacter* tend to be reduced in these patients. The gut microbe changes are associated with alterations in fecal concentrations of SCFAs and urinary concentrations of amino acids and ammonia. Conflicting reports exists as to whether antibiotics and probiotics are useful in treating bacterial imbalances observed in ASD patients (discussed in more detail below). Further studies are needed with larger datasets to perform side-by side comparisons of gut microbiota, fecal and urinary analysis for bacterial SCFAs, amino acids, and ammonia in ASD patients with and without GI symptoms and those who have and have not received antibiotic and probiotic treatment. It is also important that repeated analyses are done over time for each patient to monitor how these microbiome/metabolic parameters correlate with disease symptomology. Based on reports suggesting environmental transmission, siblings should also be assayed. To determine the temporal order of events and possibly establish causation, it would be useful if fecal and urinary samples were obtained from all babies (including those who will develop ASD and healthy individuals) at the time of birth.

Other Microbiomes

Emphasis of current animal model and human studies has been to link gut microbiota changes and ASD. The neurological effects of microbiomes inhabiting other systems have been for the most part overlooked. Microbial communities in other systems can undergo dynamic fluctuations in response to various intrinsic and extrinsic factors. These may in turn affect brain function (2012b; Ding and Schloss, 2014). Other body regions known to harbor unique microbes include the skin, oral cavity and associated structures, respiratory system, and vagina.

There are isolated reports detailing the effects the oral cavity microbiome exerts on neural function. Oral cavity microbial shifts are associated with seizure severity in epileptic patients (Costa et al., 2014) and Alzheimer's disease (Noble et al., 2014; Shaik et al., 2014; Shoemark and Allen, 2015). Vaginal dysbiosis, such as may occur through maternal stress, might affect offspring neurobehavioral development (Jasarevic et al., 2015), but sufficient data to support this claim is currently lacking. Even so, disruptions in these and other microbiomes might hold promise in understanding and developing therapeutic intervention strategies against various neurological diseases, including ASD.

Intriguingly, a novel microbiome was discovered this past year in the placenta (Aagaard et al., 2014), a region previously considered sterile (Wassenaar and Panigrahi, 2014). It is also thought of as the primary organ to buffer the fetus against environmental insults (Rosenfeld, 2011). While initial doubts were casts whether the microbiome identified truly originated from the fetal placenta (Kliman, 2014), subsequent work by the original and other groups supports this pioneering finding. Moreover, these additional studies suggest that microbial communities within the placenta may vary according to maternal weight, gestational state, and in women afflicted with preeclampsia (Amarasekara et al., 2014; Antony et al., 2014; Doyle et al., 2014). A floodgate of further questions is raised by this discovery. First and foremost, how do alterations in the placenta microbiome contribute to later disease or health effects, i.e. developmental origins of health and disease (DOHaD)? Are there potential sex differences in the placental microbiome that might also impact DOHaD effects? What other intrinsic and extrinsic maternal factors might influence the placental microbiome? In relation to the last two questions, prior work determined

maternal diet interacting with offspring sex yields unique placental epimutations and transcriptomic changes, especially for genes regulating metabolic pathways (Gallou-Kabani et al., 2010; Mao et al., 2010; Gabory et al., 2012; Gabory et al., 2013). Therefore, it is highly plausible that the placental microbiome might also be vulnerable in a sex-dependent manner to *in utero* changes. It is too early to ascertain the effects of placenta dysbiosis on neurobehavioral programming and CNS diseases. Other placental disruptions, however, have been linked with neurobehavioral disorders, especially in males (Mueller and Bale, 2008).

Future work is needed to determine how alterations in these other microbiomes might contribute to neurological diseases, including ASD. Work in this area should also be directed at exploring for microbiota inhabiting other host systems and tissues, including those possessing abundant nutrients and environments hospitable for colonization by anaerobic or aerobic bacteria.

Therapeutic Modulation of Gut Dysbiosis and ASD

Based on the premise microbiome-gut-brain axis are a potentiating risk factor ASD, several interventionary measures may be conceived. As shown in Fig. 1, these include diets that facilitate the growth of "good" bacteria, prebiotics, probiotics, synbiotics, postbiotics, antibiotics, fecal transplantation, and activated charcoal (Critchfield et al.; Gilbert et al., 2013; Fond et al., 2014). The benefits of each of these adjuvant therapies will be considered.

Diet

Considerable evidence links contrasting diets to gut microbiota changes and subsequent health effects (Reviewed in (Fond et al., 2014; Luna and Foster, 2014; Salazar et al., 2014; Voreades et al., 2014)). Diets containing non-caloric artificial sweeteners (NAS) have been linked with gut

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

dysbiosis and glucose intolerance (Suez et al., 2014). The gut microbe complexity and behavioral patterns of mice provisioned for 3 months with either a rodent chow or beef diet were examined (Li et al., 2009). Beef-fed animals possessed greater gut microbial diversity and displayed superior working and reference memory, and reduced anxiety compared to those fed the chow based diet. Another study tested the effects of transplanting the microbiota from mice maintained on a HF diet to those reared on a control diet (Bruce-Keller et al., 2014). Recolonization of control mice with microbiota from the HF animals shifted the microbiota diversity and taxonomies, impaired gut barrier function, elevated circulating endotoxin levels, increased lymphocyte markers and neuroinflammation, and disturbed cerebrovascular homeostasis. A gluten-free and casein-free diet might be used to treat core and peripheral behavioral symptoms in certain ASD patients. This diet might favorably influence gut microbiota populations and intestinal barrier function, (Whiteley et al., 2012; Pedersen et al., 2014; Whiteley, 2014). As with other proposed treatments, this remedy needs to be experimentally tested.

Prebiotics

Prebiotics include carbohydrates, such as inulin and various oligosaccharides, and other food ingredients indigestible by the host. The compounds may preferentially promote bacterial colonies capable of fermenting them into SCFAs that then affect the gut and distal target organs (Saulnier et al., 2008; Fond et al., 2014). No studies to date have considered whether prebiotic treatment alone improves ASD clinical signs. One study administered two prebiotic treatments (fructo-oligosaccharids, FOS and Bimuno® galacto-oligosaccharides, B-GOS) to healthy volunteers and then monitored their hormonal and behavioral responses (Schmidt et al., 2014). The findings revealed that the latter prebiotic formulation reduced salivary cortisol secretion,

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

indicative of a suppressed neuroendocrine stress response, and increased subject's attention span. These are two behavioral domains impacted in patients with ASD.

Probiotics/Synbiotics

The use of probiotics to treat various aliments dates back to almost a century ago when Dr. Elie Metchinkoff developed a milk-based diet fermented with bacterium he termed "*Bulgarian bacillus*". His premise was that such bacteria may stimulate the growth of advantageous bacteria at the expense of harmful microbes with the long term effect of promoting an overall healthy lifespan (Reviewed in (Fond et al., 2014)).

The term probiotic was however not employed until 1965 (Lilly and Stillwell, 1965). The widely accepted definition of probiotics today is that they are living non-pathogenic organisms that when consumed in adequate amounts may confer various health benefits to the host organism (Critchfield et al.; Caselli et al., 2013). Lactic-acid producing bacteria, such as lactobacilli, lactococci, and bifidobacteria or yeast, e.g. *Saccharomyces boulardii* comprise the majority of currently available probiotic products. Probiotics are deemed safe food products for human consumption by the US Food and Drug Administration (FDA) and European counterparts.

While not defined at the time, the first case of using such bacteria to treat neurological diseases dates to 1910, when Dr. George Porter reported that a gelatin-whey formula with living lactic acid producing bacteria alleviated depression in at-risk individuals ((Phillips, 1910), Reviewed in (Fond et al., 2014)). Approximately one-fifth of physicians today encourage the use of probiotics in ASD children, especially those plagued with GI symptoms (Golnik and Ireland, 2009).

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

Example of direct benefits conferred on the gut by probiotics include alteration of host immunity- in particular stimulating secretory IgA, restoration of normal commensal bacterial colonies and simultaneous suppression of microbial pathogens, stabilization of the intestinal mucosal barrier predominantly due to stimulation of mucin production, which reduces the likelihood of bacterial spread and absorption of harmful bacterial metabolites, and promoting the synthesis of antioxidant substances (Lutgendorff et al., 2008).

Psychobiotics refers to living organisms with beneficial effects on mental health (Dinan et al., 2013). While a handful of reports speculate on the use of potential probiotics/psychobiotics as adjuvant treatments for ASD (Critchfield et al.; Linday, 2001; Garvey, 2002; Levy and Hyman, 2005; Finegold, 2011; Borre et al., 2014; Fond et al., 2014; Ianiro et al., 2014; Reardon, 2014), definitive evidence that this complementary alternative therapy improves behavioral symptomology in ASD patients is for the most part lacking. Probiotic treatment of 19 ASD children restored the Bacteriodetes to Firmicutes ratio, *Desulfovibrio* spp, and the amount of *Bifidobacterium* spp (Tomova et al., 2015). However, the behavioral patterns of the children pre- and post-probiotic supplementation were not assessed. Another report showed urinary concentrations of D-arabinitol (DA) and the ratio of D-/L-arabinitol (DA/LA) were elevated in autistic children but probiotic treatment reversed the metabolic disruptions and improved behavioral performance (Discussed above) (Kaluzna-Czaplinska and Blaszczyk, 2012).

There are limited animal model studies in this area. Wild-type mice fed a chow based diet and provided the probiotic, *Lactobacillus helveticus*, exhibited decrease anxiety-like behaviors (Ohland et al., 2013). As discussed previously, treatment of the MIA mouse model for ASD with

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

the probiotic, *Bacteroides fragilis*, improved the mucosal barrier and gut dysbiosis, mitigated elevations in several metabolites associated with ASD, and abolished ASD-like behavioral disruptions (Hsiao et al., 2013). While much work remains to be done to determine whether probiotics should be promoted as an adjunct treatment for ASD patients, another idea that is gaining currency is the combined treatment of pre- and post-biotics, otherwise considered synbiotic therapy (Kaur et al., 2009; Firmansyah et al., 2011; Szajewska and Makrides, 2011; Fond et al., 2014). The efficacy of synbiotics in alleviating neurological diseases merits further pursuit.

Postbiotics

Another potential therapeutic approach against gut dybiosis-induced neurobehavioral disorders is to ascertain the specific metabolites or molecules altered by microbial changes and supplement such nutrients or their precursors in the diet. Examples of metabolites affected by the gut microbiota populations include amino acid derivatives and SCFAs (Fond et al., 2014; Klemashevich et al., 2014). On the other hand, certain metabolites are elevated in ASD animal models or children. Examples of such biomolecules include 4-EPS, *p*-cresol, indolepyruvate, indolyl-3-acrylolylglycine, n-acetylserine, PPA, and urinary concentrations of dimethylamine, hippurate, and phenyacetylglutamine (Yap et al., 2010; Gilbert et al., 2013; Hsiao et al., 2013; Persico and Napolioni, 2013). Therefore, tailored pre- and post-biotic diets might be conceived to prevent the bacterial synthesis of harmful metabolites and simultaneously supplement those that may be benefical.

Antibiotics

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

Antimicrobiotics (antibiotics) may either be bacteriolytic or bacteriostatic for susceptible intestinal bacteria, but such compounds may also suppress the growth of commensal and beneficial populations of bacteria. Moreover, pathogenic bacteria may rebound once the antibiotic is discontinued. For these reasons, antibiotics are generally not given much credence as a long-term therapy for ASD. One small-scale study treated eleven ASD children for 8 weeks with vancomycin, an antibiotic commonly used in the treatment of *Clostridium difficile colitis*, and found the children's communication and other behavioral scores improved significantly during the treatment period (Sandler et al., 2000). The beneficial effects though were ephemeral with behavioral impairments recurring upon termination of the antibiotic treatment.

Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) is a therapeutic remediation strategy employed to restore normal intestinal flora balance (Aroniadis and Brandt, 2013; Ianiro et al., 2014). It has been particularly successful in the case of recurrent *Clostridium difficile* infection (RCDI) (Bakken et al., 2011; Borody and Khoruts, 2012). This shotgun approach may, however, inadvertently introduce opportunistic infections into the recipient's GI system. Nonetheless, neurological improvement was reported in a single Parkinson's patient after FMT (Anathaswamy, 2011), and there is an anecdotal report of beneficial effects in two ASD children receiving FMT (Aroniadis and Brandt, 2013).

Activated charcoal (carbon)

Activated charcoal or carbon is a standard treatment in many acute oral toxicity cases as the compound binds to toxins present in the upper GI system and thereby prevents absorption across

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

the intestinal wall. Toxins produced by gut microbiota may also lead to injurious effects in the brain. Consequently, activated charcoal may be useful for microbe-induced neurobehavioral disorders, including ASD. A few studies suggest activated charcoal suppresses the growth of antibiotic-resistant intestinal bacteria (Khoder et al., 2010; Grall et al., 2013). Similar to antibiotic treatments, it is likely though that any beneficial effects of this intervention will be short-lived and disease symptomology will return upon cessation of this therapy.

Potential Mechanisms Microbiota Alterations Lead to ASD and related Neurological Disorders

The next sections will delve into some of the mechanisms by which microbiomes (especially in the gut) may impact brain function. As we are still at the nascence of understanding the interactions between microbiota and ASD, this section will for the most part employ a holistic approach to examine how dysbiosis in general affects neurological diseases.

Breaking down barriers

The intestinal epithelial (mucosal) barrier is the primary site of contact for micro-organisms, antigens, and immunogenic proteins. In this sense, it serves as the "homeland security" for the host to process and permit entry of essential extrinsic factors, while blocking transmission of detrimental microorganisms and antigens. This barrier processes over 100 tons in food-borne factors in an individual's lifetime (Alonso et al., 2014). Disruptions in this barrier are likely pivotal to gut-microbiota-brain comorbid disorders (Ait-Belgnaoui et al., 2012; Alonso et al., 2014). Leakiness of this barrier provides a portal for bacterial spread, along with increasing the

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

potential for systemic transmission of antigens, virulence factors, other pathogens, and bacterial metabolites. Such factors may then impact brain function and inflammatory processes (Alonso et al., 2014). One study suggests that probiotic treatment with *L. farciminis* restores the intestinal barrier and educes the concentration of circulating LPS levels culminating in a blunting of the hypothalamic-pituitary-axis (HPA) response and neuroinflammation (Ait-Belgnaoui et al., 2012). Probiotics may restore the mucosal barrier by directly competing and preventing translocation of pathogenic bacteria, stimulating mucosal immunity (secretory IgA), and up-regulating mucin and antioxidant expression (van Minnen et al., 2007; Lutgendorff et al., 2008; Lutgendorff et al., 2009).

The blood brain barrier (BBB) serves as a gatekeeper to minimize the chance of pathogens and foreign particles transferring across the blood vessels to reach the brain parenchyma. The integrity of this barrier is essential for normal brain development and function. It has been recently been shown that the development of the BBB is contingent upon the presence of commensal gut flora. Beginning *in utero*, the permeability of the BBB of GF mice is greater than that of SPF mice, and these differences persist through adulthood. The tight junction proteins (occludin and claudin-5) governing the endothelial-portion of this barrier are reduced in GF animals. However, there is the potential to rescue these animals even later in life as inoculation of adult GF mice with non-pathogenic gut microbiota reverses these abnormalities (Braniste et al., 2014).

Enteric nervous system and vagal nerve

The enteric nervous system (ENS) and vagal nerve provide a bidirectional communication between the gut and brain, resulting in the expansion of the concept to the “brain-gut-enteric

microbiota axis". This association between the brain and gut was first conceived to explain how the CNS may impact gastrointestinal function (Reviewed in (Mayer, 2011)). The current focus though has turned to understanding the other direction of this pathway, including transmission of gut microbes via the ENS and vagus nerve to the brain (Reviewed in (Douglas-Escobar et al., 2013; Stilling et al., 2014)).

As detailed above, behavioral patterns of GF relative to SPF mice provide clear evidence that the gut microbiota can exert neural effects. Proteins regulating synaptogenesis (synaptophysin and PSD-95) are markedly reduced in the striatum of the former mice (Diaz Heijtz et al., 2011). Vagotomy or chemical sypathectomy abolishes the behavioral differences between the two groups of mice (Bercik et al., 2011). Mice provided chronic supplementation with a probiotic (*Lactobacillus rhamnosus*) demonstrate less anxiety-like and depressive behaviors and corresponding expression changes in brain neurotransmitters. However, vagotomy ablates these neurobehavioral responses, supporting the presumption that the vagus nerve is an essential route of transmission between the gut microbiota and brain (Bravo et al., 2011).

Infection of mice with the pathogenic gut bacteria, *Campylobacter jejuni,* results in a rapid increase in anxiety-like behaviors (Lyte et al., 1998). After *C. jejuni* infection, FOS expression is acutely up-regulated in the brainstem of visceral sensory nuclei, particularly in the nucleus tractus solitarius (termination site of the vagus nerve) (Gaykema et al., 2004). A follow-up study verified that the bacterial-induced neurobehavioral responses are attributed to *C. jejuni* activation of vagal ascending pathways (Goehler et al., 2005). The intestinal pathogen, *Salmonella enterica*

subsp enterica serovar Typhimurium (*S. typhi*), also can disseminate to the brain via the vagus nerve (Wang et al., 2002).

Bacterial metabolites

Several bacterial-derived metabolites modulate neurobehavioral responses. Spermidine is one such example, and it may suppress aging and age-related memory impairment (Eisenberg et al., 2009; Gupta et al., 2013). Other bacterial metabolites are attributed with potential encephalopathic effects. Two well such characterized bacterial metabolites are D-lactic acid and ammonia. D-lactate results from an excessive carbohydrate load, and a surge in fecal D-lactate producing bacteria is associated with chronic fatigue syndrome (Sheedy et al., 2009). While some probiotics reduce generation of D-lactic acid by gut microbiota, others increase this bacterial metabolite and may exacerbate cognitive disorders (Mack, 2004; Munakata et al., 2010).

Ammonia results from bacterial urease cleavage of urea; whereupon, it circulates to the liver in the portal vein. There it is further metabolized via the urea cycle. Ammonia does not pose a threat in individuals possessing a normal portal system and functional liver. However, extra- or intra-hepatic shunts, where the blood from the intestinal system bypasses the liver and enters the systemic circulation, result in delivery and concentration of ammonia in the brain. Here, ammonia induces neurotoxic effects, otherwise termed hepatic encephalopathy (HE) (Qureshi et al., 2014). Other pathological changes include disruption of the BBB, suppression of serotonin and dopamine synthesis, and stimulation of octopamine, an atypical neurotransmitter (Skowronska and Albrecht, 2012). Increased urease-producing gut bacteria along with cirrhosis

of the liver raises the potential for HE/cognitive dysfunction (Zhang et al., 2013). Synbiotic treatment of cirrhotic patients appears to improve cognitive function (Malaguarnera et al., 2007).

Bacteria produce several volatile fatty acids comprised of two to four carbon atom chain length, hence termed short-chained fatty acids (SCFAs). The SCFAs, acetate, propionate-PPA, and butyrate-BA), result from bacterial fermentation of indigestible carbohydrates in the large intestine. Health benefits ascribed to these compounds include,. energy-supplementation for colonic epithelium, anti-inflammatory activity, and improved insulin (Segain et al., 2000; Al-Lahham et al., 2010; De Preter et al., 2011). On the other hand, animal model and human epidemiological studies suggest SCFAs may also induce neurotoxic effects, which might contribute to ASD development (Macfabe, 2013). Some of these detrimental effects are likely due to mitochondrial and epigenetic disruptions (Discussed later).

In animal models, offspring exposed during development to PPA and to a lesser extent BA exhibit behaviors disturbances resembling clinical signs observed in ASD patients (Thomas et al., 2012; Foley et al., 2014a; Foley et al., 2014b; Foley et al., 2014c). For this reason, this approach is widely used to model ASD in animals, who otherwise do not develop such disorders. Elevated SCFAs and PPA are found in the stool of ASD children (Wang, 2010; Wang et al., 2012; Wang et al., 2014).

The bacterial metabolite, 4-ethylphenylsulfate (4-EPS) was markedly elevated in the MIA offspring model of ASD (discussed above). Administration of this metabolite to WT mice recapitulated the anxiety-like phenotype observed in MIA offspring (Hsiao et al., 2013).

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

Moreover, 4-EPS is related to *p*-cresol, a putative biomarker metabolite identified in high amounts in ASD children (Persico and Napolioni, 2013). The involvement of 4-EPS in human ASD, however, remains to be elucidated.

Mitochondrial Dysfunction

Mitochondrial disease (MD) is often diagnosed in conjuction with ASD. Thirty percent of patients show biomarkers linked with this former disease (Rossignol and Frye, 2012). The distinct MD observed in autistic cases is thought to be acquired rather than genetic in origin. The SCFAs, especially PPA, which are increased in ASD-associated gut microbes (*Clostridia, Desulfovibrio, Sutterella,* and *Bacteroidetes*) might contribute to mitochondrial dysfunction (Macfabe, 2012; Frye et al., 2013; Macfabe, 2013). One mechanism by which the bacterial metabolite PPA may disrupt mitochondria is through alteration of the tricarboxylic acid (TCA) cycle via conversion of PPA to propionyl-CoA.

PPA can sequester and hinder the metabolism of carnitine, which is a quaternary ammonium cofactor required to transport long-chain fatty acids into the inner mitochondria membrane for β-oxidation and energy production (Jones et al., 2010). Chronic antibiotic administration can inhibit carnitine absorption and reabsorption by the intestines and kidney, respectively (Pochini et al., 2008). The limited circulating carnitine may eventually be depleted if bound for prolonged periods to unprocessed fatty acids, as it would occur when mitochondrial fatty-acid beta oxidation is suppressed (Haas et al., 2008). Abnormal mitochondrial fatty acid oxidation manifests as increased circulating concentrations of acyl-carnitine, a potential biomarker for this disorder. Carnitine is reduced in ASD children (Mostafa, 2005). In contrast, long-chain and very

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

long-chain fatty acids and acyl-carnitine are increased in these patients (Pastural et al., 2009; Frye, 2012). Similar metabolite changes are evident in animal models of ASD treated with PPA and butyrate (Thomas et al., 2010; Thomas et al., 2012).

In ASD animal models, the bacterial metabolite PPA results in a number of other changes that may directly or indirectly result in MD, including neuroinflammation, increased oxidative stress, glutathione depletion, and changes in phosophlipid/acylcarnitine profiles in the brain (MacFabe et al., 2007; Thomas et al., 2010; Thomas et al., 2012). Analagous findings have been reported in ASD patients (Chauhan and Chauhan, 2006; James et al., 2006; Al-Gadani et al., 2009; El-Ansary et al., 2010; Wegiel et al., 2010).

Mitochondria are vital for all animal cells, especially in the brain. Microbiome-induced MD can result in ASD and other neurobehavioral deficits via several mechanisms. A few examples will be considered. Neural synapses require mitochondria for ATP production, calcium maintenance, and plasticity (Mattson and Liu, 2002). Neurons with high firing rates, e.g. GABAergic interneurons, are affected by MD (Anderson, 2008). GABA neurons are required for cerebral cortex processing of sensory information, which is affected in ASD children (Anderson, 2008). The reactive oxygen species generated with MD may induce necrosis of nervous tissue and impede synaptic transmission (Frye, 2014). A link between reactive oxygen species, mitochondrial disturbances in brain tissue, and ASD has been reported (Rose et al., 2012).

Neuroendocrine mechanisms

Microbes have evolved the ability to eavesdrop on host neurotransmitter and hormonal conversations and even exploit such host factors to their advantage. Gut bacteria may even

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

interject into the conversation by producing a range of their own neurotransmitters, which mimic those of the host and thus alter neural pathways, such as gamma-amino butyrate (GABA), norepinephrine (NE), serotonin, and dopamine (al Mardini et al., 1991; Li and Cao, 2010; Barrett et al., 2012; Cryan and Dinan, 2012). This inter-kingdom communication is extraordinary but may be more parasitic on the part of the bacteria than mutualistic.

Bacterial colonies generally self-regulate their own growth through quorum sensing molecules. Stress-related neurochemicals produced by the host including, catecholamines- NE, epinephrine, and adrenaline, act upon these bacterial signaling pathways, which increases the proliferative rate of microbial colonies (Lyte, 2004; Karavolos et al., 2011; Lyte, 2014). *Helicobacter pylori* utilizes L-DOPA for growth promotion but at the host's expense. Antibiotic elimination of the pathogen increases the availability of L-DOPA and may thus be beneficial in neurocognitive disorders (Lyte, 2010). A pilot study with stressed college students preparing for a final examination provides additional evidence that the neuroendocrine state of the host can impact gut microbe composition, as reduced numbers of fecal lactic acid bacteria were present in these individuals (Knowles et al., 2008).

Host neuroendocrine stress responses markedly affect an assortment of bacterial virulence factors. Under culture conditions, NE and dopamine increase motility of various *Vibrio* strains (Pande et al., 2014). However, co-treatment with catecholamine receptor antagonists mitigated this effect. *Vibrio hareyi* treated with NE or DOPA produce more harmful siderophores, exhibit enhanced swimming motility, and up-regulation of genes mediating flagellar activity, biofilm formation, and exopolysaccharide production. Co-administration with an α-adrenergic, bacterial-derived catecholamine receptor antagonist, and dopaminergic antagonists neutralized these

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

effects (Yang et al., 2014). GABA affects the virulence of *Pseudomonas aeruginosa* (Dagorn et al., 2013). Increased production of neuroendocrine stress hormones promotes hemolysis and release of hemolysin E by *S. typhi* (Karavolos et al., 2011).

Gut microbiota can directly influence the host HPA axis (Sudo et al., 2004). Elevated serum concentrations of ACTH and corticosterone were detected in GF mice. Early exposure to SPF feces alleviated the hormonal abnormalities, but transplantation at later stages was unable to reverse these effects. Hippocampal concentrations of 5-hydroxytryptamine and its primary metabolite 5-hydroxyindoleacetic acid were also increased in GF animals (Clarke et al., 2013). Circulating concentrations of tryptophan (a serotonin precursor) is upregulated in these animals. Besides behavioral abnormalities, GF rats have up-regulated hypothalamic mRNA expression of *Crf* and decreased *Gr* mRNA in the hippocampus and reduced dopaminergic turnover rate in the frontal cortex, hippocampus, and striatum (Crumeyrolle-Arias et al., 2014). Mice provided the probiotic, *L. rhamosus* (JB-1) demonstrated decreased anxiety- and desperation- like behaviors, blunted stress-induced response to rising corticosterone concentrations, and altered expression of $Gaba_ar$ and $Gaba_br$ in several brain regions (Bravo et al., 2011). Another study suggests that bacterial virulence factors alone can modulate the HPA axis with LPS from *S. typhi* directly activating the host HPA axis, noradrenergic, and indoleaminergic systems (Dunn et al., 2003).

Epigenetic Alterations

The term epigenetics has become vastly overused to define broadly any transcriptomic change occurring independent of a DNA mutation. The field of neuroepigenetics was born under these auspices and is currently invoked as the genesis of many neurological disorders not explained by

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

genetics alone, including ASD (Wilkinson and Campbell, 2013; Berko et al., 2014; Ladd-Acosta et al., 2014; Lesseur et al., 2014; Tordjman et al., 2014; Wong et al., 2014). Alterations in the gut microbiome might trigger epigenetic changes leading to downstream behavioral manifestations (Mischke and Plosch, 2013; Kumar et al., 2014; Stilling et al., 2014). Microbiota Epimutations Neuro Development (MEND) may thus be a descriptive acronym to describe this phenomenon.

Microbiota may shape the epigenome in several ways. Bacterial-derived SCFAs, including butyric acid (BA), PPA, and acetic acid govern key epigenetic-regulating enzymes. Of these, BA is considered one of the most potent SCFA inhibitors of histone deacetylases-HDAC (Candido et al., 1978; Davie, 2003), which remove the acetyl group from histone proteins, allowing for the proteins to re-associate with DNA and block DNA transcription. Other SCFAs, including PPA, lactate, and pyruvate, also act as weak HDAC inhibitors (Thangaraju et al., 2006; Waldecker et al., 2008; Latham et al., 2012). In contrast, acetate up-regulates the histone acetyl transferase (HAT) substrate availability (Stilling et al., 2014). Inflammatory responses within the intestinal mucosa are homeostatically-regulated by gut microbiota in an HDAC3-dependent manner (Alenghat et al., 2013).

Commensal gut microbiota synthesize the B-vitamins, folate and vitamin B12, vital for methylation of DNA and histone proteins (LeBlanc et al., 2013). A recent study with pregnant women linked gut microbiota profiles, especially for Firmicutes and Bacteroidetes, and leukocyte DNA methylation patterns for genes regulating lipid metabolism and obesity (Kumar et al., 2014). In mice, a maternal methyl supplemented diet resulted in gut dysbiosis, associated changes in colonic mucosal DNA methylation and transcriptomic patterns and colitis in the

offspring (Schaible et al., 2011). Microbiota appear to orchestrate other host epimutations, such as chromatin rearrangements, alterations in non-coding RNAs, and RNA splicing factors (Bierne et al., 2012), although further work is needed to validate such effects. One study suggests that pathogenic bacteria might usurp control of host gene expression by broadly suppressing RNA polymerase II, enzyme required for synthesis of coding and non-coding RNAs (Lutay et al., 2013). Intriguingly, there has been recent evidence that some endosymbiotic bacteria produce small non-coding RNAs with the potential to exert cross-kingdom communication and affect host processes (Mayoral et al., 2014). There are presumably more such examples awaiting discovery, especially in commensal gut and other organ microbes, which have so closely evolved and adapted to their host environments. It is provocative to contemplate how such bacterial non-coding RNAs might impact host health and disease. We are at the infancy of understanding how host microbiota and their products influence the host epigenome. Mechanistic insight into these two revolutionary fields may yield key biomarkers for early diagnosis and preventative/therapeutic remediation strategies for various diseases, including ASD.

Discussion

Microbiota populations contained within various mammalian host systems, especially the gastrointestinal region, may affect host health and disease. There are various mechanisms by which the microbiota or their products impacts the brain. Some such methods involve commandeering control of host hormonal and epigenetic systems to the advantage of the bacteria. Yet, we are dependent upon microbes for certain key nutrients and promotion of normal gut development, neurobehavioral patterns, and immunological function. Whether the scale is tipped in favor of health or disease likely depends upon the diversity of microbes present, select

populations, and absolute amount of bacteria harbored within the gut and other systems. These variations may alter metabolite profiles, virulence factors, immunological responses, and other responses impacting neurological function.

While there are select animal model and human studies implicating gut microbiota alterations and development of ASD, it is still premature to render definitive conclusions and establish causation. More work with larger cohorts and other animal models is needed to confirm the initial findings and ascertain the potential underlying mechanisms. Other studies should also explore whether shifts in other organ system's microbiomes is associated with these heterogeneous class of diseases. If dysbiosis is shown to be a precipitating factor in ASD, then several potential therapeutic approaches ranging from prebiotics, postbiotics, synbiotics, fecal transplantation, and other strategies to alter the microbiomes or products may be useful adjuvant treatments in these cases. Preliminary data provides initial support for their usage, but all of these potential therapies need to undergo rigorous testing before such huge claims can be made on their efficacy. In sum, the data to date provide some evidence linking the microbiota-gut-brain axis to ASD, but this field is still at the primordial stages.

Authorship Contribution:

Wrote or contributed to the writing of the manuscript: Rosenfeld

References

(2012a) A framework for human microbiome research. *Nature* **486:**215-221.

(2012b) Structure, function and diversity of the healthy human microbiome. *Nature* **486:**207-214.

Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, and Versalovic J (2014) The placenta harbors a unique microbiome. *Sci Transl Med* **6:**237ra265.

Adams JB, Johansen LJ, Powell LD, Quig D, and Rubin RA (2011) Gastrointestinal flora and gastrointestinal status in children with autism--comparisons to typical children and correlation with autism severity. *BMC gastroenterology* **11:**22.

Ait-Belgnaoui A, Durand H, Cartier C, Chaumaz G, Eutamene H, Ferrier L, Houdeau E, Fioramonti J, Bueno L, and Theodorou V (2012) Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. *Psychoneuroendocrinology* **37:**1885-1895.

Al-Gadani Y, El-Ansary A, Attas O, and Al-Ayadhi L (2009) Metabolic biomarkers related to oxidative stress and antioxidant status in Saudi autistic children. *Clin Biochem* **42:**1032-1040.

Al-Lahham SH, Peppelenbosch MP, Roelofsen H, Vonk RJ, and Venema K (2010) Biological effects of propionic acid in humans; metabolism, potential applications and underlying mechanisms. *Biochim Biophys Acta* **1801:**1175-1183.

al Mardini H, al Jumaili B, Record CO, and Burke D (1991) Effect of protein and lactulose on the production of gamma-aminobutyric acid by faecal Escherichia coli. *Gut* **32:**1007-1010.

Alenghat T, Osborne LC, Saenz SA, Kobuley D, Ziegler CG, Mullican SE, Choi I, Grunberg S, Sinha R, Wynosky-Dolfi M, Snyder A, Giacomin PR, Joyce KL, Hoang TB, Bewtra M, Brodsky IE, Sonnenberg GF, Bushman FD, Won KJ, Lazar MA, and Artis D (2013) Histone deacetylase 3 coordinates commensal-bacteria-dependent intestinal homeostasis. *Nature* **504:**153-157.

Alonso C, Vicario M, Pigrau M, Lobo B, and Santos J (2014) Intestinal barrier function and the brain-gut axis. *Adv Exp Med Biol* **817:**73-113.

Amarasekara R, Jayasekara RW, Senanayake H, and Dissanayake VH (2014) Microbiome of the placenta in pre-eclampsia supports the role of bacteria in the multifactorial cause of pre-eclampsia. *J Obstet Gynaecol Res*.

Anathaswamy A (2011) Faecal transplant eases symptoms of Parkinson's. *New Scientist* **2796:**8-9.

Anderson MP, Hooker, B.S., Herbert, M.R. (2008) Bridging from cells to cognition in autism pathophysiology: biochemical pathways to defective brain function and plasticity. *Am J Biochem Biotechnol* **4:**167-176.

Antony KM, Ma J, Mitchell KB, Racusin DA, Versalovic J, and Aagaard K (2014) The Preterm Placental Microbiome Varies in Association with Excess Maternal Gestational Weight Gain. *Am J Obstet Gynecol*.

Aroniadis OC and Brandt LJ (2013) Fecal microbiota transplantation: past, present and future. *Curr Opin Gastroenterol* **29:**79-84.

Bakken JS, Borody T, Brandt LJ, Brill JV, Demarco DC, Franzos MA, Kelly C, Khoruts A, Louie T, Martinelli LP, Moore TA, Russell G, and Surawicz C (2011) Treating Clostridium difficile infection with fecal microbiota transplantation. *Clinical*

gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association **9:**1044-1049.

Barrett E, Ross RP, O'Toole PW, Fitzgerald GF, and Stanton C (2012) gamma-Aminobutyric acid production by culturable bacteria from the human intestine. *J Appl Microbiol* **113:**411-417.

Beard CM, Panser LA, and Katusic SK (2011) Is excess folic acid supplementation a risk factor for autism? *Med Hypotheses* **77:**15-17.

Bello SC (2007) Autism and environmental influences: review and commentary. *Rev Environ Health* **22:**139-156.

Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, Deng Y, Blennerhassett P, Macri J, McCoy KD, Verdu EF, and Collins SM (2011) The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology* **141:**599-609, 609.e591-593.

Berko ER, Suzuki M, Beren F, Lemetre C, Alaimo CM, Calder RB, Ballaban-Gil K, Gounder B, Kampf K, Kirschen J, Maqbool SB, Momin Z, Reynolds DM, Russo N, Shulman L, Stasiek E, Tozour J, Valicenti-McDermott M, Wang S, Abrahams BS, Hargitai J, Inbar D, Zhang Z, Buxbaum JD, Molholm S, Foxe JJ, Marion RW, Auton A, and Greally JM (2014) Mosaic epigenetic dysregulation of ectodermal cells in autism spectrum disorder. *PLoS Genet* **10:**e1004402.

Bespalova IN and Buxbaum JD (2003) Disease susceptibility genes for autism. *Ann Med* **35:**274-281.

Bierne H, Hamon M, and Cossart P (2012) Epigenetics and bacterial infections. *Cold Spring Harbor perspectives in medicine* **2:**a010272.

Bolte ER (1998) Autism and Clostridium tetani. *Med Hypotheses* **51:**133-144.

Borody TJ and Khoruts A (2012) Fecal microbiota transplantation and emerging applications. *Nature reviews Gastroenterology & hepatology* **9:**88-96.

Borre YE, Moloney RD, Clarke G, Dinan TG, and Cryan JF (2014) The impact of microbiota on brain and behavior: mechanisms & therapeutic potential. *Adv Exp Med Biol* **817:**373-403.

Braniste V, Al-Asmakh M, Kowal C, Anuar F, Abbaspour A, Toth M, Korecka A, Bakocevic N, Guan NL, Kundu P, Gulyas B, Halldin C, Hultenby K, Nilsson H, Hebert H, Volpe BT, Diamond B, and Pettersson S (2014) The gut microbiota influences blood-brain barrier permeability in mice. *Sci Transl Med* **6:**263ra158.

Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, Bienenstock J, and Cryan JF (2011) 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:**16050-16055.

Bruce-Keller AJ, Salbaum JM, Luo M, Blanchard Et, Taylor CM, Welsh DA, and Berthoud HR (2014) Obese-type Gut Microbiota Induce Neurobehavioral Changes in the Absence of Obesity. *Biol Psychiatry*.

Buie T, Campbell DB, Fuchs GJ, 3rd, Furuta GT, Levy J, Vandewater J, Whitaker AH, Atkins D, Bauman ML, Beaudet AL, Carr EG, Gershon MD, Hyman SL, Jirapinyo P, Jyonouchi H, Kooros K, Kushak R, Levitt P, Levy SE, Lewis JD, Murray KF, Natowicz MR, Sabra A, Wershil BK, Weston SC, Zeltzer L, and Winter H (2010) Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: a consensus report. *Pediatrics* **125 Suppl 1:**S1-18.

Candido EP, Reeves R, and Davie JR (1978) Sodium butyrate inhibits histone deacetylation in cultured cells. *Cell* **14:**105-113.

Caselli M, Cassol F, Calo G, Holton J, Zuliani G, and Gasbarrini A (2013) Actual concept of "probiotics": is it more functional to science or business? *World J Gastroenterol* **19:**1527-1540.

Chaste P and Leboyer M (2012) Autism risk factors: genes, environment, and gene-environment interactions. *Dialogues Clin Neurosci* **14:**281-292.

Chauhan A and Chauhan V (2006) Oxidative stress in autism. *Pathophysiology : the official journal of the International Society for Pathophysiology / ISP* **13:**171-181.

Christensen J, Gronborg TK, Sorensen MJ, Schendel D, Parner ET, Pedersen LH, and Vestergaard M (2013) Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. *JAMA* **309:**1696-1703.

Clarke G, Grenham S, Scully P, Fitzgerald P, Moloney RD, Shanahan F, Dinan TG, and Cryan JF (2013) The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Mol Psychiatry* **18:**666-673.

Clemente JC, Ursell LK, Parfrey LW, and Knight R (2012) The impact of the gut microbiota on human health: an integrative view. *Cell* **148:**1258-1270.

Collins SM and Bercik P (2009) The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. *Gastroenterology* **136:**2003-2014.

Constantino JN, Przybeck, T., Friesen, D., and Todd, R.D. (2000) Reciprocal social behavior in children with and without pervasive developmental disorders. *J Dev Behav Pediatr* **21:**2-11.

Costa AL, Yasuda CL, Shibasaki W, Nahas-Scocate AC, de Freitas CF, Carvalho PE, and Cendes F (2014) The association between periodontal disease and seizure severity in refractory epilepsy patients. *Seizure* **23:**227-230.

Critchfield JW, van Hemert S Fau - Ash M, Ash M Fau - Mulder L, Mulder L Fau - Ashwood P, and Ashwood P The potential role of probiotics in the management of childhood autism spectrum disorders.

Crumeyrolle-Arias M, Jaglin M, Bruneau A, Vancassel S, Cardona A, Dauge V, Naudon L, and Rabot S (2014) Absence of the gut microbiota enhances anxiety-like behavior and neuroendocrine response to acute stress in rats. *Psychoneuroendocrinology* **42:**207-217.

Cryan JF and Dinan TG (2012) Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci* **13:**701-712.

Currenti SA (2010) Understanding and determining the etiology of autism. *Cell Mol Neurobiol* **30:**161-171.

Dagorn A, Hillion M, Chapalain A, Lesouhaitier O, Duclairoir Poc C, Vieillard J, Chevalier S, Taupin L, Le Derf F, and Feuilloley MG (2013) Gamma-aminobutyric acid acts as a specific virulence regulator in Pseudomonas aeruginosa. *Microbiology* **159:**339-351.

Dai ZL, Li XL, Xi PB, Zhang J, Wu G, and Zhu WY (2012) Metabolism of select amino acids in bacteria from the pig small intestine. *Amino Acids* **42:**1597-1608.

Davie JR (2003) Inhibition of histone deacetylase activity by butyrate. *J Nutr* **133:**2485s-2493s.

De Angelis M, Piccolo M, Vannini L, Siragusa S, De Giacomo A, Serrazzanetti DI, Cristofori F, Guerzoni ME, Gobbetti M, and Francavilla R (2013) Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. *PLoS One* **8:**e76993.

De Preter V, Geboes KP, Bulteel V, Vandermeulen G, Suenaert P, Rutgeerts P, and Verbeke K (2011) Kinetics of butyrate metabolism in the normal colon and in ulcerative colitis: the effects of substrate concentration and carnitine on the beta-oxidation pathway. *Aliment Pharmacol Ther* **34:**526-532.

de Theije CG, Wopereis H, Ramadan M, van Eijndthoven T, Lambert J, Knol J, Garssen J, Kraneveld AD, and Oozeer R (2014) Altered gut microbiota and activity in a murine model of autism spectrum disorders. *Brain Behav Immun* **37:**197-206.

Desbonnet L, Clarke G, Shanahan F, Dinan TG, and Cryan JF (2014) Microbiota is essential for social development in the mouse. *Mol Psychiatry* **19:**146-148.

Deth R, Muratore C, Benzecry J, Power-Charnitsky VA, and Waly M (2008) How environmental and genetic factors combine to cause autism: A redox/methylation hypothesis. *Neurotoxicology* **29:**190-201.

Diaz Heijtz R, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, Hibberd ML, Forssberg H, and Pettersson S (2011) Normal gut microbiota modulates brain development and behavior. *Proc Natl Acad Sci U S A* **108:**3047-3052.

Dickson RP, Erb-Downward JR, Prescott HC, Martinez FJ, Curtis JL, Lama VN, and Huffnagle GB (2014) Cell-associated bacteria in the human lung microbiome. *Microbiome* **2:**28.

Dinan TG, Stanton C, and Cryan JF (2013) Psychobiotics: a novel class of psychotropic. *Biol Psychiatry* **74:**720-726.

Ding T and Schloss PD (2014) Dynamics and associations of microbial community types across the human body. *Nature* **509:**357-360.

Douglas-Escobar M, Elliott E, and Neu J (2013) Effect of intestinal microbial ecology on the developing brain. *JAMA pediatrics* **167:**374-379.

Doyle RM, Alber DG, Jones HE, Harris K, Fitzgerald F, Peebles D, and Klein N (2014) Term and preterm labour are associated with distinct microbial community structures in placental membranes which are independent of mode of delivery. *Placenta* **35:**1099-1101.

Dunn AJ, Ando T, Brown RF, and Berg RD (2003) HPA axis activation and neurochemical responses to bacterial translocation from the gastrointestinal tract. *Ann N Y Acad Sci* **992:**21-29.

Eisenberg T, Knauer H, Schauer A, Buttner S, Ruckenstuhl C, Carmona-Gutierrez D, Ring J, Schroeder S, Magnes C, Antonacci L, Fussi H, Deszcz L, Hartl R, Schraml E, Criollo A, Megalou E, Weiskopf D, Laun P, Heeren G, Breitenbach M, Grubeck-Loebenstein B, Herker E, Fahrenkrog B, Frohlich KU, Sinner F, Tavernarakis N, Minois N, Kroemer G, and Madeo F (2009) Induction of autophagy by spermidine promotes longevity. *Nat Cell Biol* **11:**1305-1314.

El-Ansary A, Al-Daihan S, Al-Dbass A, and Al-Ayadhi L (2010) Measurement of selected ions related to oxidative stress and energy metabolism in Saudi autistic children. *Clin Biochem* **43:**63-70.

Finegold SM (2011) Desulfovibrio species are potentially important in regressive autism. *Med Hypotheses* **77:**270-274.

Finegold SM, Dowd SE, Gontcharova V, Liu C, Henley KE, Wolcott RD, Youn E, Summanen PH, Granpeesheh D, Dixon D, Liu M, Molitoris DR, and Green JA, 3rd (2010) Pyrosequencing study of fecal microflora of autistic and control children. *Anaerobe* **16:**444-453.

Finegold SM, Molitoris D, Song Y, Liu C, Vaisanen ML, Bolte E, McTeague M, Sandler R, Wexler H, Marlowe EM, Collins MD, Lawson PA, Summanen P, Baysallar M, Tomzynski TJ, Read E, Johnson E, Rolfe R, Nasir P, Shah H, Haake DA, Manning P, and Kaul A (2002) Gastrointestinal microflora studies in late-onset autism. *Clin Infect Dis* **35:**S6-s16.

Firmansyah A, Dwipoerwantoro PG, Kadim M, Alatas S, Conus N, Lestarina L, Bouisset F, and Steenhout P (2011) Improved growth of toddlers fed a milk containing synbiotics. *Asia Pacific journal of clinical nutrition* **20:**69-76.

Foley KA, MacFabe DF, Kavaliers M, and Ossenkopp KP (2014a) 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. *Behav Brain Res* **278c:**244-256.

Foley KA, MacFabe DF, Vaz A, Ossenkopp KP, and Kavaliers M (2014b) 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. *Int J Dev Neurosci* **39:**68-78.

Foley KA, Ossenkopp KP, Kavaliers M, and Macfabe DF (2014c) Pre- and neonatal exposure to lipopolysaccharide or the enteric metabolite, propionic acid, alters development and behavior in adolescent rats in a sexually dimorphic manner. *PLoS One* **9:**e87072.

Fombonne E (2005) Epidemiology of autistic disorder and other pervasive developmental disorders. *J Clin Psychiatry* **66 Suppl 10:**3-8.

Fond G, Boukouaci W, Chevalier G, Regnault A, Eberl G, Hamdani N, Dickerson F, Macgregor A, Boyer L, Dargel A, Oliveira J, Tamouza R, and Leboyer M (2014) The "psychomicrobiotic": Targeting microbiota in major psychiatric disorders: A systematic review. *Pathol Biol (Paris)*.

Forsythe P and Kunze WA (2013) Voices from within: gut microbes and the CNS. *Cell Mol Life Sci* **70:**55-69.

Frank DN and Pace NR (2008) Gastrointestinal microbiology enters the metagenomics era. *Curr Opin Gastroenterol* **24:**4-10.

Frye RE (2012) Biomarkers of abnormal energy metabolism in children with autism spectrum disorder. *NAJ Med Sci* **5:**141-147.

Frye RE (2014) Metabolic and mitochondrial disorders associated with epilepsy in children with autism spectrum disorder. *Epilepsy & behavior : E&B*.

Frye RE, Melnyk S, and Macfabe DF (2013) Unique acyl-carnitine profiles are potential biomarkers for acquired mitochondrial disease in autism spectrum disorder. *Translational psychiatry* **3:**e220.

Gabory A, Ferry L, Fajardy I, Jouneau L, Gothie JD, Vige A, Fleur C, Mayeur S, Gallou-Kabani C, Gross MS, Attig L, Vambergue A, Lesage J, Reusens B, Vieau D, Remacle C, Jais JP, and Junien C (2012) Maternal diets trigger sex-specific divergent trajectories of gene expression and epigenetic systems in mouse placenta. *PLoS ONE* **7:**e47986.

Gabory A, Roseboom TJ, Moore T, Moore LG, and Junien C (2013) Placental contribution to the origins of sexual dimorphism in health and diseases: sex chromosomes and epigenetics. *Biol Sex Differ* **4:**5.

Galland L (2014) The gut microbiome and the brain. *Journal of medicinal food* **17:**1261-1272.

Gallou-Kabani C, Gabory A, Tost J, Karimi M, Mayeur S, Lesage J, Boudadi E, Gross MS, Taurelle J, Vige A, Breton C, Reusens B, Remacle C, Vieau D, Ekstrom TJ, Jais JP, and

Junien C (2010) Sex- and diet-specific changes of imprinted gene expression and DNA methylation in mouse placenta under a high-fat diet. *PLoS ONE* **5:**e14398.

Gareau MG, Wine E, Rodrigues DM, Cho JH, Whary MT, Philpott DJ, Macqueen G, and Sherman PM (2011) Bacterial infection causes stress-induced memory dysfunction in mice. *Gut* **60:**307-317.

Garvey J (2002) Diet in autism and associated disorders. *The journal of family health care* **12:**34-38.

Gaykema RP, Goehler LE, and Lyte M (2004) Brain response to cecal infection with Campylobacter jejuni: analysis with Fos immunohistochemistry. *Brain Behav Immun* **18:**238-245.

Gilbert JA, Krajmalnik-Brown R, Porazinska DL, Weiss SJ, and Knight R (2013) Toward effective probiotics for autism and other neurodevelopmental disorders.

Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, and Nelson KE (2006) Metagenomic analysis of the human distal gut microbiome. *Science* **312:**1355-1359.

Goehler LE, Gaykema RP, Opitz N, Reddaway R, Badr N, and Lyte M (2005) Activation in vagal afferents and central autonomic pathways: early responses to intestinal infection with Campylobacter jejuni. *Brain Behav Immun* **19:**334-344.

Golnik AE and Ireland M (2009) Complementary alternative medicine for children with autism: a physician survey. *J Autism Dev Disord* **39:**996-1005.

Gondalia SV, Palombo EA, Knowles SR, Cox SB, Meyer D, and Austin DW (2012) Molecular characterisation of gastrointestinal microbiota of children with autism (with and without gastrointestinal dysfunction) and their neurotypical siblings. *Autism Res* **5:**419-427.

Gore AC, Martien KM, Gagnidze K, and Pfaff D (2014) Implications of prenatal steroid perturbations for neurodevelopment, behavior, and autism. *Endocr Rev* **35:**961-991.

Grall N, Massias L, Nguyen TT, Sayah-Jeanne S, Ducrot N, Chachaty E, de Gunzburg J, and Andremont A (2013) Oral DAV131, a charcoal-based adsorbent, inhibits intestinal colonization by beta-lactam-resistant Klebsiella pneumoniae in cefotaxime-treated mice. *Antimicrob Agents Chemother* **57:**5423-5425.

Gupta VK, Scheunemann L, Eisenberg T, Mertel S, Bhukel A, Koemans TS, Kramer JM, Liu KS, Schroeder S, Stunnenberg HG, Sinner F, Magnes C, Pieber TR, Dipt S, Fiala A, Schenck A, Schwaerzel M, Madeo F, and Sigrist SJ (2013) Restoring polyamines protects from age-induced memory impairment in an autophagy-dependent manner. *Nat Neurosci* **16:**1453-1460.

Haas RH, Parikh S, Falk MJ, Saneto RP, Wolf NI, Darin N, Wong LJ, Cohen BH, and Naviaux RK (2008) The in-depth evaluation of suspected mitochondrial disease. *Mol Genet Metab* **94:**16-37.

Hooper LV, Midtvedt T, and Gordon JI (2002) How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annu Rev Nutr* **22:**283-307.

Hsiao EY, McBride SW, Hsien S, Sharon G, Hyde ER, McCue T, Codelli JA, Chow J, Reisman SE, Petrosino JF, Patterson PH, and Mazmanian SK (2013) Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell* **155:**1451-1463.

http://www.cdc.gov/ncbddd/dd/addmprevalence.htm.

Ianiro G, Bibbo S, Gasbarrini A, and Cammarota G (2014) Therapeutic modulation of gut microbiota: current clinical applications and future perspectives. *Current drug targets* **15:**762-770.

James SJ, Melnyk S, Jernigan S, Cleves MA, Halsted CH, Wong DH, Cutler P, Bock K, Boris M, Bradstreet JJ, Baker SM, and Gaylor DW (2006) Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics* **141b:**947-956.

Jasarevic E, Rodgers AB, and Bale TL (2015) A novel role for maternal stress and microbial transmission in early life programming and neurodevelopment. *Neurobiology of stress* **1:**81-88.

Jones KL, Will MJ, Hecht PM, Parker CL, and Beversdorf DQ (2013) Maternal diet rich in omega-6 polyunsaturated fatty acids during gestation and lactation produces autistic-like sociability deficits in adult offspring. *Behav Brain Res* **238:**193-199.

Jones LL, McDonald DA, and Borum PR (2010) Acylcarnitines: role in brain. *Prog Lipid Res* **49:**61-75.

Kaluzna-Czaplinska J and Blaszczyk S (2012) The level of arabinitol in autistic children after probiotic therapy. *Nutrition* **28:**124-126.

Kang DW, Park JG, Ilhan ZE, Wallstrom G, Labaer J, Adams JB, and Krajmalnik-Brown R (2013) Reduced incidence of Prevotella and other fermenters in intestinal microflora of autistic children. *PLoS One* **8:**e68322.

Karavolos MH, Bulmer DM, Spencer H, Rampioni G, Schmalen I, Baker S, Pickard D, Gray J, Fookes M, Winzer K, Ivens A, Dougan G, Williams P, and Khan CM (2011) Salmonella Typhi sense host neuroendocrine stress hormones and release the toxin haemolysin E. *EMBO reports* **12:**252-258.

Kaur IP, Kuhad A, Garg A, and Chopra K (2009) Probiotics: delineation of prophylactic and therapeutic benefits. *Journal of medicinal food* **12:**219-235.

Khoder M, Tsapis N, Domergue-Dupont V, Gueutin C, and Fattal E (2010) Removal of residual colonic ciprofloxacin in the rat by activated charcoal entrapped within zinc-pectinate beads. *Eur J Pharm Sci* **41:**281-288.

Klemashevich C, Wu C, Howsmon D, Alaniz RC, Lee K, and Jayaraman A (2014) Rational identification of diet-derived postbiotics for improving intestinal microbiota function. *Curr Opin Biotechnol* **26:**85-90.

Kliman HJ (2014) Comment on "the placenta harbors a unique microbiome". *Sci Transl Med* **6:**254le254.

Knowles SR, Nelson EA, and Palombo EA (2008) Investigating the role of perceived stress on bacterial flora activity and salivary cortisol secretion: a possible mechanism underlying susceptibility to illness. *Biol Psychol* **77:**132-137.

Krakowiak P, Walker CK, Bremer AA, Baker AS, Ozonoff S, Hansen RL, and Hertz-Picciotto I (2012) Maternal metabolic conditions and risk for autism and other neurodevelopmental disorders. *Pediatrics* **129:**e1121-1128.

Kumar H, Lund R, Laiho A, Lundelin K, Ley RE, Isolauri E, and Salminen S (2014) Gut microbiota as an epigenetic regulator: pilot study based on whole-genome methylation analysis. *mBio* **5**.

Ladd-Acosta C, Hansen KD, Briem E, Fallin MD, Kaufmann WE, and Feinberg AP (2014) Common DNA methylation alterations in multiple brain regions in autism. *Mol Psychiatry* **19:**862-871.

Landrigan PJ (2010) What causes autism? Exploring the environmental contribution. *Curr Opin Pediatr* **22:**219-225.

LaSalle JM (2011) A genomic point-of-view on environmental factors influencing the human brain methylome. *Epigenetics* **6:**862-869.

Latham T, Mackay L, Sproul D, Karim M, Culley J, Harrison DJ, Hayward L, Langridge-Smith P, Gilbert N, and Ramsahoye BH (2012) Lactate, a product of glycolytic metabolism, inhibits histone deacetylase activity and promotes changes in gene expression. *Nucleic Acids Res* **40:**4794-4803.

Le Galliard JF, Cote J, and Fitze PS (2008) Lifetime and intergenerational fitness consequences of harmful male interactions for female lizards. *Ecology* **89:**56-64.

LeBlanc JG, Milani C, de Giori GS, Sesma F, van Sinderen D, and Ventura M (2013) Bacteria as vitamin suppliers to their host: a gut microbiota perspective. *Curr Opin Biotechnol* **24:**160-168.

Leeming RJ and Lucock M (2009) Autism: Is there a folate connection? *J Inherit Metab Dis* **32:**400-402.

Lesseur C, Paquette AG, and Marsit CJ (2014) Epigenetic Regulation of Infant Neurobehavioral Outcomes. *Medical epigenetics* **2:**71-79.

Levy SE and Hyman SL (2005) Novel treatments for autistic spectrum disorders. *Mental retardation and developmental disabilities research reviews* **11:**131-142.

Ley RE, Peterson DA, and Gordon JI (2006) Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* **124:**837-848.

Li H and Cao Y (2010) Lactic acid bacterial cell factories for gamma-aminobutyric acid. *Amino Acids* **39:**1107-1116.

Li W, Dowd SE, Scurlock B, Acosta-Martinez V, and Lyte M (2009) Memory and learning behavior in mice is temporally associated with diet-induced alterations in gut bacteria. *Physiol Behav* **96:**557-567.

Lilly DM and Stillwell RH (1965) Probiotics: Growth-promoting factors produced by microorganisms. *Science* **147:**747-748.

Linday LA (2001) Saccharomyces boulardii: potential adjunctive treatment for children with autism and diarrhea. *J Child Neurol* **16:**387.

Lord C, Rutter, M., DiLavore, P.C., and Riski, S. (2002) *Autism Diagnostic Observation Schedule*. Western Psychological Services, Los Angeles, CA.

Luckey TD (1972) Introduction to intestinal microecology. *Am J Clin Nutr* **25:**1292-1294.

Luna RA and Foster JA (2014) Gut brain axis: diet microbiota interactions and implications for modulation of anxiety and depression. *Curr Opin Biotechnol* **32c:**35-41.

Lutay N, Ambite I, Gronberg Hernandez J, Rydstrom G, Ragnarsdottir B, Puthia M, Nadeem A, Zhang J, Storm P, Dobrindt U, Wullt B, and Svanborg C (2013) Bacterial control of host gene expression through RNA polymerase II. *J Clin Invest* **123:**2366-2379.

Lutgendorff F, Akkermans LM, and Soderholm JD (2008) The role of microbiota and probiotics in stress-induced gastro-intestinal damage. *Current molecular medicine* **8:**282-298.

Lutgendorff F, Nijmeijer RM, Sandstrom PA, Trulsson LM, Magnusson KE, Timmerman HM, van Minnen LP, Rijkers GT, Gooszen HG, Akkermans LM, and Soderholm JD (2009)

Probiotics prevent intestinal barrier dysfunction in acute pancreatitis in rats via induction of ileal mucosal glutathione biosynthesis. *PLoS One* **4:**e4512.

Lyall K, Munger KL, O'Reilly EJ, Santangelo SL, and Ascherio A (2013) Maternal dietary fat intake in association with autism spectrum disorders. *Am J Epidemiol* **178:**209-220.

Lyte M (2004) Microbial endocrinology and infectious disease in the 21st century. *Trends Microbiol* **12:**14-20.

Lyte M (2010) Microbial endocrinology as a basis for improved L-DOPA bioavailability in Parkinson's patients treated for Helicobacter pylori. *Med Hypotheses* **74:**895-897.

Lyte M (2014) The effect of stress on microbial growth. *Animal health research reviews / Conference of Research Workers in Animal Diseases* **15:**172-174.

Lyte M, Varcoe JJ, and Bailey MT (1998) Anxiogenic effect of subclinical bacterial infection in mice in the absence of overt immune activation. *Physiol Behav* **65:**63-68.

Macfabe D (2013) Autism: metabolism, mitochondria, and the microbiome. *Global advances in health and medicine : improving healthcare outcomes worldwide* **2:**52-66.

Macfabe DF (2012) Short-chain fatty acid fermentation products of the gut microbiome: implications in autism spectrum disorders. *Microbial ecology in health and disease* **23**.

MacFabe DF, Cain DP, Rodriguez-Capote K, Franklin AE, Hoffman JE, Boon F, Taylor AR, Kavaliers M, and Ossenkopp KP (2007) Neurobiological effects of intraventricular propionic acid in rats: possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders. *Behav Brain Res* **176:**149-169.

Mack DR (2004) D(-)-lactic acid-producing probiotics, D(-)-lactic acidosis and infants. *Can J Gastroenterol* **18:**671-675.

Mackie RI, Sghir A, and Gaskins HR (1999) Developmental microbial ecology of the neonatal gastrointestinal tract. *Am J Clin Nutr* **69:**1035s-1045s.

Malaguarnera M, Greco F, Barone G, Gargante MP, Malaguarnera M, and Toscano MA (2007) Bifidobacterium longum with fructo-oligosaccharide (FOS) treatment in minimal hepatic encephalopathy: a randomized, double-blind, placebo-controlled study. *Dig Dis Sci* **52:**3259-3265.

Mao J, Zhang X, Sieli PT, Falduto MT, Torres KE, and Rosenfeld CS (2010) Contrasting effects of different maternal diets on sexually dimorphic gene expression in the murine placenta. *Proc Natl Acad Sci U S A* **107:**5557-5562.

Mattson MP and Liu D (2002) Energetics and oxidative stress in synaptic plasticity and neurodegenerative disorders. *Neuromolecular medicine* **2:**215-231.

Mayer EA (2011) Gut feelings: the emerging biology of gut-brain communication. *Nat Rev Neurosci* **12:**453-466.

Mayer EA, Padua D, and Tillisch K (2014) Altered brain-gut axis in autism: comorbidity or causative mechanisms? *Bioessays* **36:**933-939.

Mayoral JG, Hussain M, Joubert DA, Iturbe-Ormaetxe I, O'Neill SL, and Asgari S (2014) Wolbachia small noncoding RNAs and their role in cross-kingdom communications. *Proc Natl Acad Sci U S A* **111:**18721-18726.

Mischke M and Plosch T (2013) More than just a gut instinct-the potential interplay between a baby's nutrition, its gut microbiome, and the epigenome. *Am J Physiol Regul Integr Comp Physiol* **304:**R1065-1069.

Mostafa GA, El-Gamal, H.A., El-Wakkad, A.S.E., El-Shorbag, O.E., Hamza, M.M. (2005) Polyunsaturated fatty acids, carnitine and lactate as biological markers of brain energy in autistic children. *Int J Child Neuropsychiatry* **2:**179-188.

Mueller BR and Bale TL (2008) Sex-Specific Programming of Offspring Emotionality after Stress Early in Pregnancy. *J Neurosci* **28:**9055-9065.

Mueller NT, Bakacs E, Combellick J, Grigoryan Z, and Dominguez-Bello MG (2014) The infant microbiome development: mom matters. *Trends in molecular medicine*.

Munakata S, Arakawa C, Kohira R, Fujita Y, Fuchigami T, and Mugishima H (2010) A case of D-lactic acid encephalopathy associated with use of probiotics. *Brain Dev* **32:**691-694.

Murgas Torrazza R and Neu J (2011) The developing intestinal microbiome and its relationship to health and disease in the neonate. *J Perinatol* **31 Suppl 1:**S29-34.

Neufeld KA, Kang N, Bienenstock J, and Foster JA (2011a) Effects of intestinal microbiota on anxiety-like behavior. *Communicative & integrative biology* **4:**492-494.

Neufeld KM, Kang N, Bienenstock J, and Foster JA (2011b) Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterol Motil* **23:**255-264, e119.

Newschaffer CJ, Croen LA, Daniels J, Giarelli E, Grether JK, Levy SE, Mandell DS, Miller LA, Pinto-Martin J, Reaven J, Reynolds AM, Rice CE, Schendel D, and Windham GC (2007) The epidemiology of autism spectrum disorders. *Annu Rev Public Health* **28:**235-258.

Noble JM, Scarmeas N, Celenti RS, Elkind MS, Wright CB, Schupf N, and Papapanou PN (2014) Serum IgG antibody levels to periodontal microbiota are associated with incident Alzheimer disease. *PLoS One* **9:**e114959.

O'Hara AM and Shanahan F (2006) The gut flora as a forgotten organ. *EMBO reports* **7:**688-693.

Ohland CL, Kish L, Bell H, Thiesen A, Hotte N, Pankiv E, and Madsen KL (2013) Effects of Lactobacillus helveticus on murine behavior are dependent on diet and genotype and correlate with alterations in the gut microbiome. *Psychoneuroendocrinology* **38:**1738-1747.

Palmer C, Bik EM, DiGiulio DB, Relman DA, and Brown PO (2007) Development of the human infant intestinal microbiota. *PLoS Biol* **5:**e177.

Pande GS, Suong NT, Bossier P, and Defoirdt T (2014) The catecholamine stress hormones norepinephrine and dopamine increase the virulence of pathogenic Vibrio anguillarum and Vibrio campbellii. *FEMS microbiology ecology* **90:**761-769.

Parracho HM, Bingham MO, Gibson GR, and McCartney AL (2005) Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. *J Med Microbiol* **54:**987-991.

Pastural E, Ritchie S, Lu Y, Jin W, Kavianpour A, Khine Su-Myat K, Heath D, Wood PL, Fisk M, and Goodenowe DB (2009) Novel plasma phospholipid biomarkers of autism: mitochondrial dysfunction as a putative causative mechanism. *Prostaglandins Leukot Essent Fatty Acids* **81:**253-264.

Pedersen L, Parlar S, Kvist K, Whiteley P, and Shattock P (2014) Data mining the ScanBrit study of a gluten- and casein-free dietary intervention for children with autism spectrum disorders: behavioural and psychometric measures of dietary response. *Nutr Neurosci* **17:**207-213.

Persico AM and Napolioni V (2013) Urinary p-cresol in autism spectrum disorder. *Neurotoxicol Teratol* **36:**82-90.

Phillips J (1910) The treatment of melancholia by the lactic acid bacillus. *Journal of Mental Science* **56:**422-431.

Pochini L, Galluccio M, Scumaci D, Giangregorio N, Tonazzi A, Palmieri F, and Indiveri C (2008) Interaction of beta-lactam antibiotics with the mitochondrial carnitine/acylcarnitine transporter. *Chem Biol Interact* **173:**187-194.

Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Dore J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Bork P, Ehrlich SD, and Wang J (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464:**59-65.

Qureshi MO, Khokhar N, and Shafqat F (2014) Ammonia levels and the severity of hepatic encephalopathy. *Journal of the College of Physicians and Surgeons--Pakistan : JCPSP* **24:**160-163.

Reardon S (2014) Gut-brain link grabs neuroscientists. *Nature* **515:**175-177.

Rhee SH, Pothoulakis C, and Mayer EA (2009) Principles and clinical implications of the brain-gut-enteric microbiota axis. *Nature reviews Gastroenterology & hepatology* **6:**306-314.

Rizzo TA, Silverman BL, Metzger BE, and Cho NH (1997) Behavioral adjustment in children of diabetic mothers. *Acta Paediatr* **86:**969-974.

Rogers EJ (2008) Has enhanced folate status during pregnancy altered natural selection and possibly Autism prevalence? A closer look at a possible link. *Med Hypotheses* **71:**406-410.

Rose S, Melnyk S, Pavliv O, Bai S, Nick TG, Frye RE, and James SJ (2012) Evidence of oxidative damage and inflammation associated with low glutathione redox status in the autism brain. *Translational psychiatry* **2:**e134.

Rosenfeld CS (2011) Periconceptional influences on offspring sex ratio and placental responses. *Reprod Fertil Dev* **24:**45-58.

Rossignol DA and Frye RE (2012) Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. *Mol Psychiatry* **17:**290-314.

Rutter M, Le Couteur, A., and Lord, C. (2003) *Autism Diagnostic Interview-Revised*. Western Psychological Services, Los Angeles, CA.

Salazar N, Arboleya S, Valdes L, Stanton C, Ross P, Ruiz L, Gueimonde M, and de Los Reyes-Gavilan CG (2014) The human intestinal microbiome at extreme ages of life. Dietary intervention as a way to counteract alterations. *Front Genet* **5:**406.

Sandler RH, Finegold SM, Bolte ER, Buchanan CP, Maxwell AP, Vaisanen ML, Nelson MN, and Wexler HM (2000) Short-term benefit from oral vancomycin treatment of regressive-onset autism. *J Child Neurol* **15:**429-435.

Saulnier DM, Gibson GR, and Kolida S (2008) In vitro effects of selected synbiotics on the human faecal microbiota composition. *FEMS microbiology ecology* **66:**516-527.

Schaible TD, Harris RA, Dowd SE, Smith CW, and Kellermayer R (2011) Maternal methyl-donor supplementation induces prolonged murine offspring colitis susceptibility in association with mucosal epigenetic and microbiomic changes. *Hum Mol Genet* **20:**1687-1696.

Schmidt K, Cowen PJ, Harmer CJ, Tzortzis G, Errington S, and Burnet PW (2014) Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. *Psychopharmacology (Berl)*.

Segain JP, Raingeard de la Bletiere D, Bourreille A, Leray V, Gervois N, Rosales C, Ferrier L, Bonnet C, Blottiere HM, and Galmiche JP (2000) Butyrate inhibits inflammatory responses through NFkappaB inhibition: implications for Crohn's disease. *Gut* **47:**397-403.

Shaik MM, Ahmad S, Gan SH, Abuzenadah AM, Ahmad E, Tabrez S, Ahmed F, and Kamal MA (2014) How do periodontal infections affect the onset and progression of Alzheimer's disease? *CNS & neurological disorders drug targets* **13:**460-466.

Sheedy JR, Wettenhall RE, Scanlon D, Gooley PR, Lewis DP, McGregor N, Stapleton DI, Butt HL, and KL DEM (2009) Increased d-lactic Acid intestinal bacteria in patients with chronic fatigue syndrome. *In Vivo* **23:**621-628.

Sherman MP, Zaghouani H, and Niklas V (2015) Gut microbiota, the immune system, and diet influence the neonatal gut-brain axis. *Pediatr Res* **77:**127-135.

Shoemark DK and Allen SJ (2015) The Microbiome and Disease: Reviewing the Links between the Oral Microbiome, Aging, and Alzheimer's Disease. *J Alzheimers Dis* **43:**725-738.

Shultz SR, Aziz NA, Yang L, Sun M, MacFabe DF, and O'Brien TJ (2014) Intracerebroventricular injection of propionic acid, an enteric metabolite implicated in autism, induces social abnormalities that do not differ between seizure-prone (FAST) and seizure-resistant (SLOW) rats. *Behav Brain Res* **278c:**542-548.

Shultz SR, Macfabe DF, Martin S, Jackson J, Taylor R, Boon F, Ossenkopp KP, and Cain DP (2009) 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:**33-41.

Shultz SR, MacFabe DF, Ossenkopp KP, Scratch S, Whelan J, Taylor R, and Cain DP (2008) 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:**901-911.

Skowronska M and Albrecht J (2012) Alterations of blood brain barrier function in hyperammonemia: an overview. *Neurotoxicity research* **21:**236-244.

Song Y, Liu C, and Finegold SM (2004) Real-time PCR quantitation of clostridia in feces of autistic children. *Appl Environ Microbiol* **70:**6459-6465.

Stilling RM, Dinan TG, and Cryan JF (2014) Microbial genes, brain & behaviour - epigenetic regulation of the gut-brain axis. *Genes Brain Behav* **13:**69-86.

Stumpf RM, Wilson BA, Rivera A, Yildirim S, Yeoman CJ, Polk JD, White BA, and Leigh SR (2013) The primate vaginal microbiome: comparative context and implications for human health and disease. *Am J Phys Anthropol* **152 Suppl 57:**119-134.

Sudo N, Chida Y, Aiba Y, Sonoda J, Oyama N, Yu XN, Kubo C, and Koga Y (2004) Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J Physiol* **558:**263-275.

Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA, Maza O, Israeli D, Zmora N, Gilad S, Weinberger A, Kuperman Y, Harmelin A, Kolodkin-Gal I, Shapiro H, Halpern Z, Segal E, and Elinav E (2014) Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature* **514:**181-186.

Sullivan EL, Nousen EK, and Chamlou KA (2012) Maternal high fat diet consumption during the perinatal period programs offspring behavior. *Physiol Behav*.

Szajewska H and Makrides M (2011) Is early nutrition related to short-term health and long-term outcome? *Ann Nutr Metab* **58 Suppl 1:**38-48.

Thangaraju M, Gopal E, Martin PM, Ananth S, Smith SB, Prasad PD, Sterneck E, and Ganapathy V (2006) SLC5A8 triggers tumor cell apoptosis through pyruvate-dependent inhibition of histone deacetylases. *Cancer Res* **66:**11560-11564.

Thomas RH, Foley KA, Mepham JR, Tichenoff LJ, Possmayer F, and MacFabe DF (2010) Altered brain phospholipid and acylcarnitine profiles in propionic acid infused rodents: further development of a potential model of autism spectrum disorders. *J Neurochem* **113:**515-529.

Thomas RH, Meeking MM, Mepham JR, Tichenoff L, Possmayer F, Liu S, and MacFabe DF (2012) The enteric bacterial metabolite propionic acid alters brain and plasma phospholipid molecular species: further development of a rodent model of autism spectrum disorders. *Journal of neuroinflammation* **9:**153.

Tomova A, Husarova V, Lakatosova S, Bakos J, Vlkova B, Babinska K, and Ostatnikova D (2015) Gastrointestinal microbiota in children with autism in Slovakia. *Physiol Behav* **138:**179-187.

Tordjman S, Somogyi E, Coulon N, Kermarrec S, Cohen D, Bronsard G, Bonnot O, Weismann-Arcache C, Botbol M, Lauth B, Ginchat V, Roubertoux P, Barburoth M, Kovess V, Geoffray MM, and Xavier J (2014) Gene x Environment interactions in autism spectrum disorders: role of epigenetic mechanisms. *Frontiers in psychiatry* **5:**53.

van Minnen LP, Timmerman HM, Lutgendorff F, Verheem A, Harmsen W, Konstantinov SR, Smidt H, Visser MR, Rijkers GT, Gooszen HG, and Akkermans LM (2007) Modification of intestinal flora with multispecies probiotics reduces bacterial translocation and improves clinical course in a rat model of acute pancreatitis. *Surgery* **141:**470-480.

Voreades N, Kozil A, and Weir TL (2014) Diet and the development of the human intestinal microbiome. *Front Microbiol* **5:**494.

Waldecker M, Kautenburger T, Daumann H, Busch C, and Schrenk D (2008) Inhibition of histone-deacetylase activity by short-chain fatty acids and some polyphenol metabolites formed in the colon. *J Nutr Biochem* **19:**587-593.

Walker WA (2013) Initial intestinal colonization in the human infant and immune homeostasis. *Ann Nutr Metab* **63 Suppl 2:**8-15.

Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, and Conlon MA (2011) Low relative abundances of the mucolytic bacterium Akkermansia muciniphila and Bifidobacterium spp. in feces of children with autism. *Appl Environ Microbiol* **77:**6718-6721.

Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, and Conlon MA (2012) Elevated fecal short chain fatty acid and ammonia concentrations in children with autism spectrum disorder. *Dig Dis Sci* **57:**2096-2102.

Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, and Conlon MA (2013) Increased abundance of Sutterella spp. and Ruminococcus torques in feces of children with autism spectrum disorder. *Molecular autism* **4:**42.

Wang L, Christophersen, C., Sorich, M., Gerber, C., Angley, M., Conlon, M. (2010) Gut bacterial and fermentation profiles are altered in children with autism. *J Gastroeternol Hepatol* **25 (Suppl. 3):**116-119.

Wang L, Conlon MA, Christophersen CT, Sorich MJ, and Angley MT (2014) Gastrointestinal microbiota and metabolite biomarkers in children with autism spectrum disorders. *Biomarkers in medicine* **8:**331-344.

Wang X, Wang BR, Zhang XJ, Xu Z, Ding YQ, and Ju G (2002) Evidences for vagus nerve in maintenance of immune balance and transmission of immune information from gut to brain in STM-infected rats. *World J Gastroenterol* **8:**540-545.

Wassenaar TM and Panigrahi P (2014) Is a foetus developing in a sterile environment? *Lett Appl Microbiol* **59:**572-579.

Wegiel J, Kuchna I, Nowicki K, Imaki H, Wegiel J, Marchi E, Ma SY, Chauhan A, Chauhan V, Bobrowicz TW, de Leon M, Louis LA, Cohen IL, London E, Brown WT, and Wisniewski T (2010) The neuropathology of autism: defects of neurogenesis and neuronal migration, and dysplastic changes. *Acta Neuropathol* **119:**755-770.

Whiteley P (2014) Nutritional management of (some) autism: a case for gluten- and casein-free diets? *Proc Nutr Soc***:**1-6.

Whiteley P, Shattock P, Knivsberg AM, Seim A, Reichelt KL, Todd L, Carr K, and Hooper M (2012) Gluten- and casein-free dietary intervention for autism spectrum conditions. *Frontiers in human neuroscience* **6:**344.

Wilkinson B and Campbell DB (2013) Contribution of long noncoding RNAs to autism spectrum disorder risk. *Int Rev Neurobiol* **113:**35-59.

Williams BL, Hornig M, Buie T, Bauman ML, Cho Paik M, Wick I, Bennett A, Jabado O, Hirschberg DL, and Lipkin WI (2011) Impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children with autism and gastrointestinal disturbances. *PLoS One* **6:**e24585.

Williams BL, Hornig M, Parekh T, and Lipkin WI (2012) 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**.

Wong CC, Meaburn EL, Ronald A, Price TS, Jeffries AR, Schalkwyk LC, Plomin R, and Mill J (2014) Methylomic analysis of monozygotic twins discordant for autism spectrum disorder and related behavioural traits. *Mol Psychiatry* **19:**495-503.

Yang Q, Anh ND, Bossier P, and Defoirdt T (2014) Norepinephrine and dopamine increase motility, biofilm formation, and virulence of Vibrio harveyi. *Front Microbiol* **5:**584.

Yap IK, Angley M, Veselkov KA, Holmes E, Lindon JC, and Nicholson JK (2010) Urinary metabolic phenotyping differentiates children with autism from their unaffected siblings and age-matched controls. *J Proteome Res* **9:**2996-3004.

Zhang Z, Zhai H, Geng J, Yu R, Ren H, Fan H, and Shi P (2013) Large-scale survey of gut microbiota associated with MHE Via 16S rRNA-based pyrosequencing. *Am J Gastroenterol* **108:**1601-1611.

DMD Fast Forward. Published on April 7, 2015 as DOI: 10.1124/dmd.115.063826
This article has not been copyedited and formatted. The final version may differ from this version.

Figure Legend:

Fig. 1. Diagram of dietary and potential therapeutic factors that can affect the gut-microbiome-brain axis. Diets, including those enriched with legumes can affect nutrient availability and prebiotics available to the gut microbiota. Probiotics may colonize the gut and shift the bacterial populations within this system to the so called “good bacteria”. The data to date on potential therapeutic benefit of probiotics for ASD are mixed. Antibiotics have also been proposed as a potential treatment for ASD, but they may indiscriminately destroy both “good” and “bad” bacteria. Antiobiotics are also unlikely to be a viable long term remedy for autism and other neurobehavioral disorders, as the effects on gut microbe populations are generally transient upon discontinuation of the antibiotic. Postbiotics include supplementation of bacterial-produced amino acids and SCFAs. While some of these metabolites might be beneficial, others may induce behavioral deficits. Fecal microbial transplantation has been proposed and tested for improving neurobehavioral impairments and may be a long term option. The downside is that this approach might also introduce pathogenic bacteria into the host intestinal system. Activated charcoal has been proposed to be a useful adjuvant in treating gut-microbiome-brain disorders under the premise that it will be bind bacterial toxins and products. This treatment is unlikely to be a sustainable long term treatment for ASD and chronic neurological disorders.

Table 1: Animal models linking gut microbiota changes to ASD-like behavioral disruptions

Publication(s)	Animal Model(s)	Type of Treatment(s)	Major Findings
(Sudo et al., 2004)	Germ-free (GF) and Specific Pathogen Free (SPF) mice	• The gut of GF mice was reconstituted with *Bifidobacterium infantis* via oral administration to the parents with transmission to the offspring at the neonatal stage. • GF mice received fecal transplantation from SPF animals (0.5ml of 1 $X10^{-2}$ dilution of fresh SPF mouse feces one or three weeks prior to the stress protocol at 9 or 17 weeks of age).	• Adult GF mice subjected to restraint stress exhibited hypersecretion of ACTH and corticosterone. • Adult GF mice had suppression of neurotropin, BDNF, and NR2A in the cerebral cortex and hippocampus. • Reconstitution with *B. infantis* alleviated the exaggerated HPA responses. • Feces from SPF partially reversed the hormonal abnormalities in GF mice, but only if done early in life.
(Diaz Heijtz et al., 2011; Gareau et al., 2011; Neufeld et al., 2011b; Clarke et al., 2013; Desbonnet et al., 2014)	GF & conventionally colonized (CC) mice	• No treatment for (Diaz Heijtz et al., 2011; Gareau et al., 2011; Neufeld et al., 2011b; Clarke et al., 2013). • Post-weaning bacterial colonization (details not specified) for (Desbonnet et al., 2014).	• Combined studies indicate GF mice demonstrate reduced anxiety-like but increased exploratory behaviors, cognitive deficits in non-spatial and working memory tasks, and males, especially, avoid social situations. • Post-weaning bacterial colonization mitigates the avoidance of social situations by GF mice (Desbonnet et al., 2014).
(Foley et al., 2014a)	Rats	• Pregnant P_0 rats were treated with the bacterial metabolite, propionic acid (PPA, 500 mg/ kg body weight, bw subcutaneously, sc on gestational days-GD 12 to 16) or the lipopolysaccharide (LPS, 50 mg/ kg bw sc on gestational day-GD 15 or 16). Control pregnant P_0 rats received vehicle control on GD 12-16 or 15-16.	• F_1 male and female offspring derived from the treated rats showed impairments in olfactory-mediated social recognition, persistence in examining a novel object, hyperlocomotion, and disruptions in social behaviors.
(Foley et al., 2014b; Foley et al., 2014c)	Rats	• Pregnant P_0 rats were treated with the bacterial metabolite, propionic acid (PPA, 500 mg/ kg body weight, bw subcutaneously, sc on GD 12-16) or the lipopolysaccharide (LPS, 50 mg/ kg bw sc on GD 12 or GD 15-16), and these treatments were continued during the post-natal period. Control pregnant P_0 rats received vehicle control on GD 12, 12-16, or GD 15-16.	• F_1 males subjected to prenatal LPS treatment were hypersensitive to in acoustic startle testing. • F_1 females exposed to pre and post-natal PPA (double hit) were sensitized in this test. • F_1 females exposed to PPA during the pre-natal period had reduced pre-pulse inhibition, but similar effects were not observed in males. • F_1 males and females exposed to prenatal PPA treatment spent less time in the center of the open field maze, suggestive of increased anxiety-like behaviors. • Anxiogenic behaviors were also exhibited by F_1 females exposed to PPA pre- and post-natally when tested in the elevated plus maze (EPM). • This "double-hit" female group also engaged in more repetitive behaviors in the open-field tests.
(Thomas et al., 2012)	Rats	• Rats (47 to 49 days of age) were exposed twice daily to intraventricular injection of PPA (4.0 μL of a 0.26 M solution) for an acute period (8 days).	• Treated rats exhibited hyperlocomotion and architectural changes in the brain.
(Shultz et al., 2008)	Male Rats	• Adult male rats were assigned to one of four treatments: 1. PPA (4 μl of 0.26 M solution) 2. Sodium acetate (SA; 4 μl of 0.26 M solution); 3. 1-propanol (PROP; 4 μl of 0.26 M solution) 4. PBS vehicle control (4 μl of 0.1 M solution).	• PPA-treated rats demonstrated social behavioral deficits evidenced by increased mean distance apart and less time spent in proximity to other animals, reduced playful bouts, and altered response to playful intentions from companions. • Another SCFA, sodium acetate, induced similar impairments but no effects were detected with 1-propanol (alcohol analog of PPA).

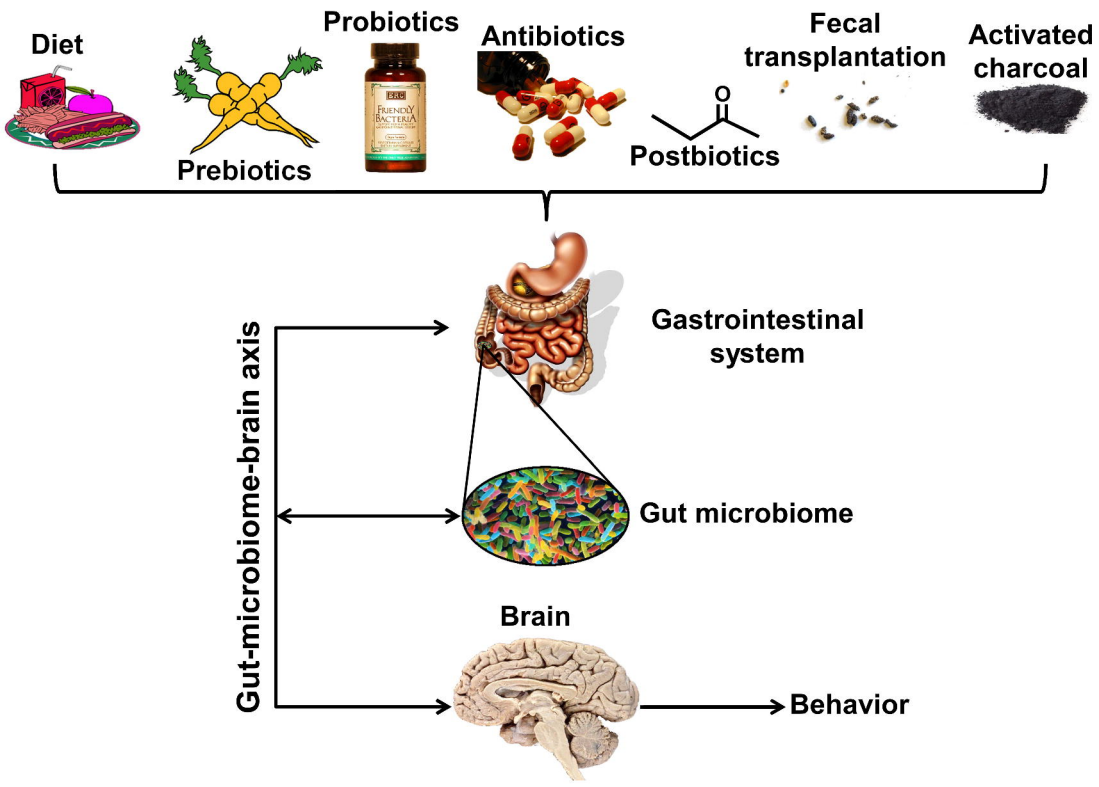
			• Reactive astrogliosis (neuroinflammation) occurred in the brains of PPA-treated rats.
(Shultz et al., 2014)	Seizure-prone (FAST) and Seizure-resistant (SLOW) male rats	• Nine week old male rats were assigned to one of four groups: 1. FAST + PPA (4μL, 0.26 M) 2. SLOW + PPA (*n* = 14) 3. FAST + PBS vehicle control (4μL) 4. SLOW + PBS	• PPA induced social abnormalities in FAST and SLOW rat strains. • PPA treatment resulted in neuroinflammation (astrogliosis) in the corpus callosum and cerebral cortex compared PBS treatment. • Neuroinflammatory effects were more prominent in FAST compared to SLOW rats.
(Shultz et al., 2009)	Male Rats	• Adult male rats were assigned to one of five treatment groups: 1. PPA-5 (4 μl of 0.26 M solution) 2. PPA-3 (4 μl of 0.26 M solution) 3. SA (4 μl of 0.26 M solution) 4. PROP (4 μl of 0.26 M solution) 5. PBS (4 μl)	• PPA treated rats showed impairments in spatial acquisition and reversal training in the water maze test and beam task • Neuroinflammation was increased in the brains of PPA-treated rats.
(de Theije et al., 2014)	Mice	• Pregnant P_0 mice were treated on GD 11 with valproic acid (600 mg/kg bw sc) or phosphate buffered saline (controls).	• F_1 valproic-acid treated offspring demonstrate social behavioral deficits. • The composition of SCFAs is changed in the F_1 treated offspring. • Gut dysbiosis with changes in the Bacteroidetes and Firmicutes phyla and Desulfovibrionales results in F_1 exposed offspring. • F_1 exposed male offspring had alterations in Alistipes, Enterohaabdus, Mollicutes, and Erysipelotrichalis genera. • Gut microbiome disturbances are more pronounced in F_1 males than female offspring.
(Hsiao et al., 2013)	Maternal Immune Activation (MIA) Mouse Model & Wild-Type (Naïve) Mice	• Pregnant P_0 mice were treated on GD 12.5 with saline (control) or the immunostimulant polyinosinic:polycytidylic acid (Poly I:C, 20 mg/kg bw via intraperitoneal-ip injection). This latter treatment results in MIA offspring. • MIA offspring were orally treated with *Bacteroides fragilis* (ATCC 9343) or vehicle every other day for 6 days at weaning. 1 X 10^{10} CFU of freshly grown *B. fragilis* or 1.5% sodium bicarbonate was administered in sugar-free applesauce. • Wild-type mice were treated with the bacterial metabolite (4-ethylphenylsulfate, 4-EPS, 30 mg/kg bw via ip injection from 3 to 6 weeks of age)	• MIA offspring demonstrated disruption of the intestinal barrier • ~8% of bacterial metabolites was altered in MIA offspring with a "leaky" gut. • MIA offspring exhibited communication deficits, stereotypic, anxiety-like, and sensorimotor behaviors. • Supplementation of MIA offspring with *B. fragilis* restored the intestinal barrier and mitigated the gut dysbiosis, metabolomics changes (including for 4-EPS), and behavioral changes. • Wild-type mice treated with 4-EPS exhibit anxiety-like behaviors observed in other ASD animal models.

Table 2: Human Epidemiological studies linking gut microbiota changes to ASD symptomology

Publication	Cohort Population	Type of Analysis and/or Treatment(s)	Major Findings
(Gondalia et al., 2012)	ASD children without gastrointestinal (GI) dysfunction (n= 23); ASD children with GI dysfunction (n= 28); Neurotypical siblings (n= 53)	• Bacterial tag-encoded FLX amplicon pyrosequencing was performed on the stool samples from all three groups.	• Firmicutes (70%), Bacteroidetes (20%), and Proteobacterias (4%) comprised the major microbiota present in the stool regardless of disease state and sociodemographic features. • No evidence was found linking gut disease, dysbiosis, and ASD symptoms.
(Song et al., 2004)	ASD children (n= 15); Non-related Controls (n= 8)	• Group and species-specific primers were designed to target the 16S rRNA genes forquantitative realtime PCR (qRT-PCR) analysis on the stool samples.	• C. bolteae and clusters I and XI were elevated in the stool of ASD children.
(Finegold et al., 2002)	ASD children (n= 13); Non-related Controls (n= 8)	• Bacterial cultures were performed on the stool samples.	• 9 species of Clostridium were present in ASD but not control children, where 3 unique species were identified. • Non-spore-forming anaerobes and microaerophilic bacteria were abudant in ASD but lacking in control children
(Parracho et al., 2005)	ASD children (n= 58); Control siblings (n= 12); Non-related controls (n= 10)	• Fluorescent in situ hybridization (FISH) analysis was performed on the stool samples	• *C. histolyticum* (Clostrium clusters I and II) is abundant in ASD children. • Non-autistic siblings possess intermediate levels of the intestinal microbe.
(Finegold et al., 2010)	ASD children (n= 33); Control siblings (n= 7); Non-related controls (n= 8)	• Bacterial tag encoded FLX amplicon pyrosequencing (bEFAP) procedure was used to analyze the stool samples.	• Phyla changes: Bacteroidetes and Protebacteria were increased in ASD children; whereas Firmcutes and Actinobacteria were less abundant in the stool of this group. • Genus changes: *Delsufovibrio*, *Bacteroides*, *Alkaliflexus*, *Acetanaerobacterium*, and *Parabacteroides* were elevated in ASD children; whereas, *Clostridum*, *Weissella*, *Turicibacter*, *Anaerofilum*, *Pseudoramibacter*, *Ruminococcus*, and *Streptococcus* were decreased in this group.
(Tomova et al., 2015)	ASD children (n= 10); Control siblings (n= 9); Non-related controls (n= 10)	• qRT-PCR analysis was performed on the stool samples. • Probiotic supplementation of one "Children Dophilus" capsule, which contains three strains of *Lactobacillus* (60%), 2 strains of *Bifidumbacteria* (25%), and one strain of *Streptococcus* (15%) was provided orally to the ASD group for 3X a day for 4 months	• ASD children showed significant reduction in the Bacteroidetes/Firmcutes ratio but increased amount of *Lactobacillus* spp. • *Delsulfovibrio* spp showed a trend to be increased in this group, especially with increasing autistic severity. • Clinical severity of GI symptoms was positively correlated with autism severity. • Probiotic supplementation of the autistic children corrected the imbalanced Bacteroidetes/Firmicutes ratio, suppressed *Delsufovibrio* spp, and increased the amount of *Bifidobacterium* spp present in the stool.
(Williams et al., 2011)	ASD children with GI dysfunction (n= 15); Non-related controls with GI symptoms only (n= 7)	• qRT-PCR with human mRNA samples for SI, MGAM, LCT, SGLT1, GLUT2, Vilin, and CDX2. • Pyrosequencing of intestinal microbiota. • qRT-PCR of Bacteroidete and Firmicute 16s rRNA genes from intestinal biopsies.	• ASD children exhibit decreased expression of disaccharidases, hexose transporters, and the transcription factor CDX2. • The host transcriptomic changes correlated with the degree of gut dysbiosis observed in this ASD child cohort. • ASD children showed decrease amounts of *Bacteroidetes* and ratio of *Bacteroidetes* to Firmcutes, and greater preponderance of *Betaproteobacteria* in the intestinal biopsy samples
(Wang et al., 2013)	ASD children (n=	• qPCR on the stool samples.	• ASD children with and without GI disorders showed high amounts of fecal

	23); Control siblings (n= 22); Non-related controls (n= 9)		*Sutterella* spp. • Ruminococcus torques was elevated in the stool of children with ASD and GI symptoms compared to those without any such disorders.
(De Angelis et al., 2013)	ASD children (n= 10); Non-related controls (n= 10)	• (bEFAP) procedure was used to analyze the stool samples. • Measurement of free amino acid and volatile organic compounds in the stool samples.	• Phylum changes exist in Bacteroidetes, Firmicutes, Fusbobacteria, and Verrucomicrobia in ASD compared to health children. • Caloramator, *Sarcina*, and Clostridium genera are greater in ASD children. • Variations within the Lachnospracease family were observed. • ASD children possessed high amounts of fecal Bacteroidetes genera, select *Alistipes* and *Akkermansia* species, but Sutterellaceae. • Enterobacteriaceae, Eubacteriaceae and *Bifidobacterium* species were reduced in this group. • Levels of free amino acids and volatile organic compounds within the stool were affected in this group
(Wang et al., 2011)	ASD children (n= 23); Control siblings (n= 22); Non-related controls (n= 9)	• qPCR on the stool samples	• Bifidobacteria species was reduced and mucolytic bacterium, *Akkermansia muciniphilia* was increased in the stool of ASD children.
(Williams et al., 2012)	ASD children with GI dysfunction (n= 15); Non-related controls with GI symptoms only (n= 7)	• Pyrosequencing and qPCR of ileal and cecal biopsies	• *Sutterella* spp. (*wadsworthensis* and *stercoicanis*) predominated in the gut microbiota of ASD children with concurrent GI dysfunction, but these species were absent in children with GI symptoms only
(Kang et al., 2013)	ASD children (n= 20); Non-related controls (n= 20)	• Pyrosequencing of the stool samples.	• Presence of ASD rather than GI symptoms may be a better predictor of a less diverse gut microbiota composition. • The genera *Prevotella*, *Coprococcus*, and unclassified *Veillonellaceae* were reduced in the stool of ASD children.
(Adams et al., 2011)	ASD children (not on probiotic supplement and those taking a daily probiotic, n= 58); Non-related controls (n= 39)	• Bacterial culture of the stool samples. • Concentrations of Lysozyme, lactoferrin, secretory IgA, elastase, short chain fatty acids (SCFAs) were measured in the stool.	• There was a positive correlation with GI symptoms and ASD clinical severity. • Decreased number of SCFAs, specifically acetate, propionate, and valerate, were identified in ASD children, especially those consuming a daily probiotic. • The stool of ASD children contained less *Bifidobacter* but greater amounts of *Lactobacillus*. • Lysozyme was suppressed in ASD children.
(Sandler et al., 2000)	Regressive-onset autistic children (n= 11)	• The children received a 12-week treatment of oral vancomycin (125 mg 4 times a day for a daily dose of 500 mg). • Once antibiotic treatment was discontinued, the children received probiotic supplementation with a mixture of *L. acidophilus*, *L. bulgaricus*, and *B. bifidum* (40 X 10^9 colony forming units-CFU/mL) • Psychological evaluations were performed before and after the antibiotic treatment.	• The vancomycin treatment improved the behavioral scores of ASD children, but the beneficial effects were transient with the behavioral symptoms recurring upon discontinuation of the antibiotic
(Wang et al., 2012)	ASD children (n= 23); Non-related controls (n= 31)	• Concentrations of SCFAs, phenols, and ammonia were measured in the stool samples.	• Fecal SCFAs were significantly higher in ASD children. • Acetic, butyric, isobutryic, valeric, and isovaleric acids were elevated in the stool of ASD children; whereas, caproic acid was reduced. • The ASD group had greater ammonia concentration in the stool.

(Yap et al., 2010)	ASD children (n= 39 with 35 males and 4 females); Control siblings (n= 28 with 14 males and 14 females); Non-related controls (n= 34 with 17 males and 17 females)	• H NMR spectroscopy and pattern recognition methods were used to measure the concentration of free amino acids and bacterial metabolites in the urine.	• The free amino acids glutamate and taurine are elevated in the urine of ASD children • Disturbances in the patterns of bacterial metabolites dimethylamine, hippurate, and phenylacetylglutamine were also observed in this group.
(Kaluzna-Czaplinska and Blaszczyk, 2012)	ASD children with GI dysfunction (n= 22)	• Concentrations of D-arabinitol (DA), LA, and the ratio of DA/LA in the urine were determined by capillary gas chromatography/mass spectrometry before and after probiotic therapy. • The children were provided twice daily for 2 months an oral probiotic capsule containing *L. acidophilus* (strain Rosell-11, containing 5 X 10^9 CFU/gram)	• The concentration of D-arabinitol (DA) in the urine was higher in ASD children compared to controls before and after probiotic supplementation. • The probiotic therapy appeared to partially mitigate the elevated urinary concentrations of DA and ratio of DA/LA as there was a noticeable improvement in the behaviors of ASD children, particularly in concentration and carrying out orders


Diet
Prebiotics
Probiotics
FRIENDLY BACTERIA
Antibiotics
O
Postbiotics
Fecal transplantation
Activated charcoal
Gut-microbiome-brain axis
Gastrointestinal system
Gut microbiome
Brain
Behavior