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Gut Microbiota and Autism: Key Concepts and Findings

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Abstract There is an emerging body of evidence linking the intestinal microbiota with autism spectrum disorders (ASD). Studies have demonstrated differences in the composition of gut bacteria between children with ASD and controls. Certain intestinal bacteria have been observed in abundance and may be involved in the pathogenesis of ASD; including members of the *Clostridium* and *Sutterella* genus. Evidence from animal models suggest that certain microbial shifts in the gut may produce changes consistent with the clinical picture of autism, with proposed mechanisms including toxin production, aberrations in fermentation processes/products, and immunological and metabolic abnormalities. In this article, we review studies examining the relationship between intestinal bacteria and ASD, and discuss bacterial species that may be implicated and proposed mechanisms.

Keywords Autism · Autism spectrum disorder (ASD) · Regressive autism · Gastrointestinal symptoms · Microbiota · Microbiome

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Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impaired communication and social interactions and restricted interests and behaviors. Based on recent estimates, 1 in 68 children currently live with ASD in the United States (Centers for Disease Control and Prevention 2014). Families of these children face numerous significant emotional, social, and financial burdens. The causes of ASD are largely unknown, except in the rare instance when identifiable genetic abnormalities give rise to a syndrome with ASD features, such as seen in Fragile X. While there is likely a strong multi-genetic basis to ASD (DiCicco-Bloom et al. 2006), emerging research points to the important contributions of non-genetic factors and a conceptualization of ASD as having multiple underlying causes given the great clinical heterogeneity and variable symptomatic course (Arndt et al. 2005). Thus, given this heterogeneity, research into the etiology of ASD is increasingly being focused on subgroups of ASD individuals who share a defining group of characteristics, such as children with regressive autism and gastrointestinal (GI) symptoms.

Many studies in the last several years reveal the central role of the gut microbiota in the post-natal development and maturation of the immune and endocrine systems, with disruption of this important balance associated with many disease states occurring throughout host life. Dysbiosis, the state of unbalanced and disrupted microbial communities, has been implicated in the etiology and pathogenesis of a wide range of medical conditions, including autoimmune disorders such as rheumatoid arthritis, type I diabetes, and inflammatory bowel disease; allergies such as asthma and eczema; and other illnesses such as necrotizing enterocolitis in infants, systemic infections following

cancer chemotherapy, dental caries, and obesity (Hooper et al. 2012; Penders et al. 2007; Taur et al. 2012). In each of these disease states, the structure and composition of the hosts' microbiota has been shown to impact each of these clinical entities. This exciting new field continues to link gut microbes with disease states, and those in psychiatry are no exception. There is now increasing evidence of a brain–gut–microbe connection, with dysbiosis linked to depression, anxiety, and most compellingly, with autism. In this manuscript we will review the emerging data of links between gut microbiota and ASD.

The Human Microbiota

The human body serves as the natural ecosystem for a large number of bacteria and other microorganisms. The entirety of these microorganisms has been termed the human microbiota; the entirety of genes from this collective has been termed the human microbiome. Studies in the last decade examining the human microbiota have shown that an immense population of bacteria exists within the human body, with diversity and uniqueness far greater than previously conceived (Eckburg et al. 2005).

Study of the human microbiota has been made possible through significant advances in molecular approaches which allow researchers to study microbial populations in great detail. For instance, DNA sequence data can now be obtained from entire microbial communities simultaneously in a single reaction using next generation sequencing (NGS) technologies, which is the term describing the collective of approaches used to obtain sequence data in parallel fashion, at a fraction of the cost compared with conventional sequencing methods. Based on current estimates, the approximate cost of sequencing a million bases of DNA has decreased from approximately $2500 in 2003 to a mere $0.06 in 2013, 10 years later (Wetterstrand 2013). Analyzing stool samples, a non-invasive route of collection, is the typical route by which information regarding the composition of the distal gut microbiome is obtained. Prior to this, characterization of the microbiota typically relied primarily on culture-based methods, which provided a biased view of only a very small subset of microorganisms comprising the microbiota. Another important advance is in metabolomics, in which the complete set of small molecule metabolites in a particular environment are quantitatively measured. Metabolomic profiles can be measured using platforms based in mass spectrometry. In the human intestinal tract, metabolites are closely linked to the microbiota, and quantitative assessments of these signatures have added to existing understanding of the microbiota. Because of these breakthroughs, the importance of the human microbiota is only beginning to be realized at this time.

Studies have shown that under normal circumstances, the intestinal microbiota is largely comprised of anaerobic bacteria that are essentially non-pathogenic and serve a variety of functions that are beneficial to the human host, such as absorption of nutrients, production of short-chain fatty acids and vitamins, amino acid synthesis from ammonia or urea, detoxification of xenobiotics, stimulation and development of host immunity, prevention of overgrowth or infection with pathogenic bacteria, and maintenance of colonic wall health (Hooper et al. 2012; Sekirov et al. 2010). Some of these commensal organisms live exclusively in the human intestinal tract and cannot be found in any other ecologic environment, suggesting a deep and long-lived relationship with their human hosts, with the full clinical implications yet to be discovered and realized.

Evidence has shown the importance of the evolution of GI microbiota composition over the first few years of life. At birth, the human gut is initially sterile; microbial colonization begins immediately afterwards, and is influenced by the route of delivery, maternal transfer, diet, environmental factors, and antibiotic usage (Sekirov et al. 2010). Over the course of the first year of life, an idiosyncratic intestinal microbiome is stabilized within each infant. In adulthood, eubiosis, the state of healthy and balanced gut communities, is marked by the predominance of the phyla Firmicutes and Bacteroidetes, followed by Actinobacteria and Proteobacteria (Human Microbiome Project 2012).

The Relationship Between Microbiome, Gut, and Brain

There is evidence that close connections exist between the microbiome, gut, and brain, and that cross-communication occurs regularly. For instance, the CNS exerts control over the gut microbiome composition through peptides which are sent upon satiation and thus affect nutrient availability. In another example, the hypothalamic–pituitary–adrenal (HPA) axis releases cortisol, which regulates intestinal motility and integrity. Immune and neural pathways regulate secretion of mucin from intestinal epithelial cells, which is known to exert control over microbial populations within the gut (Wang and Kasper 2014). In the other direction, the gut microbiota has been shown to control CNS activities through a variety of neural, endocrine, immune, and metabolic mechanisms (Wang and Kasper 2014).

Preclinical studies support the premise of a brain–gut–microbiome connection. Psychological stress has been shown to induce changes in gut microbial composition, using an animal model of early life stress induced by maternal separation (O'Mahony et al. 2009, 2011). Intestinal infection with *Campylobacter jejuni* was shown to elevate anxiety behaviors in mice; a bacterial signal carried by

vagal sensory neurons was hypothesized to be the mechanism (Goehler et al. 2008).

ASD and Gastrointestinal (GI) Symptoms

The hypothesized connection between ASD and the microbiota likely stemmed in part from frequent observation of GI symptomatology. A marked proportion of children with ASD suffer from gastrointestinal (GI) symptoms that can be similar to those of irritable bowel syndrome (IBS) (Buie et al. 2010). Symptoms include diarrhea, constipation, vomiting, reflux, abdominal pain/discomfort, flatus, and unusually foul-smelling stools (Nikolov et al. 2009). Clinical studies have reported abnormalities such as altered GI motility and increased intestinal permeability (de Magistris et al. 2010). A recent multicenter study of over 14,000 individuals with ASD revealed a higher prevalence of inflammatory bowel disease (IBD) and other GI disorders in patients with ASD as compared to controls (Kohane et al. 2012). The precise prevalence of GI symptoms in children with autism is not clear, with prior estimates ranging widely, from 9 to 70% (Buie et al. 2010). These variations may be due to differences in study populations and definition of GI symptomatology. What is not in dispute is that GI symptoms are a significant problem in autism and may be important contributors to behavioral difficulties (Buie et al. 2010).

Individuals with ASD who have GI symptoms may display significantly higher measures of irritability, anxiety, and social withdrawal when compared to those without GI symptoms (Nikolov et al. 2009). Problem behavior such as self-injury and aggression, as well as sleep disturbances and irritability may be behavioral manifestations of abdominal pain or discomfort in persons with ASD (Buie et al. 2010). Studies have also shown a strong correlation between GI dysfunction and autism severity, across all domains including speech, social, and behavioral (Adams et al. 2011). Although this finding does not define a cause-effect relationship, it nonetheless underscores the importance of alleviating GI symptoms in children with autism. While GI symptomatology could be viewed as nothing more than a frequently occurring exacerbating factor facing ASD patients, it could also be that these symptoms point to a much more fundamental relationship: that the gut plays a pivotal role in the etiology of ASD in a subset of patients.

Hypothesized Role of *Clostridium*

Given the observation that the onset of neurobehavioral symptoms and chronic diarrhea appeared to occur after repeated courses of antibiotics in a subset of children with the regressive form of ASD, a species of toxin-producing *Clostridium* was proposed as a possible cause. In regressive autism, a period of normal or near normal early development is followed by a cessation of all further development and a loss of previously acquired communication and/or social skills, which usually occurs between 15 and 30 months of age (Barger et al. 2013). Once development has regressed, these children then generally follow the typical ASD developmental trajectory (Stefanatos 2008). This hypothesis was formally proposed by Ellen Bolte, the mother of a child with regressive autism (Bolte 1998). She and other parents of children with regressive-onset autism anecdotally observed onset of neurobehavioral changes following a pattern of repeated courses of antibiotics (often given for chronic otitis media) with subsequent chronic diarrhea.

The rationale for the *Clostridium* hypothesis is based on similarities with the known behavior of other *Clostridium* species that cause toxin-mediated disease. Clostridia represent a very heterogeneous group of anaerobic, spore-forming, largely gram-positive bacilli; many of these bacteria are felt to be beneficial members of a healthy intestinal microbiota, and are completely nonpathogenic. However, certain *Clostridium* species, such as *Clostridium tetani* and *Clostridium perfringens*, can produce potent toxins that are known to cause a variety of human diseases. In some instances, the trigger for toxin release can be related to disruption of the intestinal microbiota. *Clostridium difficile* is known to cause a toxin-mediated diarrheal illness that typically follows antibiotic administration. In botulism, the neuroparalytic illness caused by toxin produced by *Clostridium botulinum*, some disease forms are thought to be triggered by disturbances of the intestinal microbiota such as antibiotics (specifically, adult intestinal toxemia). In infant botulism, neonates are thought to be susceptible due to an immature intestinal microbiota.

The *Clostridium* hypothesis was further supported by the work of Sandler and Finegold (Sandler et al. 2000) in which children with regressive autism were treated with a 6-week course of oral vancomycin, an antibiotic with known activity against clostridia. Significant improvement in neurobehavioral symptoms (with some even scoring within neuro-typical range) was observed in eight of the ten children studied as well as improvement in gastrointestinal symptoms, providing evidence in favor of a toxin-producing *Clostridium* as a potential cause of regressive autism. A gradual regression in bowel and behavioral symptoms occurred in all subjects following the discontinuation of vancomycin. This relapse could be explained by the fact that many clostridia can convert into spore-form, which are highly resistant to antibiotics, but can then later germinate into vegetative, infective forms and resume toxin production. Moreover, the fact that oral vancomycin's

effects are confined to the intestinal tract and not systemically absorbed provides further support for involvement of the intestinal microbiota. Interestingly, minocycline, an antibiotic that readily crosses the blood–brain barrier, was administered to children with ASD for its potential direct neuro-protective effects, but no significant benefits were observed (Pardo et al. 2013). However, a main limitation of the vancomycin study was the design as it was an open label study with non-blind assessments and their results have yet to be replicated in any subsequent controlled trials.

Subsequent studies continue to support the *Clostridium* hypothesis, although the exact species responsible has not been fully elucidated (Fig. 1). A ten-fold higher level of cultured *Clostridium* species in the feces of children with ASD with GI symptoms compared with healthy controls, as well as a notable lack of anaerobic bacteria has been observed (Finegold et al. 2002). One clostridial species observed in high abundance was classified as *C. clostridioforme* but then later found to be a distinct novel species, and was renamed *C. bolteae*, in honor of Ellen Bolte (Song et al. 2003). *C. bolteae* was again observed in high comparative abundance in ASD patients, in another study by the same group (Song et al. 2004). These investigators also found greater abundances in clostridial clusters I and XI (which include toxin-producing clostridia such as *C. perfringens* and *C. difficile*) with a decrease in clusters

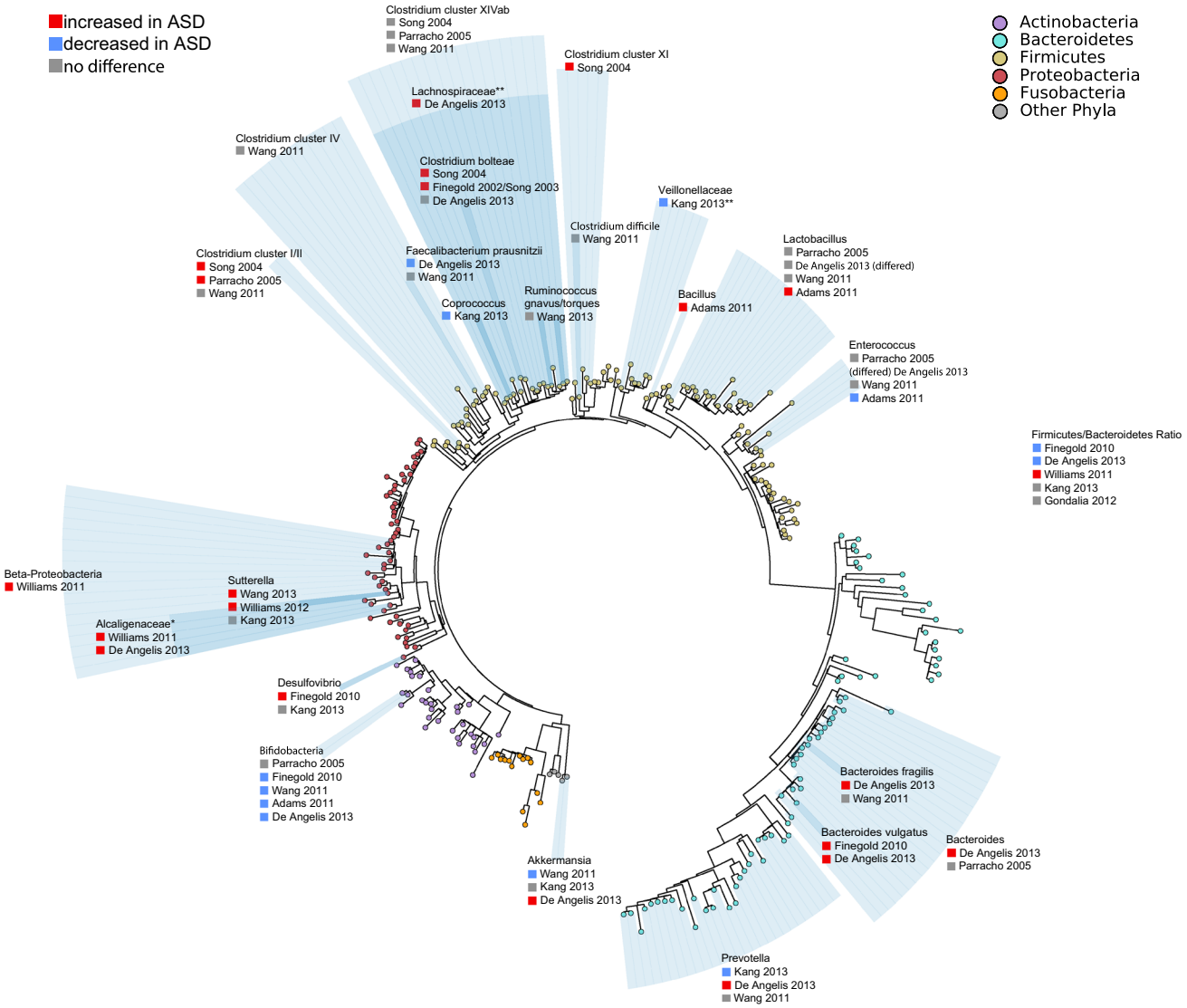

Fig. 1 Overview of selected microbiome studies of ASD. Results of studies are shown on a phylogenetic tree. Tree tips (*circles*) represent bacterial species known to colonize humans; color denotes phylum-level classification. Bacterial taxa (*blue shaded areas*) studied are shown, at various levels of classification. Study findings (*squares*) are listed for each bacterial taxon; *color* denotes direction of change observed in ASD. Studies vary considerably by design, sample size, molecular approach, and analysis. *Sutterellaceae is a new family originally from Alicaligenaceae. **Correlation was with unclassified Veillonellaceae

XIVab (thought to consist of beneficial clostridial species). Another study by Parracho et al. (2005) identified higher levels of *Clostridium histolyticum* group, which are known producers of toxin, in patients with ASD compared with healthy controls. Moreover, this study included children with ASD with and without GI symptoms and found that within the ASD group, high level of *Clostridium* species were significantly associated with GI problems.

Differences in the Prevalence of *Sutterella* and Other Species

Sutterella, a genus of anaerobic gram negative bacteria within the Proteobacteria phylum, has also been hypothesized to play a role in ASD. A significantly higher prevalence of *Sutterella* species in biopsies taken from the GI tract of ASD children with GI disturbances compared to controls with GI disturbances has been found (Williams et al. 2012) (Fig. 1). These findings suggest that *Sutterella* is a major component of the intestinal microbiota in over half of the children with autism and GI dysfunction and is absent in children with only GI dysfunction. Wang et al. (2013) also demonstrated elevated numbers of *Sutterella*, as well as *Ruminococcus torques*, in the feces of children with ASD as compared to community controls. (Kang et al. 2013) found reduced abundance of *Prevotella* (a member of the Bacteroidetes phylum) in the intestinal microbiota of children with ASD. Since *Prevotella* and other Bacteroidetes are typically thought to be associated with good colonic health, the manifestations of ASD may originate in a compositional shift from healthy microbes to increases in *Sutterella*, which can be found in lesser abundance in healthy humans but may exist as a potential pathogen under certain circumstances. Unlike *Clostridia*, no mechanism for ASD has been put forth. However, *Sutterella* does exist within the Betaproteobacteria class, making it closely related to other known pathogens such as *Neisseria gonorrhoeae* and *meningitidis, Bordetella pertussis*, and *Burkholderia cepacia*. The organisms are closely related genetically but can cause a wide variety of illnesses.

Other microbes observed in high compositional abundance in ASD children include: *Akkermansia muciniphilia*, (De Angelis et al. 2013; Kang et al. 2013) *Desulfovibrio*, (Finegold et al. 2010) and *Faecalibacterium prausnitzii* (De Angelis et al. 2013) (Fig. 1). However, repeat studies examining these organisms did not show significant differences (Kang et al. 2013; Wang et al. 2011). Moreover, the biologic plausibility of these organisms as pathogens with potential role in neurodevelopmental disease is not obvious; for example, *Akkermansia muciniphilia* and *Faecalibacterium prausnitzii* are both thought to be biomarkers of healthy gut flora. At the higher level of phylum, microbiota studies have noted overall compositional shifts between Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria, but also have not been particularly reproducible or mechanistically meaningful (De Angelis et al. 2013; Finegold et al. 2010; Kang et al. 2013; Williams et al. 2011). These conflicting results may underscore the complexity of community relationships within the microbiota, but also may highlight the current challenges of microbiota data analysis, where the risk of false positive discovery can be difficult to avoid. In general, microbiome studies currently vary greatly in terms of their analytic approach to high dimension data, and there is no single consensus as to what exactly constitutes a clinically meaningful microbial shift, whether it be in terms of which metric to use or what magnitude of change to consider significant. Supporting studies with adjunctive approaches such as metabolomics may serve to support a causal pathway that is biologically plausible to verify if these organisms truly play a mechanistic role in the development of ASD.

Proposed Mechanisms

There have been a variety of proposed mechanisms, including dysbiosis-induced breakdown in gut integrity, production of toxins, aberrations in fermentation processes or products, and immunological and metabolic abnormalities.

Factors Influencing Dysbiosis

As discussed above with the *Clostridium* hypothesis, the use of antibiotics has been proposed as a mechanism by which the normal flora is disrupted, leading to deleterious effects. Another proposed mechanism for dysbiosis is that deficient disaccharidase and hexose transporter leads to an altered carbohydrate milieu in the distal cecum/ileum, which then leads to dysbiosis by providing an additional grown substrate for bacteria (Williams et al. 2011).

Gut Permeability

The epithelial gut barrier is maintained by tight junctions that control the flow of molecules between the GI tract and bloodstream (Hollander 1999). Microbiota and their ligands are crucial in maintaining the cell–cell junctions critical to barrier integrity, with GI barrier defects seen with dysbiosis (Hsiao et al. 2013). Compromised integrity of the epithelial barrier has been termed "leaky gut" and has been linked to a wide range of intestinal and systemic disorders (Fasano 2012). Increased intestinal permeability may allow for the passage of bacteria, toxins, and metabolites and can lead to immune activation.

Immune Activation/Dysfunction

One consequence of increased intestinal permeability is the increase in circulating bacteria-derived lipopolysaccharide (LPS) which triggers both an immunological and inflammatory response characterized by increased systemic pro-inflammatory cytokines (Qin et al. 2007). Indeed, the possible role for immune dysfunction in ASD has been described for many years, and numerous immune abnormalities have been reported in ASD. Cytokines are necessary for normal neurodevelopment, and perturbations can impact this process. Increased levels of cytokines have been reported in children with autism, especially in those with the regressive subtype of the disorder (Ashwood et al. 2011). In ASD, increased plasma level of pro-inflammatory cytokines such as IL-1B, IL-6, IL-8, and IL-12p40 as well as macrophage migration inhibitory factor (MIF) and platelet derived growth factor (PDGF) have been found, with elevated levels of these cytokines in the plasma associated with poor communication and impaired social communication (Onore et al. 2012). Moreover, changes in peripheral immune profiles, notably the levels of TGF beta, p-selectin, and MIF, have been associated with the severity of stereotypy, hyperactivity and deficits in communication (Ashwood et al. 2011).

A key finding has been that of marked neuroinflammation in postmortem brain specimens from individuals with ASD (Li et al. 2009; Morgan et al. 2010). In a rigorously conducted study that analyzed autopsy and cerebrospinal fluid (CSF) samples of individuals with ASD, a neuroinflammatory response involving excess microglial activation and increased proinflammatory cytokine profiles as compared to non-ASD controls was found (Vargas et al. 2005). Microglia are the phagocytic cells of the CNS and play important roles not only in immune surveillance of the CNS, but also in the synaptogenesis and developmental apoptosis seen in normal CNS development (Bessis et al. 2007; Paolicelli et al. 2011). Furthermore, emerging research points to the role of microglia deficits and neurodevelopmental disorders, with a reduction of microglia during early postnatal period associated with weak synaptic transmission, decreased functional brain connectivity, deficits in social interactions, and increased repetitive behavior phenotypes in a mouse model (Zhan et al. 2014).

Bacterial-Derived Toxin, e.g. Phenols

Another proposed mechanism is that an increase in circulating bacterial-derived toxins, with dissemination possibly ameliorated by increased intestinal permeability, leads to neurotoxic effects. Phenol compounds have been the subject of recent interest, and of note, some phenols have been found to be related to specific bacteria (e.g. *C. difficile, F. prausnitzii, Bifidobacterium*) (Nicholson et al. 2012) and are postulated to have neurotoxic effects (Finegold et al. 2002). Polyphenols are metabolized via the gut microbiota into simpler phenol compounds. These phenol compounds are then further transformed in the liver and released in the urine (van Duynhoven et al. 2011). Overall, phenol compounds [e.g., phenol, 4-(1,1-dimethylethyl)-phenol, *para*-cresol] are increased in the feces of children with Pervasive developmental disorder not otherwise specified (PDD-NOS) and, especially, ASD (De Angelis et al. 2013). Urinary *para*-cresol (*p*-cresol) and its conjugated derivative *p*-cresylsulfate have also been found to be elevated in a sample of autistic children under 8 years of age (Gabriele et al. 2014; Persico and Napolioni 2013).

Environmental exposure to *p*-cresol and subsequent absorption through the skin and the gastrointestinal and respiratory systems is common, but the most widespread source of this compound is through gut bacteria which express *p*-cresol synthesizing enzymes not found in human cells (Persico and Napolioni 2013). *p*-Cresol is one of the metabolites of the amino acids tyrosine and phenylalanine (Vanholder et al. 1999). In particular, *C. difficile* (Selmer and Andrei 2001), as well as certain strains of *Lactobacillus* (Yokoyama and Carlson 1981), are able to push the fermentation of tyrosine up to the formation of *p*-cresol through the expression of *p*-hydroxyphenylacetate decarboxylase. *p*-Cresol is postulated to worsen autism severity and gut dysfunction, with gut infection, antibiotics, and abnormal intestinal permeability included as potential sources of *p*-cresol excess in ASD (Persico and Napolioni 2013).

Metabolomic Aberrations

The metabolome, which is the complete set of chemical compounds involved in an organism's metabolism, is regulated by the types of substrate entering the colon as well as by the composition of the microbiota. The metabolites produced by intestinal microbes play a crucial role in health and disease. One proposed mechanism is that fermentation processes or utilization of fermentation products may be altered in children with ASD as compared to children without ASD, thereby impacting neurodevelopment and behavior.

Propionic Acid (PPA) and Other Short Chain Fatty Acids (SCFAs)

Dietary carbohydrates, specifically resistant starches and dietary fiber, are substrates for fermentation that produce SCFAs as end products, with the rate and amount of SCFA production dependent on the composition of the microbiota, the substrate source, and gut transit time (Wong et al.

2006). ASD-associated bacteria (Clostridia, Bacteroidetes, *Desulfovibrio*) play an important role in the production of PPA and its related SCFAs, which after readily crossing the gut–blood and blood–brain barriers, can then induce widespread effects on gut, brain, and behavior (Macfabe 2012). They impact CNS function via changes in neurotransmitter synthesis and release, mitochondrial function, immune activation, lipid metabolism, and gene expression (Shultz et al. 2009). Excessive SCFAs may have negative effects, with conditions resulting from elevations in PPA and SCFAs, such as propionic and methylmalonic acidemia, collectively marked by developmental delay and regression, seizures, metabolic acidosis, and gastrointestinal symptoms, with symptom severity associated with PPA levels (Macfabe 2012).

In animal models, intraventricular injection of PPA has been shown to impair social behavior and cognition, induce abnormal behaviors, restrict interests, as well as induce a neuroinflammatory response (Shultz et al. 2009, 2008; Thomas et al. 2012). In human subjects, elevated levels of fecal SCFA and ammonia (a protein fermentation by-product that can be adverse to host health) have been found in children with ASD (Wang et al. 2012).

Dysregulated Metabolism of Free Amino Acids (FAA)

Dysregulated metabolism of free amino acids (FAA) has been observed in children with autism and pervasive developmental disorder-not otherwise specified (PDD-NOS), with fecal samples from children with ASD containing the highest levels of total free amino acids (De Angelis et al. 2013). Some FAA, in particular glutamate, act as neurotransmitters and impact the central nervous system. Glutamate has been implicated in the pathophysiology of some neuropsychiatric disorders, with glutamate excess found to lead to neuronal cell death (Sheldon and Robinson 2007). Glutamatergic neurotransmission has thus been hypothesized to be involved in ASD given the major role of glutamate in brain development (Shimmura et al. 2011). Conversely, decreased levels of glycine, serine, threonine, alanine, histidine, and the glutamyl amino acids have also been found in ASD children which also points towards abnormal amino acid metabolism (Ming et al. 2012).

Other Metabolomic Aberrations

Metabolomic studies of urine from individuals with ASD have identified molecules associated with the microbiome such as dimethylamine, hippuric acid and phenylacetylglutamine (Yap et al. 2010). Decreased plasma levels of *p*-hydroxyphenylacetate, a metabolite associated with Bifidobacteria and lactobacilli, which is known to serve as an antioxidant has also been observed in children with ASD (West et al. 2014). Levels of the organic acid taurine as well as the levels of antioxidants such as carnosine have been found to be significantly lower in ASD children which further points towards metabolomics abnormalities as well as increased oxidative stress (Ming et al. 2012).

Microbiome Reconstitution as a Potential Therapy

In a notable study, Hsiao et al. (2013) demonstrated that microbial shifts within the gut of a mouse resulted in changes in serum metabolites and subsequent onset of autism-like behaviors. Moreover, GI barrier defects were seen with dysbiosis, further underscoring the crucial role of microbiota and their ligands in maintaining the cell–cell junctions critical to barrier integrity. Administration of *Bacteroides fragilis*, a beneficial bacterium, reversed the physiological, neurological, metabolic, and immunological abnormalities, such as ameliorating abnormal communicative, stereotyped, sensorimotor, and anxiety behaviors, as well as correcting intestinal permeability defects, and cytokine alterations. These findings support a microbiome–gut–brain connection in ASD and suggest that reconstitution of beneficial microbes, whether it be through probiotics, bacteriotherapy with defined consortia, or fecal microbiota transplantation, may serve to improve symptoms of ASD by repairing an aberrant microbiota.

At the time of writing, there are little to no completed clinical studies that have examined the benefits of microbiome reconstitution in ASD. A similar situation exists for other psychiatric conditions like depression and anxiety in which there is limited evidence supporting the use of probiotics (Pirbaglou et al. 2016). Of note, there are several ongoing clinical trials that are currently investigating microbial replenishment strategies for ASD, including probiotics (Santocchi et al. 2016; The University of Texas Health Science Center and Health 2016), prebiotics (University of California 2014), and fecal microbiota transplantation (University, Arizona, Clinical, and Institute 2014).

Conclusion

There is an increasing body of evidence demonstrating the clinical importance of microbes habituating the intestinal tract; compelling links between dysbiosis and many disease states are being formed. Within neurobehavioral disorders, it seems that at least a subset of the cases comprising ASD are connected to, and perhaps dependent on, the health and well-being of the intestinal microbiota. However, it remains to be determined whether dysbiosis is secondary to altered neural regulation of key gut functions or if it signifies primary aberrations that impact brain development

and function. Examination must also be made into possible confounding factors, for example children with autism may have diets that differ from children without autism, with ingestion of a high fat diet shown to lead to obesity-associated changes in gut microbiota. Thus, further studies will be needed in order to come to a consensus as to the exact nature of this relationship between gut microbiota and autism. Moreover, the mechanisms involved in the pathophysiology has not yet been elucidated, and thus studies investigating mechanisms, including metabolomics to further examine the role of neuroactive metabolites, are crucial. As more insight is gained, novel approaches to therapeutic management may become available and will potentially help to alleviate the burden of this disorder.

Author Contributions HD was the primary writer. YT and JW participated in the drafting and editing of the manuscript. The final version of the manuscript was approved by all authors.

Compliance with Ethical Standards

Conflict of interest Dr. Ding declares that she has no conflict of interest. Dr. Taur declares that he has no conflict of interest. Dr. Walkup has received free drug/placebo from the following pharmaceutical companies for National Institute of Mental Health funded studies Eli Lilly (2003), Pfizer (2007), Abbott (2005). Dr. Walkup was paid for a one time consultation with Shire (2011). Dr. Walkup is a paid speaker for the Tourette Syndrome-Center for Disease Control and Prevention outreach educational programs; American Academy of Child and Adolescent Psychiatry, American Psychiatric Association. Dr. Walkup receives royalties for books on Tourette syndrome from Guilford Press and Oxford Press. Dr. Walkup receives grant funding from the Hartwell Foundation and the Tourette Syndrome Association, and is an unpaid advisor to Anxiety Disorders Association of America, Consumer Reports, and Trichotillomania Learning Center.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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