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The microbiome of autism spectrum disorder: the implications on co-morbid symptomatology and the role in therapeutics

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Thesis

THE MICROBIOME OF AUTISM SPECTRUM DISORDER: THE IMPLICATIONS ON CO-MORBID SYMPTOMATOLOGY AND THE ROLE IN THERAPEUTICS

by

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Fairy tales are more than true: not because they tell us that dragons exist, but because they tell us dragons can be beaten.
–C.K. Chesterton

DEDICATION

I would like to dedicate this work to my family who have pushed me to be the woman I am today; especially my mother who introduced me to science and showed me there are no limits to what can be achieved. I, also, want to thank my brother for being the inspiration of this thesis and having the beautiful mind that he has, while being absolutely his authentic, true self.

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I want to think my thesis readers for their time and effort to help make this thesis what it is and for making an impact as women in the field of medicine.

THE MICROBIOME OF AUTISM SPECTRUM DISORDER: THE IMPLICATIONS ON CO-MORBID SYMPTOMATOLOGY AND THE ROLE IN THERAPEUTICS

LAURYN BRASCH

ABSTRACT

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by impairment in social communication and repetitive behavior. The etiology of ASD is believed to be multifactorial, yet much about causation remains unknown. There is a growing interest in the role of the intestinal microbiome, how it may differ in the ASD population and how the intestinal microbiota may impact central nervous system (CNS) function. This process involves bidirectional communication between the gut and the CNS and is referred to as the gut-brain axis or the microbiome-gut-brain axis. The gut-brain axis comprises the autonomic and enteric nervous systems, the vagus nerve, and the hypothalamic-pituitary-adrenal axis. While the exact mechanism of this pathway is currently unknown, there is growing evidence to suggest that gut microbiota plays an essential role in the brain, behavior, and cognitive development.

When comparing the microbiome in ASD to that observed in neurotypical individuals, there is a good deal of variability. Research has shown that distinct bacteria are associated with GI symptoms, Autism severity, and other systemic repercussions on the host's health. However, because of the inconsistency in microbiome composition, there is not a signature biomarker of ASD.

It is still unknown if the diagnosis of ASD causes the gut biome dysbiosis or if the

disruption in the biome worsens and contributes to the ASD core symptomology. Individuals with ASD are five times more likely to exhibit atypical eating patterns and six to eight times more likely to have gastrointestinal problems, including diarrhea, constipation, bloating, and gastroesophageal reflux disease when compared with neurotypical subjects. These GI disorders have been reported to be associated with anxiety problems, somatic complaints, sleep abnormalities, and externalizing problems in many ASD individuals. The microbiome in ASD is believed to be atypical and these atypicalities may contribute to some of the brain, behavior and cognitive features associated with ASD. This paper will review what is known about the microbiome in ASD and possible implications for treatment and interventions.

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5-HT	Serotonin
25(OH)D	25-hydrooxyvitamin D
ABA	Applied Behavior Analysis
ADHD	Attention Deficit Hyperactivity Disorder
ASD	Autism Spectrum Disorder
CARS	Childhood Autism Rating Scale
CDC	Center of Disease Control
CFU	Colony-Forming Unit
CMA	Chromosomal Microarray Analysis
CNS	Central Nervous System
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
FDA	Food and Drug Administration
FMT	Fecal Microbiota Transfer
GABA	Gamma Amino Butyric Acid
GCPR	G Protein Coupled Receptor
GERD	Gastroesophageal Reflux Disease
GI	Gastrointestinal
GSRS	Gastrointestinal Symptom Rating Scale
Hu-mAb	Human Monoclonal Antibodies
IL	Interleukin
Keto	Ketogenetic
MAMP	Microbe Associated Molecular Patterns
NIH	National Institute of Health
NT	Neurotypical
OTU	Operational Taxonomic Units
SCFA	Short Chain Fatty Acids
TD	Typical Development

WHAT IS AUTISM SPECTRUM DISORDER?

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by impairment in social communication and repetitive behavior (*Autism Spectrum Disorder Fact Sheet | National Institute of Neurological Disorders and Stroke*, n.d.). The impairment severity of autism is now recognized as a spectrum because ASD individuals present different symptomology and co-morbidities that can span from mild to severe, and many individuals with ASD will require some degree of lifelong care and support (Fattorusso et al., 2019; Masini et al., 2020). Symptoms typically begin to become evident during early development. Children are typically screened and assessed for ASD at their 18 and 24-month checkup as recommended by the National Institute of Health (NIH). Some early symptomologies may include failure to speak a single-word by 16 months or two-word-phrases by 24 months, poor eye contact, and/or lack of social responsiveness. The NIH also reports that some later indicators of ASD may include impaired ability to make friends with peers, abnormally intense fixations, and inflexibility to deviate from a specific routine (*Autism Spectrum Disorder Fact Sheet | National Institute of Neurological Disorders and Stroke*, n.d.)

Recent data from the Center of Disease Control, CDC, has estimated that 1 out of 44 children in the United States may be diagnosed with ASD (CDC, 2022b). The increasing trend in diagnosis can be seen in Figure 1. Figure 1 displays how, since the year 2000, the CDC has reported that the diagnostic rate of ASD has increased by 241%. It is said that the increasing diagnosis may, at least in part, be related to increased awareness, recognition, and assessments (Genovese & Butler, 2020).

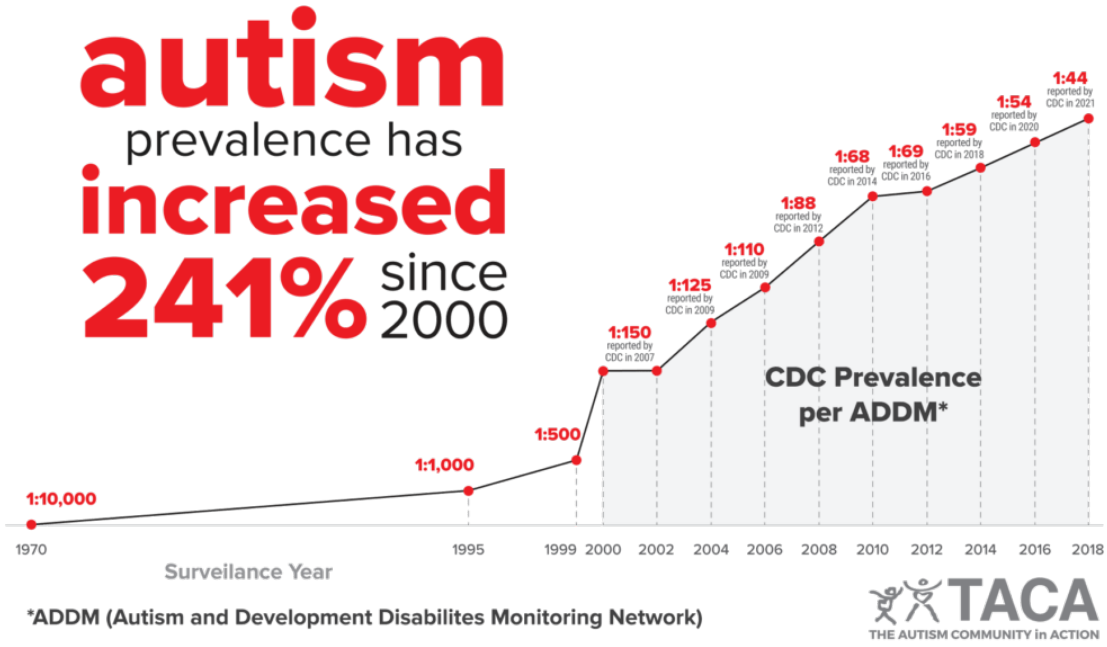


Figure 1: The increased diagnosis rate of autism spectrum disorder. Reproduced from The Autism Community in Action (Autism Prevalence in the United States, 2021). https://tacanow.org/about-autism/autism-prevalence-2021/

The cause of ASD is unknown. However, current research suggest that the etiology of ASD maybe multifactorial and may involve complex environmental and genetic factors (Genovese & Butler, 2020). Figure 2 summarizes some of the potential pre- and post- natal factors that may contribute to ASD, as well as the potential genetic predispositions. Also, per Figure 2, all these proposed hypotheses converge to state that there is a possible impairment in a biological process. ASD is known to affects all races, ethnicities, and socioeconomic groups (CDC, 2022b).

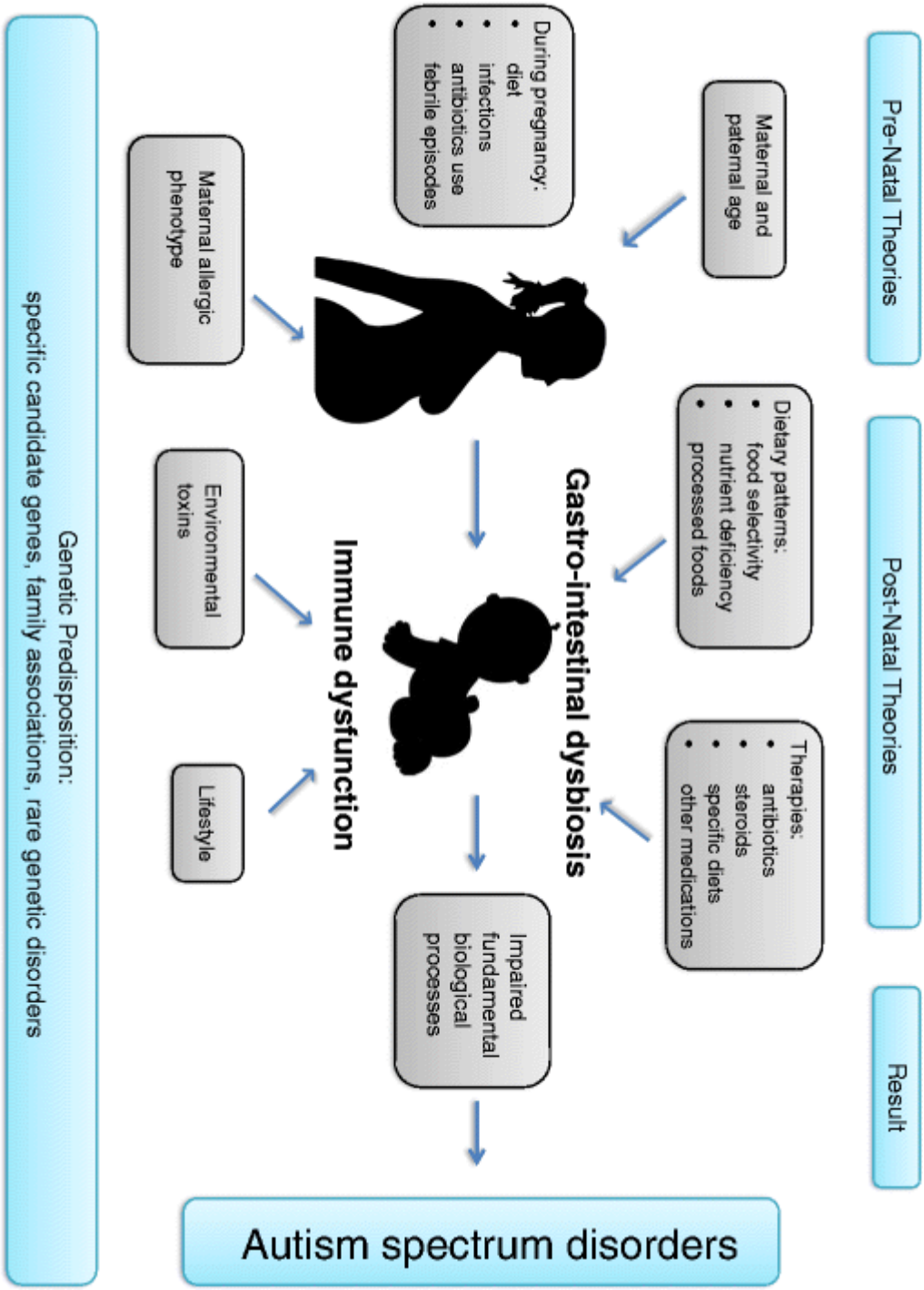


Figure 2 : The theorized complex etiology of Autism Spectrum Disorder. Reproduced by European Journal of Pediatrics (Noriega & Savelkoul, 2014). https://link.springer.com/article/10.1007/s00431-013-2183-4

Several genes have been associated with the genetic component of ASD. While the genetic component of ASD is not fully understood, it is known that there is a hereditary aspect to the genetic epidemiology of ASD in some cases. As noted previously, the CDC has estimated that 2% of the general population may be at risk of autism diagnosis (1 in 44 or circa 2%). However, this statistic escalates to a high of 25-30% if another child within the family has been already diagnosed with ASD (Bai et al., 2019; Genovese & Butler, 2020; Wu et al., 2015). Furthermore, twin studies have demonstrated that the heritability estimates in ASD to be as high as 70% to 90% (Constantino & Todd, 2003; Folstein & Rutter, 1977; Genovese & Butler, 2020).

Case-control studies on human populations and animal models, have been reported that there are over 800 genes associated with autism (Masini et al., 2020). Genes that are found to associated with ASD are both common and rare genetic variants (Thapar & Rutter, 2021). Out of the 800+ genes, the most commonly identified genes appear to be those involved in the encoding for chromatin remodeling and transcriptional regulation, cell proliferation, as well as synaptic architecture and functionality (Masini et al., 2020).

Chromosomal microarray analysis (CMA) has demonstrated the highest diagnostic yield for individuals with ASD. However, a survey conducted by a large autism center reported that less than 10% of ASD confirmed individuals had undergone any type of genetic evaluation (Ho et al., 2016).

While genetic factors appear to play a significant role in the etiology of ASD, environmental factors also appear to influence the prevalence of ASD. A number of studies have cited maternal health as a risk factor for ASD, including deficiencies in

micronutrients such as vitamin D and iron, as these substances may have impact on fetal development (Masini et al., 2020). In addition, two meta-analyses have demonstrated that a maternal viral or bacterial infection during pregnancy may increase the risk of ASD, potentially due to an immune-inflammatory response (Chen et al., 2016; Wu et al., 2015; Zerbo et al., 2015). It has been suggested that the proinflammation cytokines released into the maternal bloodstream can cross the placenta and lead to aberrant neurogenesis that alters synapses and brain development, resulting in ASD behavioral symptoms (Robinson-Agramonte et al., 2022). Additional factors have been reported to include maternal medications such as Valproic acid, extreme prematurity of the infant, and maternally derived anti-brain autoantibodies (Meltzer & Van de Water, 2017).

Some studies have suggested that teratogens and toxic exposures can pose as environmental risks that increase the prevalence of ASD. Toxic xenobiotics and brominated flame retardants has been found to led to mitochondrial dysfunction, which has a documented association with ASD. (Masini et al., 2020). It has been reported that 10-20% of patients diagnosed with ASD have been found to have a mitochondrial dysfunction (Genovese & Butler, 2020; Hu et al., 2019; Rossignol & Frye, 2011). However, whether mitochondrial disorders are causative or associated to ASD remains unknown.

THE GUT-BRAIN-AXIS

It has been estimated that the average human has an intestine that houses an ecosystem with over one hundred billion microorganisms, 7,000 strains of microbiota, and over 1,000 species of microbiota. Within this ecosystem, data indicates that the predominant specie is anaerobic bacteria as well as viruses, bacteriophages, protozoa, archaea, and fungi (Larroya-García et al., 2019; Parekh et al., 2015). Furthermore, research suggest that only a third of the biome is consistent across populations while two-thirds comprise unique and specific microbiota on an individual basis (Larroya-García et al., 2019).

One crucial role of the microbiota within the gut has been found to aide in the synthesis and digestion of essential nutrients that humans cannot naturally make nor digest, such as riboflavin, thiamine, and folate (Vuong & Hsiao, 2017).

It is now recognized that a complex communication network exists from the gut to the CNS. This proposed network has been named the gut-brain axis or the microbiome-gut-brain axis. Figure 3 summarizes this proposed bidirectional communicational route between the gut microbiota and the brain. While the exact mechanism of this pathway is currently unknown, the present evidence suggests that gut microbiota can play an essential role in brain, behavior and cognitive development by producing hormones, immune factors, and metabolites (H.-X. Wang & Wang, 2016a). The gut-brain axis is believed to encompass the autonomic and enteric nervous system, the Vagus nerve, and the hypothalamic-pituitary-adrenal axis (Larroya-García et al., 2019). Based on the topical review by the Chinese Medical Journal, the Vagus nerve may

be the most important neural connection between the gut microbe and the CNS (Dinan & Cryan, 2017; Sun et al., 2020). To support this relationship, a pre-clinical study has suggested that the communication between the Vagus nerve and GI tract may have behavioral effects integrated in the CNS which was demonstrated by means of a vagotomy (Bercik et al., 2011). Afferent fibers of the vagus nerve may be stimulated by microbiota and their metabolites, which then could be integrated into the central nervous system and, in return, could lead to local or systemic effects (Bonaz et al., 2018; Van Der Zanden et al., 2009; Warner, 2019).

One of the systemic effects may be hormone secretion, a function that has been demonstrated by a study that reported direct stimulation of the Vagus nerve from the gut that resulted in the secretion of hormones (Haase et al., 2018). It is thought that the gut microbiota can be considered an endocrine organ because of the physiological responses it triggers on a local and systematic level (Zhang & Davies, 2016). Tryptophan, an essential amino acid, is produced by specific bacteria or is exogenously consumed. Research has suggested three major pathways that tryptophan can take in the GI tract, and an important one involving 5-HT production. 5-HT (serotonin) is said to be produced by enterochromaffin cells (Agus et al., 2018). Data suggest that 90% of the body's serotonin is in the gut rather than the brain (Agus et al., 2018; Larroya-García et al., 2019). 5-HT receptors found in human and animal studies may modulate gut motility, vasodilation, inflammation, and gut-brain signaling by activating the Vagus nerve, nutrient absorption rate, and platelet functions (Mawe & Hoffman, 2013).

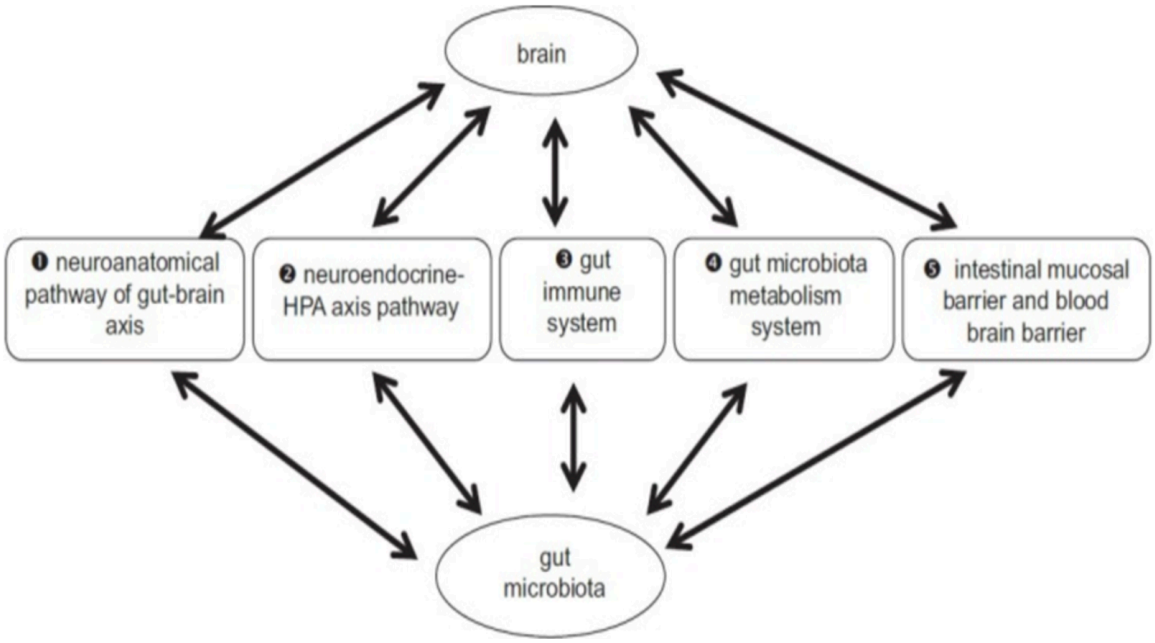


Figure 3: The proposed five communicational routes of the gut microbiota-brain axis. (1-5) Intestinal mucosal barrier and blood brain barrier (5) is the important base for neuroendocrine-HPA axis pathway (2), gut immune system (3), and the gut microbiota metabolism system (4). Substances produced by neuroendocrine-HPA axis pathway (2), gut immune system (3), and the gut microbiota metabolism system (4) can enter the system's circulation and brain through the intestinal mucosal barrier and the blood brain barrier system can play an effect on the gut microbiota. HPA = Hypothalamic-pituitary adrenal. Reproduced from the Chinese Medical Journal. (H.-X. Wang & Wang, 2016). https://journals.lww.com/cmj/Fulltext/2016/10050/Gut_Microbiota_brain_Axis.16.aspx

Additionally, the gut-brain axis is believed to play a role in the immune system by producing microbial-associated molecular patterns (MAMPs) and other stimulatory signals to activate the innate immune system (Larroya-García et al., 2019). It is believed that short-chain-fatty-acids (SCFA) have many roles that can range from energy balance, metabolism in adipose, liver and skeletal function, gut epithelial integrity, and immune effects that may stimulate GPCRs (G Protein Coupled Receptor), Toll-Like Receptor 2, and T regulatory cells (Dinan & Cryan, 2017; Haase et al., 2018; Larroya-García et al., 2019).

The intestinal mucosal epithelial integrity is an aspect of the innate immune system that protects the body from the invasion of microbes. Evidence suggests that when this mucosal epithelium is weakened by stress and other factors, the exogenous bacterial may cause the gut to produce inflammatory cytokines (Fasano, 2012; Haase et al., 2018). These cytokines and other factors are said to travel through the body to the brain and can cross the blood-brain barrier and directly influence the brain (H.-X. Wang & Wang, 2016a). The integrity of the mucosal epithelium may partly be due to the gut microbiota influencing the regeneration of the intestinal epithelial cells. Additionally, the SCFA metabolites are reported to help produce and nourish the mucosal layer to prevent the invasion of microbes and protect against the deteriorating effects of stomach acid (Bonaz et al., 2018; H.-X. Wang & Wang, 2016a). Thus, while the mechanism by which the gut-microbiota-brain axis functions is still unknown, current data suggests that a bi-communicational network impacts many bodily functions.

THE IMPLICATION OF THE GUT-MICROBIOTA-BRAIN AXIS FOR AUTISM SPECTRUM DISORDER

How the Microbiome Can Change

The microbiota is a dynamic biome and the colonization of the gut appears to begin at birth (Dinan & Cryan, 2017). Changes in the microbiota biome can result from myriad factors and a change in the biome could, additionally, impact on the health of an individual.

The initial colonization seems to occur when the infant is exposed to the mother's bacteria composition as the fetus passes through the birth canal (Dinan & Cryan, 2017; Dominguez-Bello et al., 2010; Rutayisire et al., 2016). Recent research has noted that the diversity and colonization of the biome can be based on whether the delivery process has been vaginal or cesarean (Rutayisire et al., 2016). However, no association between the prevalence of ASD and the mode of delivery has been reported (Curran et al., 2016). A change in the biome is believed to be evoked through various dynamic factors such as diet, stress, use of antibiotics and other drugs, illness/infections, age, geography, and genetics (H.-X. Wang & Wang, 2016a). It has also been suggested, in both animal and human studies, that the host's genome may correlate with the composition of an individual's microbiome (Bai et al, 2019; Turpin et al. 2016)

Some research has suggested that antibiotics may have an impact on the composition of the microbiome in ASD. A meta-analysis has reported that a history of "early antibiotic exposure, either prenatally or postnatally, may be associated with ASD

(Lee et al., 2019). Further, Lee et al. (2019) has reported that, in a study of ASD children under three years of age, there appeared to be evidence of the increased use of oral antibiotics.

The microbiome can change in various ways. Regarding ASD, the composition of the microbiome in ASD can be altered in many ways. Yet, it is unsure if the atypical biome that is said to exist in ASD is due to the diagnosis of ASD that may foster the environment for specific bacterial growth or if the lifestyle of ASD individuals fosters the biome formation.

The Composition of the ASD Microbiome

When comparing the microbiome obtained and analyzed from ASD individuals in comparison with that of neurotypical individuals, it has been suggested that there are increases and decreases in the particular phylum of bacteria. Figure 4 subsection a displays a Venn diagram that shows the composition of Operational Taxonomic Units (OTU), indicating the species of fecal microbiota, based on a study investigating differences between typically developing (TD) and ASD individuals. This study found 67 unique OTU for ASD, 173 unique OTU for TD, and 1082 shared OTU between the two groups. Subsection b of Figure 4 shows significantly less richness and diversity of phylum in ASD than in TD.

However, Figure 4 subsection c indicates no significant richness difference between genus OTU of the microbiota. In taxological terms, this study suggests that the broader classifications of bacteria differ more than the specific types of bacteria within

that phylum, which could potentially make therapeutic manipulation of the biome easier.

Figure 5 shows the statistically significant differences of some specific bacteria between neurotypical without GI symptoms (NT), ASD with GI symptoms (ASD-FGID), and neurotypical with GI symptoms (NT-FGID). *Clostridium, Lachnoclostridium*, and *Flavonifractor* are those that are increased in ASD individuals with gastrointestinal problems, whereas *Dorea, Blautia, and Sutterlla* are decreased in ASD individuals with gastrointestinal problems. Regarding the significant differences in the biome composition, many specific bacteria and other species have been reported to have systemic implications on health. For example, *Candida* is a fungus that has been found to be statically more frequent in ASD than in neurotypical individuals. *Candida* has been reported to lead to an extensive array of infection extending from superficial mucosal infections to life-threatening infections. (d'Enfert et al., 2020; Uthayakumar et al., 2021). It is possible that *Candida* could contribute to the increased immune-inflammatory response previously mentioned.

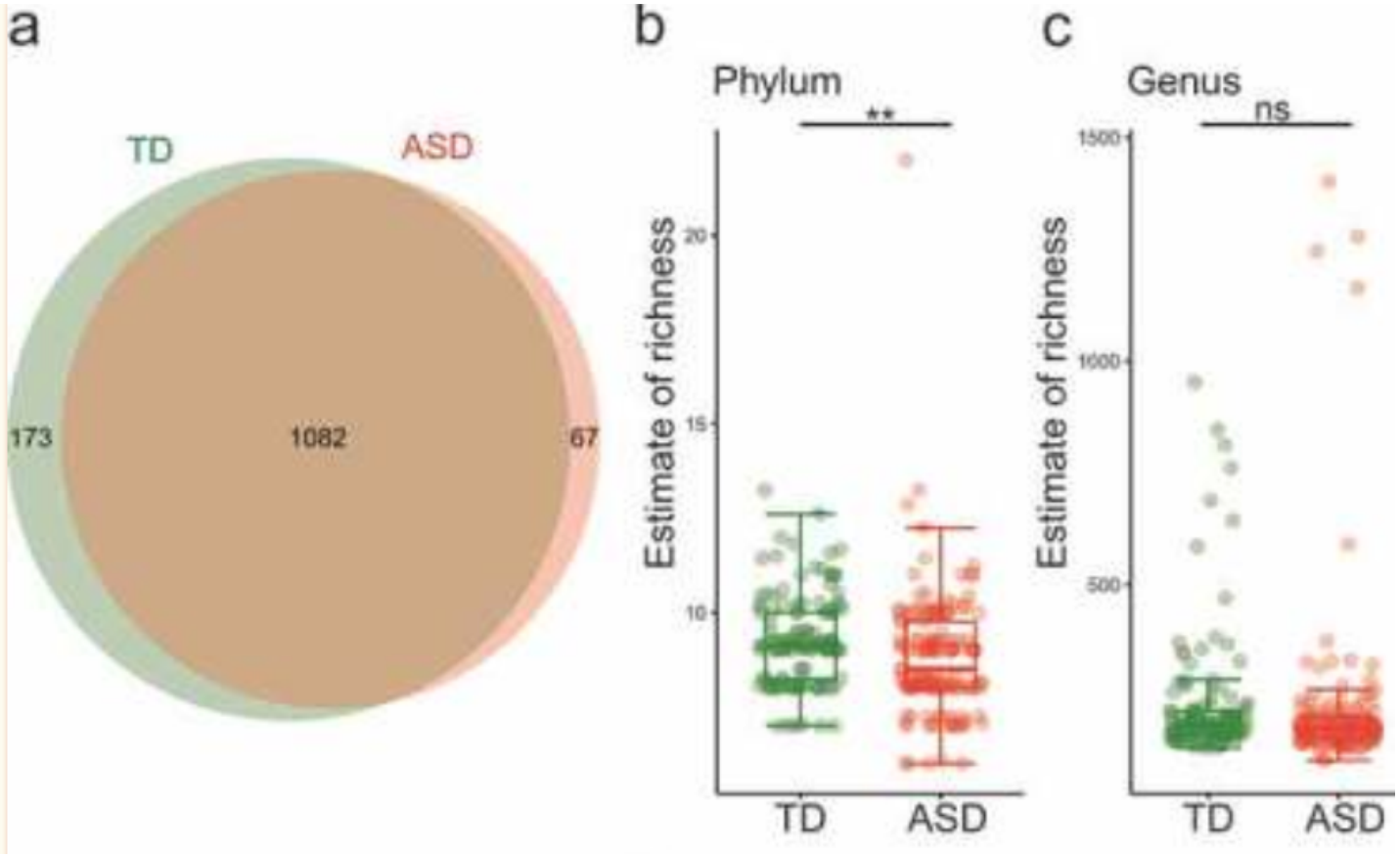


Figure 4: The difference in gut microbiota per 16S rRNA data between TD and ASD children. (A) Displays the unique and shared number of OUT in ASD and TD. (B&C) Displays the richness in phylum and genus levels per group. Reproduced from Gut Microbes (Dan et al., n.d.) https://www-ncbi-nlm-nih-gov.ezproxy.bu.edu/pmc/articles/PMC7524265/

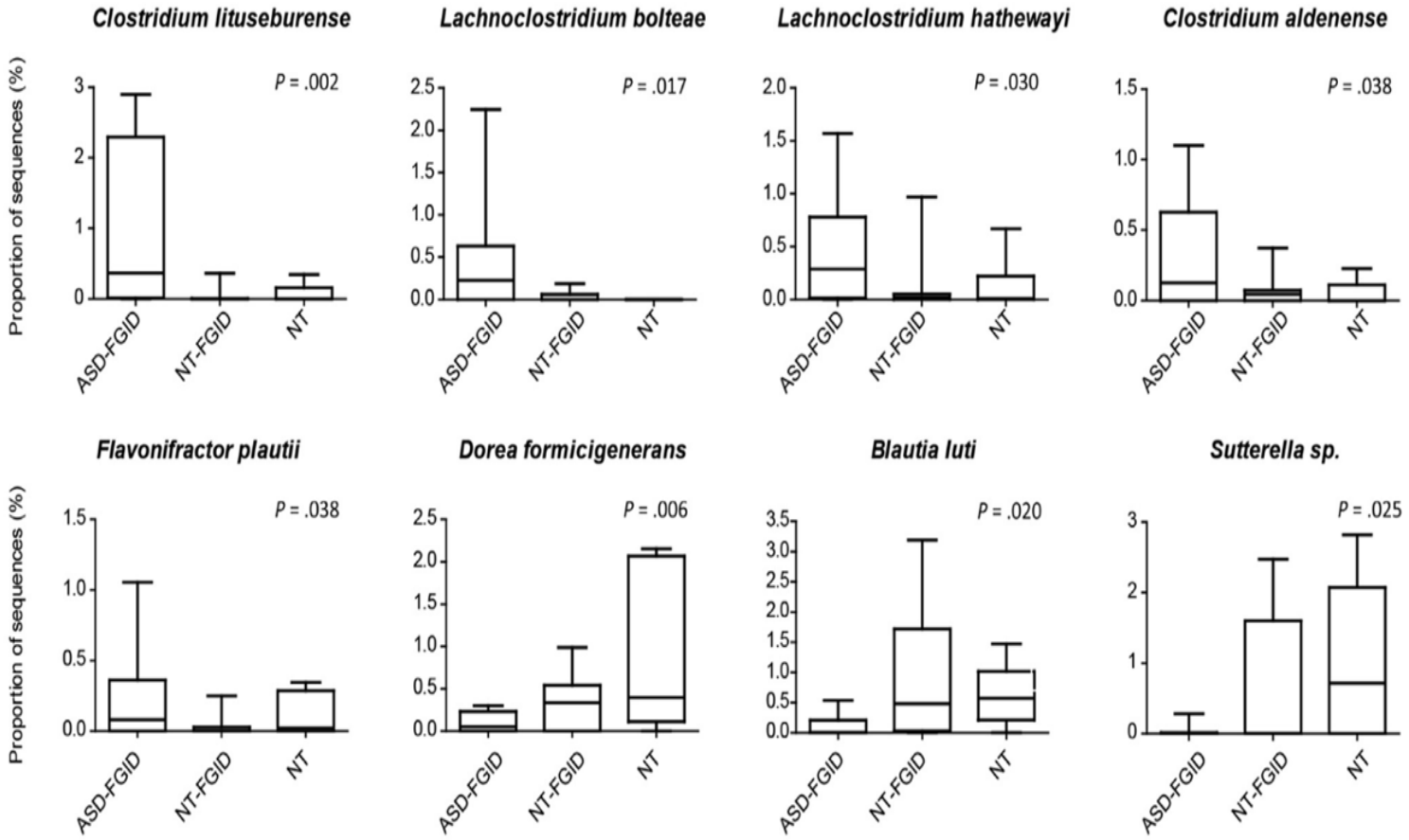


Figure 5 : Differences in OTUs related to specific bacteria associated with ASD were identified in the mucosal microbial community. Overall, an increase in Clostridiales (C lituseburense, P ¼ .002; L bolteae, P ¼ .017; L hathewayi, P ¼ .030; C aldenense, P ¼ .038; and F plautii, P ¼ .038) was seen in the ASD-FGID group compared with the NT-FGID and NT groups. A decrease in relative abundance in the ASD-FGID group was seen for D formicigenerans (P ¼.006), B luti (P ¼ .020), and Sutterella species (P ¼ .025). Plots depict the maximum and minimum (whiskers), upper and lower quartile limits (box), and median (horizontal line). Reproduced from the Cellular and Molecular Gastroenterology and Hepatology Journal (Luna et al., 2017) https://doi.org/10.1016/j.jcmgh.2016.11.008

Clostridiales have also been found to be increased in ASD, as seen in Figure 5. *Clostridiales* have been commonly implicated in irritable bowel syndrome. It has also been shown that *Clostridiales* have a role in stimulating intestinal 5-HT biosynthesis and releasing and modulating GI motility (Labus et al., 2019). *Dorea formicigenerans*, another bacterial species in the gastrointestinal track, has been found to have lower levels in ASD when compared with neurotypical individuals. *Dorea formicigenerans* is involved in mucin degradation, and therefore a decrease in this bacterium could lead to an imbalance of mucin in the gastrointestinal track (Vacca et al., 2020).

It is known that bacteria can produce p-cresol from dietary tyrosine or toluene. P-cresol has been found to be higher in children with ASD than in NT children. High levels of p-cresol have been shown to be associated with DNA damage in vitro as well as damage to the integrity of the intestinal epithelium barrier (Kang et al., 2018). P-cresol has been associated with ASD severity by acting as an inhibitor of certain neurotransmitters that require sulfonation (Sivamaruthi et al., 2020).

Despite current data, there is not yet a signature pattern of atypical microbiome composition that could be considered as a biomarker for ASD. When reviewing current research on the composition of the microbiome in ASD, there is not an agreed consensus of what specific bacteria are increased or decreased It is agreed, however, that there is a difference in the composition the microbiome in ASD overall when compared to neurotypical development.

A systemic review was conducted of 26 published studies pertaining to the changes in microbiome composition in the ASD population. The results of this review

resulted in inconsistencies of the bacteria strains that differed across all 26 studies. It is important to note, however, that this systemic review confirmed that there were 'distinguishable' patterns in the change of *Firmicutes*, *Clostridium,* and *Bifidobacterium* levels (Xu et al., 2019). Thus, the inconsistent finding to date pose a significant limitation on delineating reliable patterns of bacterial composition in ASD. Further research will be needed to describe the microbiota dysbiosis more precisely in ASD. However, the similar 'distinguishable' patterns of bacterial strains described lay the foundation for future research directed toward the identification of consistent and characteristic patterns of bacterial composition associated with ASD.

Typical Diets of ASD and the Impact on the Microbiota

It has been reported that children with ASD are five times more likely to have feeding problems than neurotypically developing children (Madra et al., 2020). These challenges include increased food selectivity and food refusal as well as decreased oral intake (Ahearn et al., 2001; Bandini et al., 2017). The term 'food selectivity' refers to a restricted intake of food that is employed by categorizing the food , such as by smell, taste or texture (Bandini et al., 2010). ASD individuals may also have ritualistic tendencies that can result in the same food being consumed consistently, potentially leading to inadequate nutritional intake (Chaidez et al., 2014). Figure 6 highlights the multiple factors that can impact on food selectivity frequently observed in ASD individuals. In 2017, Vuong and Hsias conducted three-day nutritional interview study that focused on ASD children 4-8 years of age and found that the overall total caloric for these children

intake was low.

Figure 6: The proportion of sensory characteristics that encompasses food selectivity in the ASD population. Replicated from Nutrients (Ristori et al., 2019). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6893818/

In addition, carbohydrate intake was found to be high when compared to the recommended levels. Additional dietary data related to ASD subjects has been obtained through a cohort study. This study conducted a comparative analysis of the nutritional intake of 53 ASD individuals versus that of 58 neurotypical individuals. Children receiving any specific dietary restrictions were excluded. The results from the study are shown in Table 1 and Table 2. Out of all the ASD children, all but one had inadequate fiber intake. 12 ASD children were found to have inadequate intake of 5 or more nutrients. Table 1 illustrates the degree of food selectivity and the height of refusal rates found in ASD when compared to neurotypical children, p-value <. 0001. Table 1 addresses how ASD children tend to eat a limited number of foods and the fact that the specific food is eaten at a much higher rate per day. Table 2 illustrates the evidence for decreased nutritional intake in ASD children compared to neurotypical developing children (p=0.03) (Bandini et al., 2010).

Table 1 Food Selectivity in Children with ASD and typically developing children.

	N	Typical children	N	Children with ASDs	P value *
Food refusal†: Mean (SD)					
Number of FFQ items will not eat	58	21 (18)	53	45 (26)	<.0001
Percentage of FFQ items will not eat of those offered‡	58	18.9% (15.6%)	53	41.7% (21.2%)	<.0001
Limited repertoire§: Mean (SD)	56	22.5 (4.6)	48	19.0 (5.0)	0.0003
High frequency single-food intake‖: n (%)	58	1 (1.7%)	53	4 (7.6%)	0.19

*** P value is for difference between children with ASDs and typically developing children.**
†For food refusal, minimum, median, maximum, number of foods refused for typical children was 0, 16, 79, and for children with ASDs was 1, 47, 94. The minimum, median, and maximum percent of foods refused of those offered was 0%, 14%, 62% for typical children and 1%, 41%, 74% for children with ASDs.

‡Number of foods will not eat of those offered is the percentage of foods that the child would not eat relative to the number of foods that were offered.

§Food repertoire was the number of foods eaten over a three-day period.

‖ Number of children eating a food more than 4.5 times a day
(Herman & Herman, 2022) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2936505/pdf/nihms179410.pdf

Table 2 :Frequency of nutrient inadequacy in children with autism spectrum disorders and typically developing children.

# (%) not meeting EAR*/AI† for specific nutrients	Typically developing children (n=56)‡	Children with ASDs (n=48)	P value§
Vitamin A	0 (0.0%)	5 (10.4%)	0.02
Vitamin C	5 (8.9%)	10 (20.8%)	0.10
Vitamin D	31 (55.4%)	38 (79.2%)	0.01
Vitamin E	41 (73.2%)	30 (62.5%)	0.29
Zinc	3 (5.4%)	5 (10.4%)	0.47
Calcium	24 (42.9%)	31 (64.6%)	0.03
Iron	0 (0.0%)	0 (0.0%)	NA
Fiber	56 (100%)	47 (97.9%)	0.46
# (%) nutrients inadequate			
0 nutrients	0 (0%)	0 (0%)	
1 nutrient	3 (5.4%)	3 (6.3%)	
2 nutrients	20 (35.7%)	5 (10.4%)	
3 nutrients	19 (33.9%)	21 (43.8%)	
4 nutrients	10 (17.9%)	10 (20.8%)	
5 nutrients	4 (7.1%)	5 (10.4%)	
6+ nutrients	0 (0.0%)	4 (8.3%)	
			0.03‖

*** EAR is estimated average requirement**
† AI is adequate intake ‡ 7 children (5 with ASDs, 2 typical) did not have complete 3-day food records and are not included in this table.
§ P values are for differences between children with ASDs and typically developing children.
‖ P value is from a Fisher's exact test with 11 degrees of freedom
(Herman & Herman, 2022)
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2936505/pdf/nihms179410.pdf

Another study conducted on the food selectivity of ASD children found that the typical ASD diet contained fewer fruits and vegetables (Bandini et al., 2017). In addition, this study reported an inverse relationship between decreased fruit/vegetable consumption and increased overweight/obesity prevalence in ASD (Bandini et al., 2017). While there is a decrease in fruit and vegetable consumption, it has been found that ASD children eat energy-rich foods such as cakes, fries, peanut butter, and pasta, all of which can further increase the risk of overweight/obesity (Liu et al., 2016). As stated previously, obesity can have a negative effect negative on the gut microbiome composition. Given the present data, it can be hypothesized that the difference in diet and increased risk of obesity could lead to downstream effects in the difference in microbiome diversity in ASD rather than the taxonomic difference caused by the ASD diagnosis per se(Yap et al., 2021). Alternatively, it has been demonstrated that the CNS has pathways that control the intestinal epithelial lining and motility which, in turn, may alter the gut microbiome composition (Sivamaruthi et al., 2020). Thus, dysregulation of the CNS in ASD may have an impact on the microbiome and gut biome dysbiosis.

Impact of Vitamin D Deficiency in ASD

Per the Table 2, with a $p<0.05$ to show a 95% confidence interval, ASD individuals are more likely to have a nutrient deficiency in vitamin A, vitamin D, and calcium. Vitamin D also plays a role in regulating calcium absorption and excretion, so the low vitamin D could explain why calcium is low (*Calcium and Vitamin D Combination Uses, Side Effects & Warnings*, n.d.). It is to be found that vitamin D and

the vitamin D receptor have a role in the regulation of the microbiome. It should be noted that, however, the knowledge of the biological function and mechanism of the relationship between vitamin D and the receptor is still limited. Nevertheless, research has stated that people who have Crohn's disease can have a vitamin D deficiency that can contribute to the pathogenesis (Koh et al., 2016; Mostafa & Al-Ayadhi, 2012).

Autism has a unique gut disorder related to Crohn's disease which is prevalent in the community called autistic enterocolitis (Pulikkan et al., 2019). Autistic enterocolitis is characterized by chronic inflammation and lymph node hyperplasia (Sivamaruthi et al., 2020). Therefore, it could be concluded that gastrointestinal abnormalities that exist in the ASD population can be derived from the atypical microbiome that exists because of the repercussions of lack of vitamin D from the food selectivity.

Upon further exploration, it was found that vitamin D, besides may fostering autistic enterocolitis, can have a more prominent role in ASD development and symptomology. The effects of vitamin D have been seen to have implications in ASD before food selectivity. A study found that a higher serum concentration of precursors of vitamin D, 25-hydroxyvitamin D (25(OH)D) in utero or early life may reduce the risk of autism (Mostafa & AL-Ayadhi, 2012; Saad et al., 2016). Also, a study found that serum 25(OH)D levels had an inverse relationship to the degree of severity of ASD (Mostafa & AL-Ayadhi, 2012). Lastly pertaining to vitamin D, a case-controlled cross-sectional study was conducted with 122 ASD children to assess vitamin D levels. The results concluded that 57% had vitamin D deficiency, and 30% had vitamin D insufficiency.

More investigation occurred for 83 ASD subjects with a serum level of 25(OH)D

less than 30ng/ml. For reference, Harvard Health has stated that children and adults require a serum level of 25(OH)D at a minimum is 30ng/mL, but that the recommended range is 40 to 60ng/mL (Tello, 2020). The study participants received 300 IU/kg/day of vitamin D supplements. The outcomes of the 25(OH)D deficient population on supplements included decreased ASD symptoms based on increased positive outcomes on the Childhood Autism Rating Scale (CARS) (Saad et al., 2016).

Also, like vitamin D, there was an inverse relationship between vitamin A levels and the severity of autism (Liu et al., 2016). Vitamin A has also been seen to be involved in the regulation of microbiota. Vitamin A regulates the central nervous system through retinoic acid the active metabolite that promotes intestinal immunity and the maintenance of the mucosal epithelial barrier in the GI system (Cassani et al., 2012; McCullough et al., 1999). The gut permeability is altered by microbiota dysbiosis (Martel et al., 2022). The gut epilethium acts as a line of defense against pathogens and metabolites. However, increased gut permeability, 'leaky gut,' is partly caused by increased bacterial metabolites and inflammation cytokines (Fasano, 2012; Fowlie et al., 2018; Obrenovich, 2018).

A normal epithelium barrier has tight junctions to inhibit the trans/paracellular motility. However, in a leaky gut, the tight junctions are degraded (Fasano, 2012). The result of a leaky gut can allow for bacteria, toxic metabolites, bacterial toxins, and small molecules to leak from the GI tract into the bloodstream, causing a weakened innate immune barrier (Obrenovich, 2018). When the integrity of the gut barrier is harmed, it can further impact on other autoimmune, metabolic, and mental health disorders (Martel et al., 2022). A nationwide population-based study was conducted (n=1596) to

investigate the association between ASD and autoimmune diseases. The results supported the association between allergic diseases (asthma, allergic rhinitis, atopic dermatitis, and urticaria) and autoimmune diseases (type 1 diabetes and Crohn's diseases) with ASD as a comorbidity.

Decreased vitamin A levels can impact the mucus and epithelium lining to further the leaky barrier in ASD. However, the exact underlying mechanism of the implications of vitamin A to further ASD symptomology or the leaky gut remains unspecified.

The Gastrointestinal Abnormalities in ASD and the Impacts on Behavior

Gastrointestinal Symptoms in ASD

In addition to symptoms of impairments in communication, social interaction, and stereotyped repetitive behaviors, research conducted on 760 children with ASD (24 to 60 months of age) has found that ASD children are six to eight times more likely to experience gastrointestinal disorders than neurotypical children (Chaidez et al., 2014; Fulceri et al., 2016). Gastrointestinal disorders can include diarrhea, constipation, bloating, and Gastroesophageal Reflux Disease, GERD (Ristori et al., 2019). In addition, it has been reported that these gastrointestinal dysfunctions are pathologically characterized by an increased intestinal concentration of cytokines, IgG autoantibodies, monocytes, natural killer cells, eosinophils, and lymphocytes (Robinson-Agramonte et al., 2022).

There is now evidence that ASD children who experience gastrointestinal disturbances, display increased evidence of severe anxiety, irritability, and social

withdrawal compared with those without gastrointestinal disturbances (Sun et al., 2020). Therefore, increased negative behaviors seen in some ASD children may, in some cases, be associated with gastrointestinal problems. Supporting this observation is the fact that ASD children with GI symptoms have been found to have a higher post-stress cortisol level, indicating a greater risk of anxiety (Madra et al., 2020).

A study was conducted by the Thompson Center for Autism & Neurodevelopmental Disorders in Columbia, Missouri. This study included 340 children/adolescents with ASD ranging from two to eighteen years of age. The analyzed variables were obtained from questionnaires that asked about dietary problems, nutritional problems, GI symptoms, and internalizing and externalizing symptoms. The results of this study demonstrated that 65% of these children experienced constipation, 47.9% experienced stomach pain, 29.7% experienced diarrhea, and 23.2% experienced nausea. It was also found that younger children with ASD and gastrointestinal disturbances were more likely to demonstrate aggressive problem behaviors (Ferguson et al., 2019).

Additional data from this study showed that older ASD children tended to exhibit more internalized symptoms such as anxiety and withdrawn behavior (Ferguson et al., 2019). As a result, it can be concluded that gastrointestinal disturbances can have different adverse behavioral and relational effects at varying ages.

An additional study, conducted on preschoolers, focused on the potential association between GI symptoms and behavior (Fulceri et al., 2016). Results indicated that ASD children with GI symptoms had statistically more anxiety problems, somatic

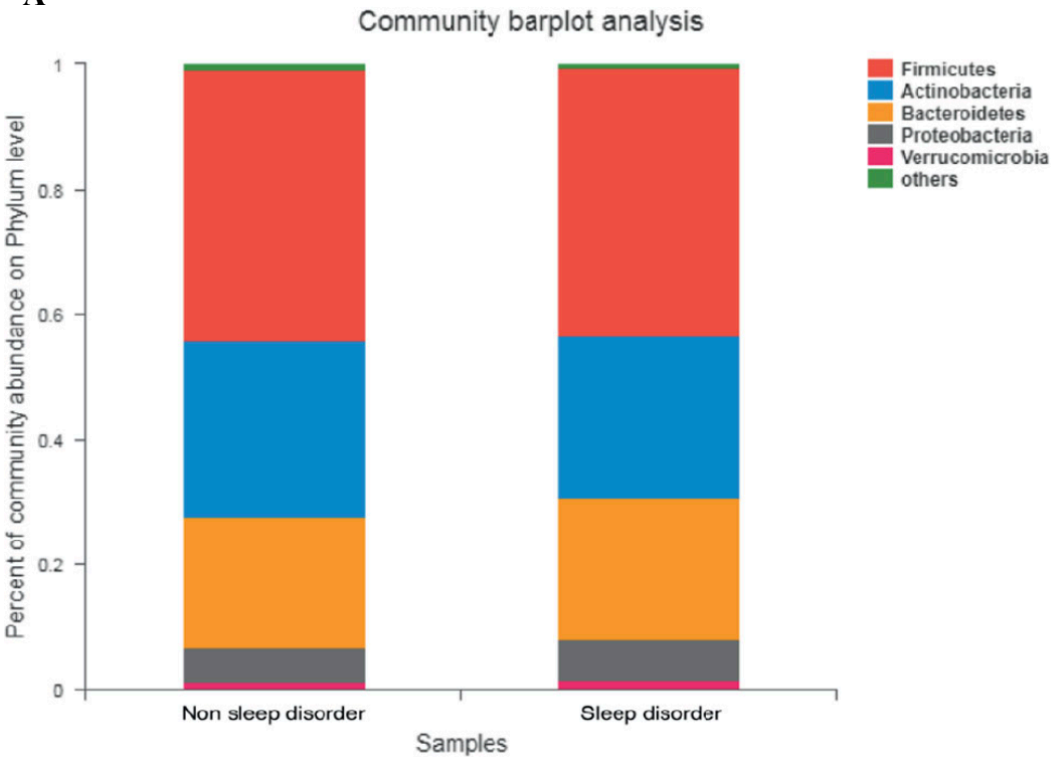
complaints, and externalizing symptoms than ASD children without GI problems. When comparing the ASD group to the neurotypical developing (NT) control group, there was no difference in the behavioral problems between NT with GI symptoms and NT without GI symptoms (Fulceri et al., 2016).

Gastrointestinal disturbances have been commonly found in ASD have been linked to abdominal discomforts that can negatively impacted sleep. In fact, comorbid sleep abnormalities affect as high as 80% of ASD children (Fulceri et al., 2016; Madra et al., 2020; Ristori et al., 2019). Additionally, there have been reports of increased gut biome dysbiosis in association with sleep fragmentation and loss due to bacterial metabolites dysregulating circadian rhythm transcription factors (Matenchuk et al., 2020). Therefore, the combination of abdominal discomfort and the dysregulation of circadian transcription can exacerbate the sleep disturbances that ASD patients experience.

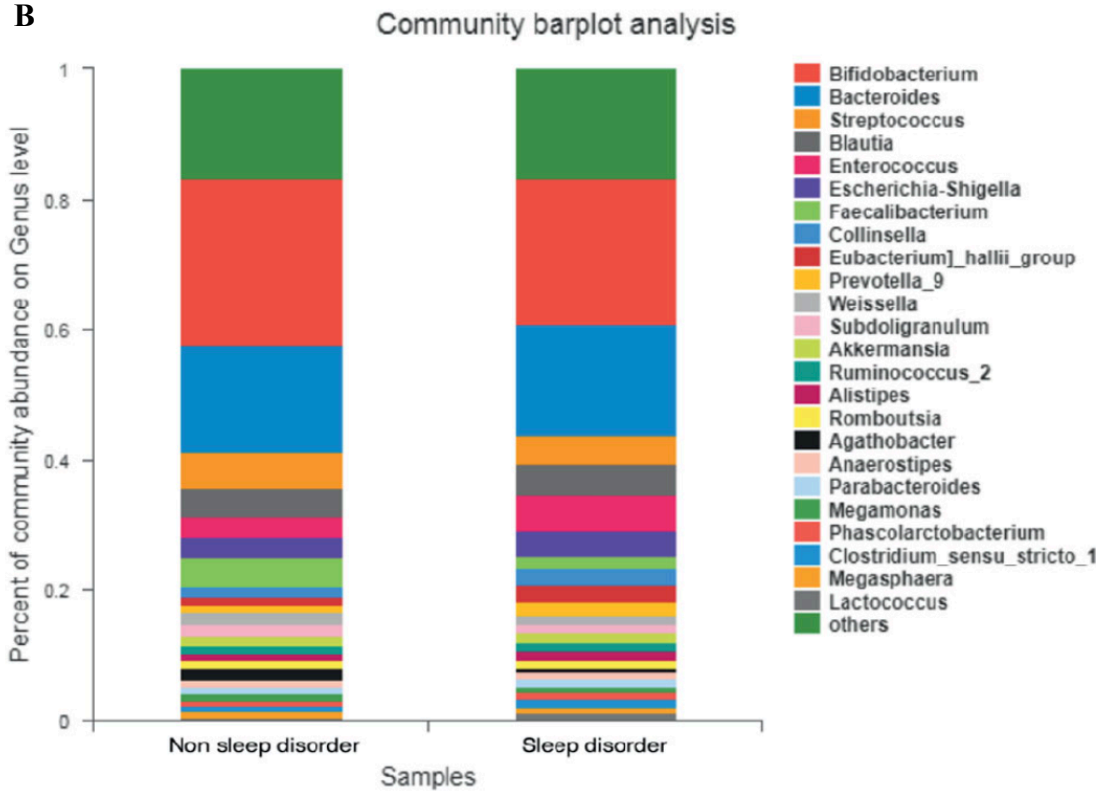
Research has shown that disordered sleep can increase the severity of ASD symptoms, including, but not limited to, stereotypical behaviors, social impairment, aggression, and self-sustained injurious behavior (Singh & Zimmerman, 2015). A study was conducted to evaluate the correlation between gut microbiota and sleep disorders in children. In this study, 120 ASD children were divided into the sleep disorder group (n=60) and a control non-sleep disorder group (n=60). The metrics used in this study included questionaries to assess autism symptoms and evaluate sleep problems as well as the use of Illumina MiSeq analysis of 16S rRNA genes that allowed the researchers to assess the microbiome composition of the ASD children. The results indicated that the bacteria composition of the two groups was distinct, and the core symptoms of ASD were

more severe within the sleep disordered group (Hua et al., 2020). The results can be seen below in Figure 7. Figure 7A shows that the predominant phyla in the ASD group with sleep disorders and the predominant phyla in the ASD group without disordered sleep show no significant difference ($p>0.05$). Figures 7B and 7C show differences within the bacteria's genera, explicitly noting that the *Faecalibacterium* and *Agathobacter* are statistically lower in the non-sleep disorder ASD group. Figure 7D displays those three main bacteria metabolites that are found to differ between the groups. In addition, levels of 3-hydroxybutyric acid and melatonin were found to be lower in the sleep disorder group, whereas serotonin levels were higher in the sleep disorder group.

A


Community barplot analysis
Percent of community abundance on Phylum level
1
0.8
0.6
0.4
0.2
0
Non sleep disorder
Sleep disorder
Samples
Firmicutes
Actinobacteria
Bacteroidetes
Proteobacteria
Verrucomicrobia
others

B


Community barplot analysis
Percent of community abundance on Genus level
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Non sleep disorder
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Streptococcus
Blautia
Enterococcus
Escherichia-Shigella
Faecalibacterium
Collinsella
Eubacterium]_hallii_group
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Weissella
Subdoligranulum
Akkermansia
Ruminococcus_2
Alistipes
Romboutsia
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Phascolarctobacterium
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Lactococcus
others

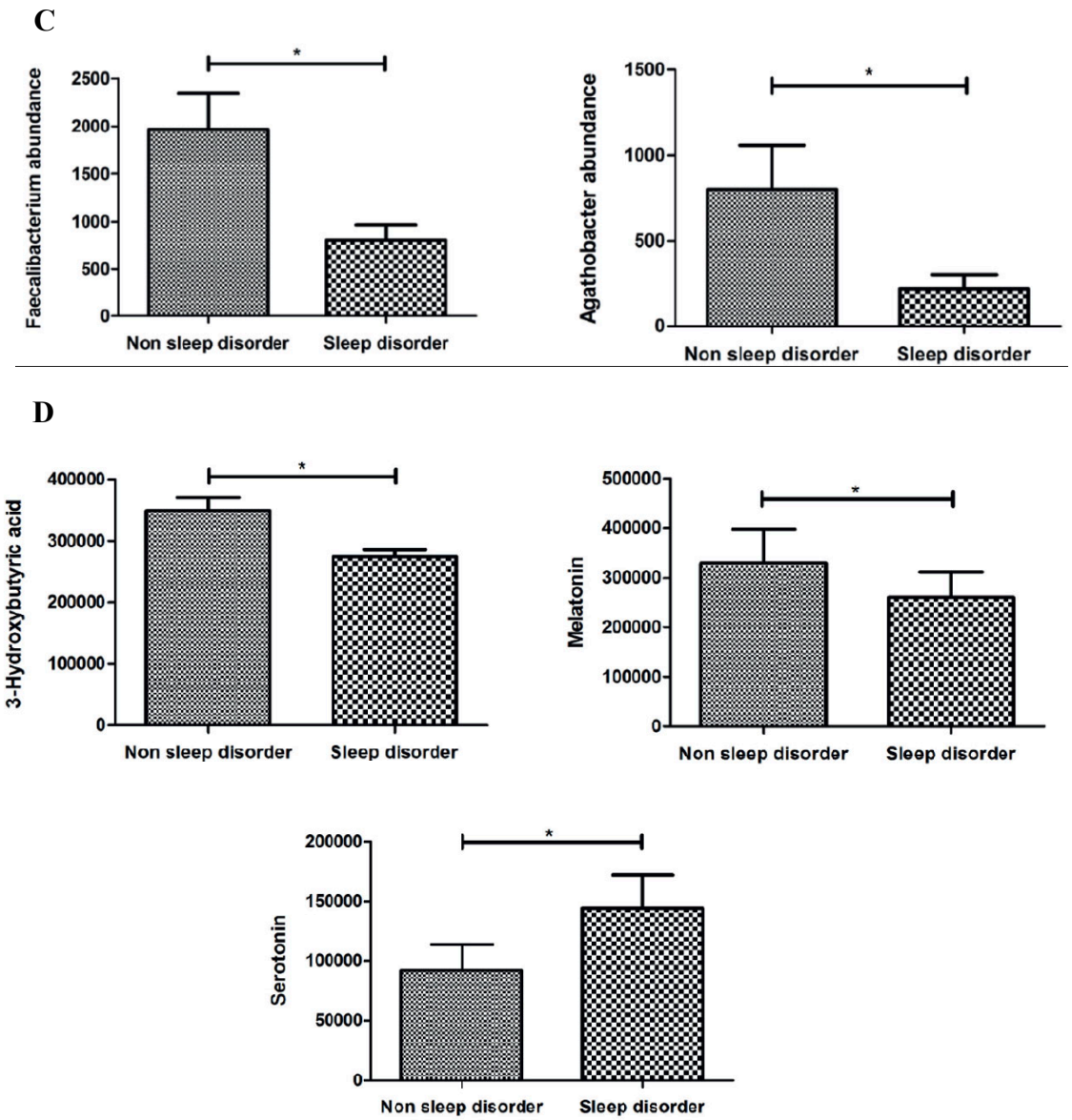

Figure 7 : The relative abundance of bacteria in feces and metabolites from ASD children with and without sleep disorders.

(A) Displays the dominant bacteria phyla with the relative abundance over 1%

(B) Displays the dominant bacteria genera with the relative abundance over 1%.

(C) Displays the difference in *Faecalibacterium* and *Agathobacter* abundance relative to the two groups at the genus level.

(D) Displays the three main difference in metabolites between ASD children with and without sleep disorders.

(* p<0.05, T test and Mann-Whitney test)

Adapted from *Frontiers in Psychiatry's The Gut Microbiota and Associated Metabolites Are Altered in Sleep Disorder of Children With Autism Spectrum Disorders.* (Liu et al., 2016)

This study also explored the relationship between metabolites and bacteria levels. Findings indicated that "3-hydroxybutyric acid levels were positively correlated with *Faecalibacterium* abundance (rs = 0.382, p = 0.000) as was the melatonin level with both *Faecalibacterium* (rs = 0.197, p = 0.036) and *Agathobacter* (rs = 0.192, p = 0.041) abundance. The 3-hydroxybutyric acid level was positively associated with melatonin level (rs = 0.782, p = 0.000)" (Hua et al., 2020).

Faecalibacterium is an anaerobic bacterium that is one of the most abundant species in the human intestinal microbiome and comprises around 5% of the total bacteria in feces. It has been widely reported that changes in the *Faecalibacterium* genus are related to metabolic and intestinal diseases. One of the metabolites produced by *Faecalibacterium* is short-chain fatty acids which help maintain the integrity of the gut's epithelium. The more profound importance of this metabolite will be elaborated on further in the next section. Furthermore*, Agathobacter* is a gram-positive bacterium that produce butyrate, acetate, hydrogen, and lactate through fermentation (Rosero et al., n.d.). The role of butyrate is to provide energy to the intestinal epithelium, to help maintain homeostasis in the microbiome colonies, and to inhibition of inflammation (Hamer et al., 2008). However, in ASD individuals, it is reported that the average level of butyrate is low (Hua et al., 2020). Thus, the conclusion from this data suggests that the change in the microbiome can cause gastrointestinal disorders by increased inflammation and decreased epithelium integrity, leading to sleep disturbances by the decreased presence of certain bacteria metabolites.

These negative behaviors associated with GI problems can negatively impact the

way of life for ASD individuals. Thus, it can be proposed that addressing the gastrointestinal disturbances associated with ASD can mitigate and relieve the painful symptoms while helping improve the way of life in these individuals and help to ease the impact on caregivers of the ASD individuals. As stated previously, ASD affects a growing number of children in the United States diagnoses. Of these children, it is estimated that43% suffer from gastrointestinal disturbances, and up to 80% of ASD children suffer from sleep disorders (Chaste & Leboyer, 2012; Singh & Zimmerman, 2015). Therefore, it is pivotal to help address and deepen the existing breadth of knowledge to help this population of children.

Gut Permeability, the Vagus Nerve, and Immune Dysregulation in ASD.

The gut-microbiota- brain axis allows the bacterial environment to influence the CNS. Consequently, as seen in ASD, the GI system's increased permeability may lead to a leaky gut and an increased risk for the autoimmune disorders and chronic inflammation due to the increased presence of cytokines (Bruce-Keller et al., 2018; Vuong & Hsiao, 2017). The leaky gut is due to the disruption of the tight junctions, the connector between epithelial cells that regulate paracellular transport (Zoccante et al., 2022). Evidence suggests that one plausible explanation is due to genetic abnormalities in tight junction gene expression in ASD, not the alteration of the gut bacterial flora (Robinson-Agramonte et al., 2022). Again, vitamin A could be another explanation of the leaky gut. The leaky gut can allow neurotoxins and cytotoxins to enter the bloodstream, affecting

neurotransmitters' functions in the brain. Thus, abnormal behaviors, such as those seen with ASD, can become apparent (Sivamaruthi et al., 2020).

To help maintain the integrity of the intestinal barrier, short-chain fatty acids (SCFA) play a crucial role (Warner, 2019). SCFA are produced by the gut microbiota, specifically *Lactobacillus* and *Bifidobacterium* (Al-Ayadhi et al., 2021; H.-X. Wang & Wang, 2016b). *Lactobacillus* and *Bifidobacterium* have been reported to enhance the prevalence of tight junctions that maintain intestinal permeability (Al-Ayadhi et al., 2021). However, individuals with ASD have been found to have lower levels of SCFA (Adams et al., 2011).

Furthermore, SCFA include fermented undigested carbohydrates metabolites that are most commonly 1. Acetate 2. Propionate or 3. Butyrate (Warner, 2019). It is suspected that SCFA, like those from the intestinal microbiota, are involved in immune T cell regulation and naïve T cell differentiation into Th1 or Th17 to increase an immune response. Within the immune system, studies have reported an imbalanced ratio of T helper cells to T suppressor cells in ASD, which may lead to an increased risk of autoimmune diseases (Robinson-Agramonte et al., 2022). Th17 cells secrete IL-17A, which is involved in numerous autoimmune and neuroinflammatory diseases, including autism (Lammert et al., 2018).

Additionally, it has been found that SCFA can cross the blood-brain barrier and influence central nervous system (CNS) activity, such as the maturation of resident CNS macrophages, and the microglia (Erny et al., 2015). Autopsied cerebellum and cerebral cortex of ASD patients have shown more microglia and astroglia, plus proinflammatory

cytokines in the cerebrospinal fluid (Vuong & Hsiao, 2017). Pre-clinical studies in mice propose that the gut microbiome can influence the blood-brain barrier and that cytokines reduce the integrity of the blood-brain barrier and lead to neuroinflammation (Al-Ayadhi et al., 2021). As previously mentioned in the latter section, *Faecalibacterium*, the bacteria associated to be negatively correlated with sleep disturbances in the ASD population, have low levels of SCFA, and *Faecalibacterium* secretes compounds such as salicylic acid that are anti-inflammatory compounds (Leylabadlo et al., 2020). The decrease in *Faecalibacterium* could help explain why there are more sleep and gut disturbances in ASD and why there is a systemic inflammatory effect in ASD.

Current literature suggests that SCFA in the gut can stimulate the Vagus nerve's afferent fibers to be integrated into the CNS (Bonaz et al., 2018). Interestingly, vagal activity can be a protective mechanism for the intestinal epithelial barrier. Consequently, low vagus activation and stimulation may lead to increased leakiness, producing systematic inflammation (Bonaz et al., 2018; Van Der Zanden et al., 2009). Systemic inflammation is consistent with the increased proinflammation cyto/chemokines: Ilβ, IL-17, IL-6, IL-5, IL-12, TNFα, and TGFβ (Robinson-Agramonte et al., 2022; Vuong & Hsiao, 2017). IL-6 has also been positively associated with ASD severity as well as social and cognitive deficits (Robinson-Agramonte et al., 2022). Additionally, IL-6 has additionally been seen in mucosal biopsies obtained from ASD children with abdominal pain and discomfort (Luna et al., 2017). In ASD, gram-negative bacteria have been discovered to increase the production of LPS and proinflammatory cytokines, which in return cause disruption of the gut-brain axis (Al-Ayadhi et al., 2021).

The disrupted communication of the gut-microbiota-brain axis from the atypical inflammation immune response has been reported in ASD's GI and systemic disturbances. Likewise, the disruptive immune response has been associated with increased depression and anxiety, which can worsen the behavior in ASD (Sun et al., 2020).

Regardless, with this dysbiosis in the gut microbiota, it is hard to say if the change in bacteria composition of ASD patients leads to the aberrant immune responses or if the composition of bacteria is a result of ASD's immune dysregulation.

Neurotransmitters Modulated by Bacterium in ASD.

Many neurotransmitters have been modulated by the expressed concentration of microbes and their metabolites (Xiao et al., n.d.). On a biochemical level, some neurotransmitters contribute to the onset and the progression of ASD, including dopamine, serotonin, GABA, Acetylcholine, glutamate, and histamine (Eissa et al., 2018). As previously mentioned, tryptophan is an essential amino acid that can be produced by specific bacteria and lead to the production of 5-HT/serotonin. In ASD patients, the plasma and fecal metabolism of tryptophan is abnormal. Furthermore, a correlation between altered levels of tryptophan and gut dysbiosis in ASD has been observed (Xiao et al., n.d.). 5-HT is known to be a critical modulator of the enteric and central nervous system development. Previous studies have reported that ASD children had elevated 5-HT compared to NT children (Dan et al., n.d.).

Bifidobacterium is a bacterium that has been reported to produce GABA, an inhibitory neurotransmitter (McCormick, 1989). An evaluation of a meta-analysis of nine studies with a population of 254 ASD patients investigated the gut microbiota and reported that the level of *Bifidobacterium* was lowered in the ASD population (Xu et al., 2019). A review of the motor cortex in ASD patients revealed that a GABAergic reduction function might lead to an excitation and inhibition imbalance that can cause the pathophysiology symptoms of ASD (Masuda et al., 2019). If *Bifidobacterium* produces GABA and is a bacterium that is typically reported lower in ASD, then the excitation and inhibition disparities of the pathophysiology symptoms may be linked to the bacteria difference.

Additionally*, Bifidobacterium* produces SCFA and GABA, both being involved in pathways that exacerbate the symptomology of ASD and pathophysiology, respectively. Thus, increasing the concentration of this bacteria and targeting the neural circuits related to GABA could be evaluated to investigate if any promising therapeutic results may be suggested (Dan et al., n.d.; Masuda et al., 2019). A study reported by the International Society for Autism Research used magnetic resonance spectroscopy to measure GABA and glutamate, an excitatory neurotransmitter and reported data that demonstrated comparable levels in all brain regions to NT (Kolodny et al., 2020). However, since there are contradicting statements within the ASD data, further research is needed to investigate the role of the GABA inotropic receptor that leads to excitation and inhibition imbalance or if *Bifidobacterium* produces a different isotype of GABA.

CURRENT THERAPEUTICS FOR ASD INVOLVING THE MICROBIOME

The Introduction to ASD therapeutics

When discussing ASD, there is no cure. Intercurrent co-morbid aspects of ASD are what most pharmaceutical interventions target, which include antipsychotics, antidepressants, and psychostimulants (Eissa et al., 2018). Since ASD is on a spectrum and the etiology is complex and unknown, designing a fundamental biomedical intervention to address the physiology is intricate and complex, and there is not yet a successfully proposed drug target mechanism.

Besides pharmacological approaches for treating ASD, there are behavioral treatments called applied behavior analysis (ABA) that focuses on promoting behaviors to improve life, social, and educational skills (CDC, 2022a). While these are current therapeutics, this thesis will analyze other therapeutics that manipulate the gut microbiome to help alleviate co-morbid symptomologies of ASD.

Microbiome ASD Therapeutics

Fecal Transplants

The discovery of the gut-microbiota-brain axis has stimulated a surge of research studying the microbiome's implication on health and disease, as in this case, autism. Therefore, some modern therapeutics have been focused onto targeting and manipulating the gut microbiome.

One potential therapeutic approach has been fecal microbiota transplants, FMT.

Interestingly, the history of FMT dates back to the 4th century but has been a prominent research topic since 2013, when the FDA approved FMT for treating Clostridium infections (J.-W. Wang et al., 2019). Besides being potentially therapeutic for ASD, FMT research has been investigated in other diseases, such as Crohn's, Parkinson's, Alzheimer's, depression, epilepsy, multiple sclerosis, psoriasis, anorexia nervosa, and some cancers (Antushevich, 2020).

The overall goal of FMT is to introduce healthy bacteria and normalize the composition of the recipient's microbiota. Consequently, the new biome will relieve the GI symptoms and subsequently improve core ASD symptomology. The sequence of events and mechanism of an FMT is illustrated in Figure 8. First, fecal matter from a screened, healthy donor is placed into a child with ASD via the route nasoduodenal, colonoscopy, or an enema. The most suggested route is colonoscopy. After the insertion of feces, an anti-diarrheal pill is given so that the new microbiota stays in the recipient for as long as possible to ensure the transfer (*Fecal Transplant*, n.d.; *Fecal Transplant*, 2022; Xiao et al., n.d.). The transfer of new microbes into the recipient has a usual success rate of around 90% (*Fecal Transplant*, n.d.).

A small, open-label clinical trial evaluated the FMT's impact on 18 ASD patients. During this 7–8-week trial, a decrease of roughly 80% of the GI symptoms was reported, derived from the Gastrointestinal Symptom Rating Scale, GSRS. ASD-related behavior following FMT were said to improve as seen through the scoring of the PGI-II test (evaluates 17 ASD-related symptoms), SRS test (assesses social skill deficits), ABC test (evaluates irritability, hyperactivity, lethargy, stereotypy, and aberrant speech), and

VABS-II test(evaluates adaptive behaviors such as communication, daily living skills, and socialization) (Kang et al., 2017). In addition, the overall diversity composition increased, including, more specifically, the abundance of *Bifidobacterium, Prevotella, and Desulfovibrio* (Kang et al., 2017). The same small, open-label clinical trial then re-evaluated the participants after two years to see if the biome change and symptoms were still present. In the long term, there was a 58% reduction in GSRS ratings relative to baseline. In addition, the CARS rating during the initial trial suggests that 83% of the participants had severe ASD. However, after the two years, only 17% were severe, 39% mild to moderate, and 44% were below the ASD diagnostic cut-off. In addition, 16 of the 18 patients gave a fecal sample, and the results were that most participants had a higher microbiota diversity compared to baseline after the year mark (Kang et al., 2019). Nevertheless, the study remarked that using antibiotics, such as vancomycin and phytochemical sulforaphane, reversed the benefit of the FMT (Kang et al., 2019).

There could be a reduction in ASD symptomology and GI disturbances because of the reduced harmful metabolites and less mucosal activation that results in the immune response. A study evaluated the immune response of FMT in patients with colitis. The results showed a significant reduction in CD8+ T cell density and a relative increase of regulatory T cells and CD4+ T cells, reducing the inflammatory response (Y. Wang et al., 2018). While this study was just for colitis, it can apply to ASD. In a previous section, it was discussed about the immune implications of ASD and the increased inflammatory responses. Therefore, this can be another mechanism that can be explicitly evaluated to see the associations FMT can have on ASD immune-inflammatory response.

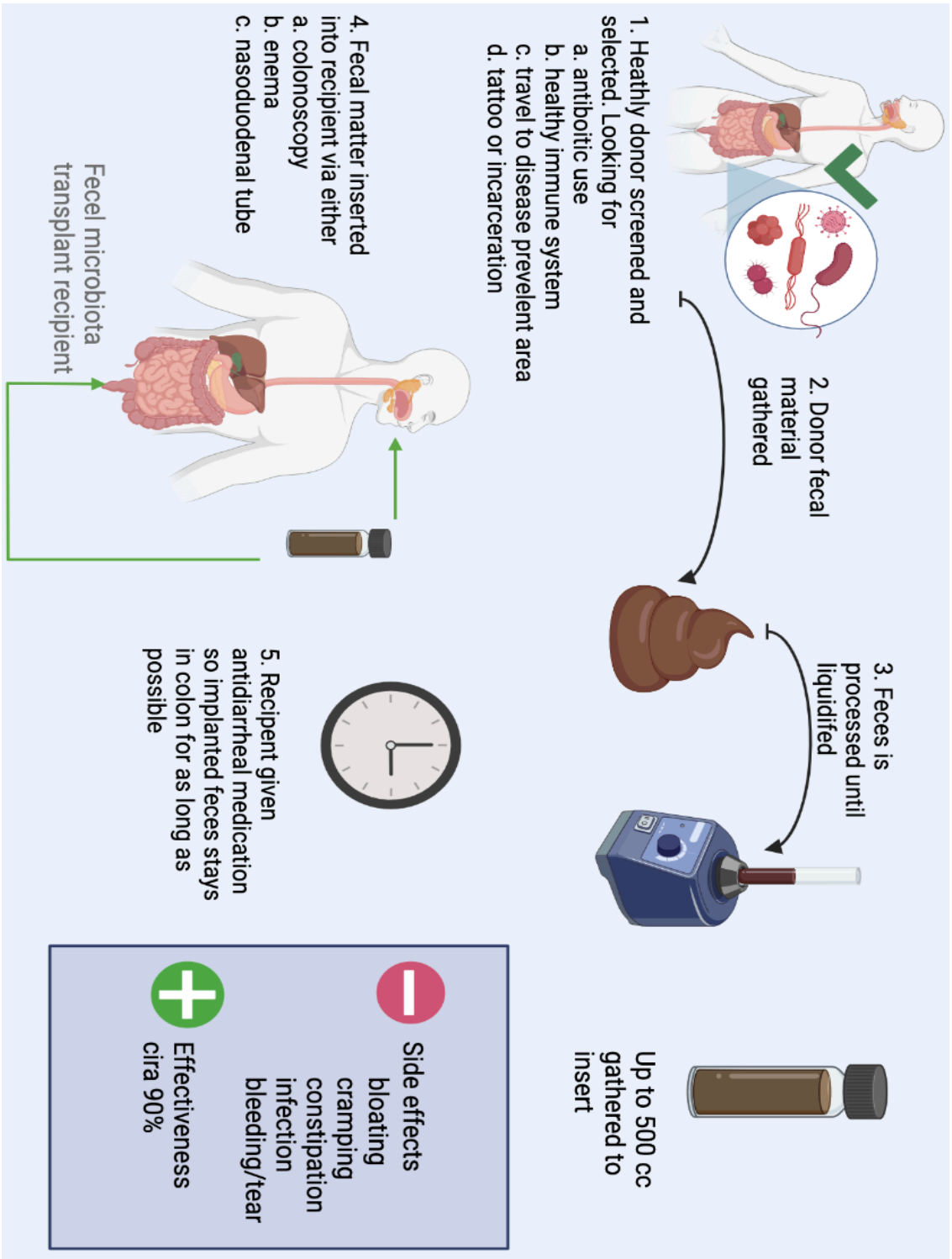

Figure 8: The sequence of fecal microbiota transplants. Created by Lauryn Brasch via BioRender. (Fecal Transplant, n.d.; Fecal Transplant, 2022)

Animal models are being utilized too to see the efficacy of ASD and FMT. One study evaluated the effectiveness of FMT on gut microbiota in a propionic acid rat model of autism. The propionic acid shrunk the diversity of the gut microbiome to try to make it comparable to ASD. It was reported that after the treatment use of FMT, the social impairments and the composition of the microbiome increased (Abujamel et al., 2022).

A meta-analysis of current human FMT studies concerning neurological disorders noted an open label, randomized, controlled study with 24 FMT ASD and 24 controlled non-FMTASD patients. The results showed an initial improvement in both ASD symptoms and GI symptoms but reported that the improvements in the symptoms were only temporary (Vendrik et al., 2020).

While human-based studies showed positive results for FMT and ASD, the sample sizes were small, and the first listed ASD FMT study had no control present and therefore are limitations, yet is a topic where future research is needed. A double-blind placebo study with a large ASD population must-see true efficacy and long-term effects. A promising insight into future research was a June 2021 article that searched on clinicaltrials.gov and reported that there are currently 13 FMT investigational studies to evaluate further the efficacy of the transfers in ASD (Tan et al., 2021). With these increased investigational studies, meta-analysis can be completed to see the feasibility, effectiveness, and safety of FMT on ASD individuals to see if this therapeutic action is beneficial. Overall, it can be seen throughout modern research that FMT relieves ASD GI abnormalities symptoms and reduces core symptomology. While the validity and reliability of the normalization of these effects in the ASD population are limited, further

investigation is needed so that there is a greater acceptance of integrating FMT into ASD therapeutic plans.

Probiotics and Prebiotics

The definition of a prebiotic was first most accurately described by Gibson and Roberfriod as" a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" (*Prebiotics and Probiotics in Digestive Health - ClinicalKey*, n.d.). That was a widely used definition in 1995. However, in 2016 the International Scientific Association for Probiotics and Prebiotics altered the definition to "a substrate that is selectively utilized by the host microorganism conferring health benefit" (*Probiotics*, n.d.). The International Scientific Association for Probiotics and Prebiotics and the Food and Agricultural Organization of the World Health Organization both define a probiotic as "a live microorganism which when administered in adequate amounts confer a health benefit on the host" (*Probiotics*, n.d.). In layman's terms, a critical difference between the two is that a probiotic is a living microorganism that is selectively introduced to increase that strain’s concentration in the gut.

In contrast, a prebiotic is abiotic to stimulate the growth of preexisting microbes in the GI tract. The most widely used method for manipulating the gut microbiome is probiotic therapy. Modern literature has shown that probiotics can cause bacteria and other microorganisms to accumulate genetic mutations that turn harmfulness into usefulness (Antushevich, 2020). Both prebiotic and probiotic use is being investigated to

help ASD core symptomology and GI disturbances. When browsing current literature on prebiotics and probiotics, there are many studies but some conflicting but common, comprehensive findings. In Table 3, 13 study findings involve prebiotics and probiotics and the impact on behavior or GI symptoms.

Table 3: Systemic review of modern literature to evaluate the effectiveness of probiotics and prebiotics in ASD.

Subject Population	Study design specifics	Probiotics or Prebiotic	Dose& Duration	Key Results
ASD children[1] (4 - 16 y.o.)	Double blind, placebo controlled, cross over	Lactobacillus plantarum WCSF1	4.5 × 10^10 CFU 1xday for 3 weeks	↑ Enterococci and Lactobacilli group. ↓ Clostridium. Improved stool consistency. Improvement in ASD behaviors.
ASD children[2] (2 - 9 y.o.)	Prospective, open label, controlled	Lactobacillus, Bifidum bacteria, and Streptococcus	One capsule of "Children Dophilus" 3xday for 4 m.o	Positive gut-brain effects, reduced inflammation, and normalized bacteria ratios
ASD children[3] (4 - 10 y.o.)	Randomized, non-control study	Strain Rosell-11	5 × 10^9 CFU/g 2xday for 2 m.o	Improvement in concentration and carrying out orders

[1] Parracho, H. M. R. T., Gibson, G. R., Knott, F., Bosscher, D., Kleerebezem, M., & McCartney, A. L. (2010). A double-blind, placebo-controlled, crossover-designed probiotic feeding study in children diagnosed with Autism Spectrum Disorders. International Journal of Probiotics and Prebiotics, 5(2), 69–74. Retrieved June 5, 2022, from https://www.proquest.com/openview/37c94a7a51197eda67d4fad3b5a8431f/1?pq-origsite=gscholar&cbl=136102

[2] (Tomova et al., 2015)

[3] (Kałużna-Czaplińska & Błaszczyk, 2012)

ASD child[4] (12 y.o.)	Case study, 10 month follow up	B. longum, B. infantis, Lactobacillus acidophilus, L. plantarum, L. paracasei, L. bulgaricus, L. delbrueckii subsp, S. thermophilus, S. salivarius subsp	VSL#3 for 5 m.o	Improvement in ASD symptoms, reduced the severity of abdominal symptoms
ASD Children[5] (2 - 8 y.o.)	A double blind, placebo-controlled, combination, intervention study	Bifidobacterium infantisBi-26, Lactobacillus rhamnosusHN001, Bifidobacterium lactisBL-04, Lactobacillus paracaseiLPC-37, and fructo-oligosaccharide	10^10CFU/pack/day and fructo-oligosaccharide (FOS)	bacterial taxonomy showed no significant variations between groups. ↑ levels of isobutyric acid, valeric acid, isovaleric acid and caproic acid. ↓ in severity of autistic symptoms. ↑ levels of Bifidobacterial and B. longum. ↓levels of Clostridium and Ruminococcin
ASD children[6] (2-11 y.o.)	randomized, double blind, controlled trial of combination treatment	Bifidobacterium infantis, prebiotic oligosaccharides (bovine colostrum product)	50g of bovine colostrum 2xday & 20 billion CFU 1xday, 4 weeks	↓ GI pain and symptoms. Improved stool consistency. ↓ aberrant behaviors. ↓ in IL-13 and TNF-α production. ↓lethargy

[4] (Grossi et al., 2016)
[5] (Y. Wang et al., 2020)
[6] (Sanctuary et al., 2019)

ADHD and Asperberger's[7] (AS)	Longitudal study. Children's gut microbiota was evaluated at 3wk, 3, 6, 12, 18, 24 m.o, and 13 years old.	Lactobacillus rhamnosus GG	N/A	probiotics reduce the risk of the development of ADHD and AS. Not directly associated with gut microbiota composition. No constant microbiota composition was distinctive in children with or without neuro-psychiatric disorders.
ASD Children[8] (5-10 y.o)	Randomized controlled study	prebiotic galactooligosaccharide (B-GOS)	B-GOS orally	↑ bifidobacterial populations. Significantly altered short-chain fatty acid production. ↑ of acetate and butyrate. ↓ propionate
ASD Children[9] (4 - 11 y.o)	randomized, placebo trial	prebiotic B-GOS and exclusion diets: gluten and casein free diets	B-GOS orally	exclusion diet: ↓ abdominal pain and bowel movement. B-GOS intervention: improvements in anti-social behavior
ASD Children[10] (5 - 9 y.o)	prospective, open-label study	Lactobacillus acidophilus, Lactobacillus rhamnosus and Bifidobacteria longum	100×10^6 CFU 1x day for 3 months	↓ body weight. ↓ gastrointestinal symptoms. Improvements in the severity of autism
342 Mothers and their children[11]	randomized, placebo-controlled trial	Lactobacillus rhamnosus strain HN001, Bifidobacterium animalis subsp.	Mother- 35 weeks to 6mt (breast feeding). Infant to	no significant differences in the neurocognitive outcomes

[7] (Pärtty et al., 2015)
[8] (Grimaldi et al., 2017)
[9] (Grimaldi, R., et al. (2018).
[10] (Shaaban et al., 2018)
[11] (Slykerman et al., 2018)

		lactis strain HN019	birth to 2 years	
ASD children[12] (3 - 16 y.o)	Prospective, open-label, controlled	Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii, Bifidobacteria longum, Bifidobacteria bifidum	3xday for 21 days	↓Bifidobacteria and Lactobacillus . ↓TNF-α
ASD individuals [13](3 - 25 y.o)	randomized, double-blind, and placebo-controlled study	Lactobacillus plantarum PS128 and Syntocinon® Spray (Oxytocin)	6×10^10 CFUs 2xday	↓ASD core socio-behavioral symptoms. ↑gut microbiome composition. ↓ GI symptoms. ↓ serum inflammatory markers

As seen in Table 3, many studies have used the fundamental aspects of probiotics and prebiotics to alter the biome to help ASD symptoms. Many studies have seen just that; however, one study that gave pregnant mothers and infants up to 2 years of a probiotic did not see a difference in the prevalence of neurocognitive disorders (Slykerman et al., 2018). Another study did see a difference in the prevalence of ADHD (Attention deficit hyperactivity disorder) and Asperger's Syndrome, a non-DSM-5 disorder that is considered on the autism spectrum, diagnosed with the use of probiotics. However, the mechanism is not related to the microbiome's composition since there was

[12] (Ng et al., 2019)
[13] (Kong et al., 2021)

not a distinct constant difference that was spotted in the age groups that were liked to neither ADHD nor ASD (Mirkovic & Gérardin, 2019; Pärtty et al., 2015).

The case study with the 12-year-old ASD male used the probiotic called VSL#3. This study reported that VSL#3 has shown to have "a direct effect on epithelial barrier function in interleukin-10… This probiotic mixture [VSL#3] maintains tight junctions, prevents apoptosis, … and regulates intestinal epithelial permeability" (Grossi et al., 2016). Another study saw the impact of the probiotics on the increase in SCFAs and inferred that the combinational treatment could help ameliorate leaky gut and dysbiosis microbiota (Y. Wang et al., 2020). The leaky gut that persists in ASD individuals may be helped using this mixture to decrease the inflammation, and immune responses and, therefore, could be a viable therapeutic if further testing proves reliable and accurate. The VSL#3 probiotic case study does have limitations because they are seldom comparable due to the small sample size. Also, this case study's conclusions are not statically significant because of the lack of a control or comparison group. The conclusion of the case-study, also, may have observational bias. While this one study alone is not universally scientifically sound, it helps provide a basis to formulate relevant hypothesizes for later research.

A universal statement cannot be stated about the results of these 13 studies because each used a different strain in its probiotic and therefore are not comparable. However, the studies that did report positive outcomes produced similar consistencies in decreased GI disturbances. This is an area of further exploration to see which strains of

probiotics yield concise results and complete a formalized plan in the therapeutics methods of ASD.

Diets and Supplements

Lastly, diets can be a therapeutic way to positively manipulate the microbiome to affect the gut-brain axis in ASD. As stated previously, ASD individuals are likely to have nutritional deficiencies from stringent food selectivity. Therefore, ensuring that a tailored diet or supplements are filled with fiber, calcium, vitamin D, and vitamin A so ASD individuals can ensure adequate nutritional levels; therefore, consequently there can diminished GI abnormalities.

The ketogenic (keto) diet has been said to help elevate ASD core symptoms. The keto diet is high in fat and low in carbohydrates (Li et al., 2021). A systemic literature review was conducted to see the efficacy of the keto diet in ASD and reported that in the seven studies evaluated, it seemed that keto diets are beneficial; however, the populations are small and cannot be generalized (Li et al., 2021). Interestingly, it was found that the keto diet has anti neuro-inflammatory affects, which might be the mechanism for why ASD symptoms decreased (Jeong et al., 2011).

Omega-3 polyunsaturated fatty acids supplements utilize the anti-inflammatory pathways as well. It is said that Omega-3 fatty acids improve clinical symptoms in youth with ADHD, ASD, and major depressive disorders, especially those with high inflammation or a low baseline omega-3 polyunsaturated fatty acids index. Also, it was

suggested that Omega-3 polyunsaturated fatty acids had been seen to improve lethargy and hyperactivity symptoms in ASD (Chang & Su, 2020).

Lastly, another diet that has been used in the ASD population is a gluten-free and casein-free diet. The mechanism in which gluten and casein affect ASD is proposed through the leaky membrane. Once through the porous membrane, the gluten and casein can undergo inadequate hydrolysis once in combination with other dietary proteins, as an effect the compound can have adverse effects on the CNS (Whiteley et al., 2013). A systemic review was conducted about the efficacy of the diet in the ASD population and cited from 15 studies. Overall, out of the 15, only eight studies have shown either beneficial improvements in ASD behavior, gastrointestinal symptoms, or intestinal permeability (González-Domenech et al., 2022). It should also be noted that long-term use of the gluten-free and casein-free diet is seen to lead to micronutrient deficiencies (Baspinar & Yardimci, 2020). In fact, it is been observed in ASD children on the casein-free diet to have slower bone development (Marí-Bauset et al., 2016). Additionally, it has been reported that peripubertal boys with ASD have lower bone mineral density than typically developing controls, and low bone mineral density has been associated with increased bone fracture (Neumeyer et al., 2015). Therefore, it is also essential to monitor serum levels to ensure nutrients are adequate. The small study patient populations and incongruent results cannot yet allow the gluten-free and casein-free diet to be a standardized plan for ASD treatment. However, further exploration can lead to promising results.

Anti-Bacterial Monoclonal Antibodies

Antibiotic therapy could be therapeutic for ASD, which can be done by targeting *Faecalibacterium* and *Clostridium*, which have been seen to have adverse GI and behavioral outcomes. It was reported in an antibiotic therapy study with a 14-year-old ASD male who completed a ten-day metronidazole course followed by a 20-day course of ketoconazole. The results were improvements in aberrant behaviors and comorbid GI symptomology; however, the long-term benefits were non-existent since the improvements dissipated after two weeks of not being on the antibiotics (Ramirez et al., 2013). Also, this model of antibiotic therapy is not sustainable due to the rapid rise of antimicrobial resistance.

Antimicrobial resistance is a global issue that leads the microorganism to resist the effects of the drug; consequently, the FDA reports that in the United States alone, there are at least 2.8 million antibiotic-resistant infections and 35,000 deaths each year from this global issue (FDA, 2022). One of the main contributors to antimicrobial resistance is the overuse of the drug, and therefore, can infer that the continuous use of antimicrobials in the ASD community is not a sustainable therapeutic because of the increased risk of infection and contributing to this global issue (*Antimicrobial Resistance*, n.d.).

Innovative techniques such as monoclonal antibodies have been evolving, and their range for therapeutic indications has rapidly expanded. Human monoclonal antibodies, Hu-mAbs, have been used for anti-cancer, autoimmune, and antiviral

indications. However, recently, Hu-mAbs have been investigated for mechanisms that target bacteria. Hu-mAbs aims to target specific bacterial populations without altering the overall microbiome composition and harming the host (Jones-Nelson et al., 2020; Nagy et al., 2017). Although the anti-bacterial monoclonal antibodies address the co-morbidity symptoms of ASD, not the underlying mechanism.

For example, Bezlotoxumab is a successful antibacterial Hu-mAbs used for recurrent C difficile infection (Kollef & Betthauser, 2021). In addition, virulence factors are some targets rather than proteins. Therefore, there is less chance of antimicrobial resistance since the bacteria does not face the direct obstacle of genetic pressure to survive by mutations (Nagy et al., 2017; Zurawski & McLendon, 2020). Another form of Hu-mAbs for bacteria is those that target gram-negative bacteria through monoclonal anti-endotoxin antibodies (Baumgartner, 1990).

The current research and findings of antibacterial and antimicrobial monoclonal antibodies is an area that deserves greater attention. This could be a non-traditional use of Hu-mAbs if the harmful, abundant bacteria in ASD are specifically targeted. The research has already stated that the rest of the microbiome would not be disturbed. The toxic metabolites from these bacteria could no longer cause GI disturbances and halt the negative implication on the gut-microbiota-brain axis that could improve core ASD symptomology and behaviors.

Additionally, some cytokines are targets for therapeutic drugs. As stated previously, ASD serum shows significant higher levels of proinflammatory cytokine levels: IL-6, IL-1β, IL-7, IL-12, IL-17 IL-6, IL-5, TNFα, and TGFβ (Robinson-

Agramonte et al., 2022; Vuong & Hsiao, 2017; Zhao et al., 2021). Therefore, targeting those cytokines can be a potential therapeutic for ASD by decreasing the inflammation and the inflammatory consequences. Currently, some anti-inflammatory agents have been used successfully in inflammatory bowel disease, which has a mechanism to block similar cytokines as ASD and protect the epithelium (Katsanos & Papadakis, 2017). Therefore, it needs to be further studied to see how these drugs could have a role in ASD symptomology since ASD is a CNS and GI inflammatory disease (Matta et al., 2019). At the moment, little to no research is looking at the association between Hu-mAbs and ASD, but this contemporary therapeutic technique may help manipulate the biome and help address the GI disturbances and atypical immune response that are co-morbid symptoms of ASD that can improve the quality of life.

While there is no FDA approved Hu-mAbs for ASD, in the future one needs to evaluate the risk-benefit profile of Hu-mAbs. There needs to be an individualized approach to any therapeutic and assess the risk-benefit profile. Again, no research has been conducted on Hu-mAbs, but it might be a pathway that could lead to benefits diminishing ASD co-morbid GI symptoms.

DISCUSSION AND CONCLUSION

The ASD population is likelier to have an atypical microbiome, leaky gut, and an increased systemic inflammatory immune response. ASD individuals can have worsened core symptomology and increased GI disturbances through the bidirectional gut-microbiota-brain axis. Throughout this thesis, there have been a lot of proposed effects of change in the biome with implications on the host's health, but even more sections of which needed more investigation.

It is clear that the microbiome in ASD is atypical and leads to systemic implications. Therefore, several modern therapeutics aim to manipulate the microbiota, including fecal microbiota transfer, probiotics, prebiotics, diet changes, and supplements. In addition, forward-thinking approaches include antibacterial monoclonal antibodies and anti-cytokine targets.

Overall, more research needs to be conducted to determine if ASD pathology leads to a leaky gut and consequently the change in the biome or if the habits of ASD lead to an atypical microbiome that fosters toxic bacteria. Nevertheless, this microbiome is an area of concern that needs to be addressed to increase the quality of life of ASD individuals, their family, and their caregivers. Within today’s data, it is showing inconclusive conclusions. Most of the studies named above have limitations because of the small population sizes, inconsistent age ranges, and mainly male dominated. Therefore, it is hard to form a generalized conclusion.

One cannot say with absolute certainty that the gut microbiota is impacting the gut-microbiota-brain-axis communication that is worsening ASD symptoms through GI

disturbances. The present-day evidence limits the generalizability and reliability. However, ample research has been conducted on this topic and there is some underlying mechanism that involves the gut and ASD that must be at play for such research to keep taking place. At the moment, one can say that the only answer available is conducting more research to find the root cause.

There is a plethora of opportunities for more investigational research on ASD and the implication of the atypical microbiota composition. More profound research will allow for more concise findings so that a standard plan of treatment of ASD can be implemented. Then that plan can advance further to promote an individual treatment plan since ASD is on a spectrum and the symptomology is unique per person. Precision and tailorized therapeutics are what modern medicine is emphasizing since treatment plans are not a one size fits all approach. Once seeing which individuals have a highly regarded theorized bacteria strain to promote disruptive ASD behavior and symptoms, an effective detailed treatment plan can address the specifics without jeopardy to the rest of the biome. For example, if research found that severe ASD is associated with high levels of *Faecalibacterium* then the treatment plan would be through Hu-mAbs because research would have hypothetically shown that be the most universal way to decrease *Faecalibacterium* is through the antibacterial monoclonal antibodies.

The futurist goal is to learn the specific repercussions of specific bacteria in ASD associated symptomology and then the impact on the bacteria with different approaches. Currently, research is not adequately addressing this approach since so much is still unknown about ASD and the gut-microbiota-brain axis. Nevertheless, modern research

displays that manipulation of the microbiome does bring promising results on the symptomology of ASD in various ways; however, there cannot yet be overwhelming support for a particular method because of the variations and inconsistencies. Regardless, the technological advancements and the increasing awareness of ASD bring hope to the future of ASD therapeutics.

BIBLIOGRAPHY

Abujamel, T. S., Al-Otaibi, N. M., Abuaish, S., AlHarbi, R. H., Assas, M. B., Alzahrani, S. A., Alotaibi, S. M., El-Ansary, A., & Aabed, K. (2022). Different Alterations in Gut Microbiota between Bifidobacterium longum and Fecal Microbiota Transplantation Treatments in Propionic Acid Rat Model of Autism. Nutrients, 14(3), 608. https://doi.org/10.3390/nu14030608

Adams, J. B., Johansen, L. J., Powell, L. D., Quig, D., & Rubin, R. A. (2011). Gastrointestinal flora and gastrointestinal status in children with autism—Comparisons to typical children and correlation with autism severity. BMC Gastroenterology, 11, 22. https://doi.org/10.1186/1471-230X-11-22

Agus, A., Planchais, J., & Sokol, H. (2018). Gut Microbiota Regulation of Tryptophan Metabolism in Health and Disease. Cell Host & Microbe, 23(6), 716–724. https://doi.org/10.1016/j.chom.2018.05.003

Ahearn, W. H., Castine, T., Nault, K., & Green, G. (2001). An assessment of food acceptance in children with autism or pervasive developmental disorder-not otherwise specified. Journal of Autism and Developmental Disorders, 31(5), 505–511. https://doi.org/10.1023/a:1012221026124

Al-Ayadhi, L., Zayed, N., Bhat, R. S., Moubayed, N. M. S., Al-Muammar, M. N., & El-Ansary, A. (2021). The use of biomarkers associated with leaky gut as a diagnostic tool for early intervention in autism spectrum disorder: A systematic review. Gut Pathogens, 13, 54. https://doi.org/10.1186/s13099-021-00448-y

Antimicrobial resistance. (n.d.). Retrieved June 5, 2022, from https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

Antushevich, H. (2020). Fecal microbiota transplantation in disease therapy. Clinica Chimica Acta, 503, 90–98. https://doi.org/10.1016/j.cca.2019.12.010

Autism Spectrum Disorder Fact Sheet | National Institute of Neurological Disorders and Stroke. (n.d.). Retrieved July 6, 2022, from https://www.ninds.nih.gov/health-information/patient-caregiver-education/fact-sheets/autism-spectrum-disorder-fact-sheet

Bandini, L. G., Anderson, S. E., Curtin, C., Cermak, S., Evans, E. W., Scampini, R., Maslin, M., & Must, A. (2010). Food selectivity in children with autism spectrum disorders and typically developing children. The Journal of Pediatrics, 157(2), 259–264. https://doi.org/10.1016/j.jpeds.2010.02.013

Bandini, L. G., Curtin, C., Phillips, S., Anderson, S. E., Maslin, M., & Must, A. (2017). Changes in Food Selectivity in Children with Autism Spectrum Disorder. Journal of Autism and Developmental Disorders, 47(2), 439–446. https://doi.org/10.1007/s10803-016-2963-6

Baspinar, B., & Yardimci, H. (2020). Gluten-Free Casein-Free Diet for Autism Spectrum Disorders: Can It Be Effective in Solving Behavioural and Gastrointestinal Problems? The Eurasian Journal of Medicine, 52(3), 292–297. https://doi.org/10.5152/eurasianjmed.2020.19230

Baumgartner, J. D. (1990). Monoclonal anti-endotoxin antibodies for the treatment of gram-negative bacteremia and septic shock. European Journal of Clinical Microbiology & Infectious Diseases, 9(10), 711–716. https://doi.org/10.1007/BF02184682

Bercik, P., Park, A. J., Sinclair, D., Khoshdel, A., Lu, J., Huang, X., Deng, Y., Blennerhassett, P. A., Fahnestock, M., Moine, D., Berger, B., Huizinga, J. D., Kunze, W., McLean, P. G., Bergonzelli, G. E., Collins, S. M., & Verdu, E. F. (2011). The anxiolytic effect of Bifidobacterium longum NCC3001 involves vagal pathways for gut–brain communication. Neurogastroenterology & Motility, 23(12), 1132–1139. https://doi.org/10.1111/j.1365-2982.2011.01796.x

Bonaz, B., Bazin, T., & Pellissier, S. (2018). The Vagus Nerve at the Interface of the Microbiota-Gut-Brain Axis. Frontiers in Neuroscience, 12. https://www.frontiersin.org/article/10.3389/fnins.2018.00049

Bruce-Keller, A. J., Salbaum, J. M., & Berthoud, H.-R. (2018). Harnessing Gut Microbes for Mental Health: Getting From Here to There. Biological Psychiatry, 83(3), 214–223. https://doi.org/10.1016/j.biopsych.2017.08.014

Calcium and vitamin D combination Uses, Side Effects & Warnings. (n.d.). Drugs.Com. Retrieved June 1, 2022, from https://www.drugs.com/mtm/calcium-and-vitamin-d-combination.html

Cassani, B., Villablanca, E. J., De Calisto, J., Wang, S., & Mora, J. R. (2012). Vitamin A and immune regulation: Role of retinoic acid in gut-associated dendritic cell education, immune protection and tolerance. Molecular Aspects of Medicine, 33(1), 63–76. https://doi.org/10.1016/j.mam.2011.11.001

CDC. (2022a, March 9). Treatment and Intervention Services for Autism Spectrum Disorder. Centers for Disease Control and Prevention. https://www.cdc.gov/ncbddd/autism/treatment.html

CDC. (2022b, March 31). Screening and Diagnosis | Autism Spectrum Disorder (ASD) | NCBDDD. Centers for Disease Control and Prevention. https://www.cdc.gov/ncbddd/autism/screening.html

Chaidez, V., Hansen, R. L., & Hertz-Picciotto, I. (2014). Gastrointestinal problems in children with autism, developmental delays or typical development. Journal of Autism and Developmental Disorders, 44(5), 1117–1127. https://doi.org/10.1007/s10803-013-1973-x

Chang, J. P.-C., & Su, K.-P. (2020). Nutritional Neuroscience as Mainstream of Psychiatry: The Evidence-Based Treatment Guidelines for Using Omega-3 Fatty Acids as a New Treatment for Psychiatric Disorders in Children and Adolescents. Clinical Psychopharmacology and Neuroscience, 18(4), 469–483. https://doi.org/10.9758/cpn.2020.18.4.469

Chaste, P., & Leboyer, M. (2012). Autism risk factors: Genes, environment, and gene-environment interactions. Dialogues in Clinical Neuroscience, 14(3), 281–292.

Chen, S., Zhong, X., Jiang, L., Zheng, X., Xiong, Y., Ma, S., Qiu, M., Huo, S., Ge, J., & Chen, Q. (2016). Maternal autoimmune diseases and the risk of autism spectrum disorders in offspring: A systematic review and meta-analysis. Behavioural Brain Research, 296, 61–69. https://doi.org/10.1016/j.bbr.2015.08.035

Constantino, J. N., & Todd, R. D. (2003). Autistic traits in the general population: A twin study. Archives of General Psychiatry, 60(5), 524–530. https://doi.org/10.1001/archpsyc.60.5.524

Curran, E. A., Cryan, J. F., Kenny, L. C., Dinan, T. G., Kearney, P. M., & Khashan, A. S. (2016). Obstetrical Mode of Delivery and Childhood Behavior and Psychological Development in a British Cohort. Journal of Autism and Developmental Disorders, 46(2), 603–614. https://doi.org/10.1007/s10803-015-2616-1

d'Enfert, C., Kaune, A.-K., Alaban, L.-R., Chakraborty, S., Cole, N., Delavy, M., Kosmala, D., Marsaux, B., Fróis-Martins, R., Morelli, M., Rosati, D., Valentine, M., Xie, Z., Emritloll, Y., Warn, P. A., Bequet, F., Bougnoux, M.-E., Bornes, S., Gresnigt, M. S., … Brown, A. J. P. (2020). The impact of the Fungus-Host-Microbiota interplay upon Candida albicans infections: Current knowledge and new perspectives. FEMS Microbiology Reviews, 45(3), fuaa060. https://doi.org/10.1093/femsre/fuaa060

Dan, Z., Mao, X., Liu, Q., Guo, M., Zhuang, Y., Liu, Z., Chen, K., Chen, J., Xu, R., Tang, J., Qin, L., Gu, B., Liu, K., Su, C., Zhang, F., Xia, Y., Hu, Z., & Liu, X. (n.d.). Altered gut microbial profile is associated with abnormal metabolism activity of Autism Spectrum Disorder. Gut Microbes, 11(5), 1246–1267. https://doi.org/10.1080/19490976.2020.1747329

Dinan, T. G., & Cryan, J. F. (2017). Gut instincts: Microbiota as a key regulator of brain development, ageing and neurodegeneration. The Journal of Physiology, 595(2), 489–503. https://doi.org/10.1113/JP273106

Dominguez-Bello, M. G., Costello, E. K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., & Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proceedings of the National Academy of Sciences of the United States of America, 107(26), 11971–11975. https://doi.org/10.1073/pnas.1002601107

Eissa, N., Al-Houqani, M., Sadeq, A., Ojha, S. K., Sasse, A., & Sadek, B. (2018). Current Enlightenment About Etiology and Pharmacological Treatment of Autism Spectrum Disorder. Frontiers in Neuroscience, 12, 304. https://doi.org/10.3389/fnins.2018.00304

Erny, D., Hrabě de Angelis, A. L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., Keren-Shaul, H., Mahlakoiv, T., Jakobshagen, K., Buch, T., Schwierzeck, V., Utermöhlen, O., Chun, E., Garrett, W. S., McCoy, K. D., Diefenbach, A., Staeheli, P., Stecher, B., Amit, I., & Prinz, M. (2015). Host microbiota constantly control maturation and function of microglia in the CNS. Nature Neuroscience, 18(7), 965–977. https://doi.org/10.1038/nn.4030

Fasano, A. (2012). Leaky Gut and Autoimmune Diseases. Clinical Reviews in Allergy & Immunology, 42(1), 71–78. https://doi.org/10.1007/s12016-011-8291-x

Fattorusso, A., Di Genova, L., Dell'Isola, G. B., Mencaroni, E., & Esposito, S. (2019). Autism Spectrum Disorders and the Gut Microbiota. Nutrients, 11(3), 521. https://doi.org/10.3390/nu11030521

FDA (Food and Drug Administration) (2022). Antimicrobial Resistance Information from FDA. https://www.fda.gov/emergency-preparedness-and-response/mcm-issues/antimicrobial-resistance-information-fda

Fecal Transplant. (n.d.). Endoscopy Center of Red Bank. Retrieved June 1, 2022, from https://endoscopycenterofredbank.com/procedure/fecal-transplant

Fecal Transplant. (2022, April 4). https://www.hopkinsmedicine.org/health/treatment-tests-and-therapies/fecal-transplant

Ferguson, B. J., Dovgan, K., Takahashi, N., & Beversdorf, D. Q. (2019). The Relationship Among Gastrointestinal Symptoms, Problem Behaviors, and Internalizing Symptoms in Children and Adolescents With Autism Spectrum Disorder. Frontiers in Psychiatry, 10, 194. https://doi.org/10.3389/fpsyt.2019.00194

Folstein, S., & Rutter, M. (1977). Infantile autism: A genetic study of 21 twin pairs. Journal of Child Psychology and Psychiatry, and Allied Disciplines, 18(4), 297–321. https://doi.org/10.1111/j.1469-7610.1977.tb00443.x

Fowlie, G., Cohen, N., & Ming, X. (2018). The Perturbance of Microbiome and Gut-Brain Axis in Autism Spectrum Disorders. International Journal of Molecular Sciences, 19(8), 2251. https://doi.org/10.3390/ijms19082251

Fulceri, F., Morelli, M., Santocchi, E., Cena, H., Bianco, T. D., Narzisi, A., Calderoni, S., & Muratori, F. (2016). Gastrointestinal symptoms and behavioral problems in preschoolers with Autism Spectrum Disorder. Digestive and Liver Disease, 48(3), 248–254. https://doi.org/10.1016/j.dld.2015.11.026

Genovese, A., & Butler, M. G. (2020). Clinical Assessment, Genetics, and Treatment Approaches in Autism Spectrum Disorder (ASD). International Journal of Molecular Sciences, 21(13), 4726. https://doi.org/10.3390/ijms21134726

González-Domenech, P. J., Diaz-Atienza, F., Gutiérrez-Rojas, L., Fernández-Soto, M. L., & González-Domenech, C. M. (2022). A Narrative Review about Autism Spectrum Disorders and Exclusion of Gluten and Casein from the Diet. Nutrients, 14(9), 1797. https://doi.org/10.3390/nu14091797

Grimaldi, R., Cela, D., Swann, J. R., Vulevic, J., Gibson, G. R., Tzortzis, G., & Costabile, A. (2017). In vitro fermentation of B-GOS: Impact on faecal bacterial populations and metabolic activity in autistic and non-autistic children. FEMS Microbiology Ecology, 93(2), fiw233. https://doi.org/10.1093/femsec/fiw233

Grimaldi, R., et al. (2018). A prebiotic intervention study in children with autism spectrum disorders (ASDs). Microbiome 6, 133. Retrieved June 5, 2022, from https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-018-0523-3

Grossi, E., Melli, S., Dunca, D., & Terruzzi, V. (2016). Unexpected improvement in core autism spectrum disorder symptoms after long-term treatment with probiotics. SAGE Open Medical Case Reports, 4, 2050313X16666231. https://doi.org/10.1177/2050313X16666231

Haase, S., Haghikia, A., Wilck, N., Müller, D. N., & Linker, R. A. (2018). Impacts of microbiome metabolites on immune regulation and autoimmunity. Immunology, 154(2), 230–238. https://doi.org/10.1111/imm.12933

Hamer, H. M., Jonkers, D., Venema, K., Vanhoutvin, S., Troost, F. J., & Brummer, R.-J. (2008). Review article: The role of butyrate on colonic function. Alimentary Pharmacology & Therapeutics, 27(2), 104–119. https://doi.org/10.1111/j.1365-2036.2007.03562.x

Herman, A., & Herman, A. P. (2022). Could Candida Overgrowth Be Involved in the Pathophysiology of Autism? Journal of Clinical Medicine, 11(2), 442. https://doi.org/10.3390/jcm11020442

Ho, K. S., Wassman, E. R., Baxter, A. L., Hensel, C. H., Martin, M. M., Prasad, A., Twede, H., Vanzo, R. J., & Butler, M. G. (2016). Chromosomal Microarray Analysis of Consecutive Individuals with Autism Spectrum Disorders Using an Ultra-High Resolution Chromosomal Microarray Optimized for Neurodevelopmental Disorders. International Journal of Molecular Sciences, 17(12), 2070. https://doi.org/10.3390/ijms17122070

Hu, Z., Xiao, X., Zhang, Z., & Li, M. (2019). Genetic insights and neurobiological implications from NRXN1 in neuropsychiatric disorders. Molecular Psychiatry, 24(10), 1400–1414. https://doi.org/10.1038/s41380-019-0438-9

Hua, X., Zhu, J., Yang, T., Guo, M., Li, Q., Chen, J., & Li, T. (2020). The Gut Microbiota and Associated Metabolites Are Altered in Sleep Disorder of Children With Autism Spectrum Disorders. Frontiers in Psychiatry, 11. https://www.frontiersin.org/article/10.3389/fpsyt.2020.00855

Jeong, E. A., Jeon, B. T., Shin, H. J., Kim, N., Lee, D. H., Kim, H. J., Kang, S. S., Cho, G. J., Choi, W. S., & Roh, G. S. (2011). Ketogenic diet-induced peroxisome proliferator-activated receptor-γ activation decreases neuroinflammation in the mouse hippocampus after kainic acid-induced seizures. Experimental Neurology, 232(2), 195–202. https://doi.org/10.1016/j.expneurol.2011.09.001

Jones-Nelson, O., Tovchigrechko, A., Glover, M. S., Fernandes, F., Rangaswamy, U., Liu, H., Tabor, D. E., Boyd, J., Warrener, P., Martinez, J., Hilliard, J. J., Stover, C. K., Yu, W., DAngelo, G., Hess, S., Cohen, T. S., & Sellman, B. R. (2020). Antibacterial Monoclonal Antibodies Do Not Disrupt the Intestinal Microbiome or Its Function. Antimicrobial Agents and Chemotherapy, 64(5), e02347-19. https://doi.org/10.1128/AAC.02347-19

Kałużna-Czaplińska, J., & Błaszczyk, S. (2012). The level of arabinitol in autistic children after probiotic therapy. Nutrition, 28(2), 124–126. https://doi.org/10.1016/j.nut.2011.08.002

Kang, D.-W., Adams, J. B., Coleman, D. M., Pollard, E. L., Maldonado, J., McDonough-Means, S., Caporaso, J. G., & Krajmalnik-Brown, R. (2019). Long-term benefit of Microbiota Transfer Therapy on autism symptoms and gut microbiota. Scientific Reports, 9(1), 5821. https://doi.org/10.1038/s41598-019-42183-0

Kang, D.-W., Adams, J. B., Gregory, A. C., Borody, T., Chittick, L., Fasano, A., Khoruts, A., Geis, E., Maldonado, J., McDonough-Means, S., Pollard, E. L., Roux, S., Sadowsky, M. J., Lipson, K. S., Sullivan, M. B., Caporaso, J. G., & Krajmalnik-

Brown, R. (2017). Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: An open-label study. Microbiome, 5(1), 10. https://doi.org/10.1186/s40168-016-0225-7

Kang, D.-W., Ilhan, Z. E., Isern, N. G., Hoyt, D. W., Howsmon, D. P., Shaffer, M., Lozupone, C. A., Hahn, J., Adams, J. B., & Krajmalnik-Brown, R. (2018). Differences in fecal microbial metabolites and microbiota of children with autism spectrum disorders. Anaerobe, 49, 121–131. https://doi.org/10.1016/j.anaerobe.2017.12.007

Katsanos, K. H., & Papadakis, K. A. (2017). Inflammatory Bowel Disease: Updates on Molecular Targets for Biologics. Gut and Liver, 11(4), 455–463. https://doi.org/10.5009/gnl16308

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. (2016). From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. Cell, 165(6), 1332–1345. https://doi.org/10.1016/j.cell.2016.05.041

Kollef, M. H., & Betthauser, K. D. (2021). Monoclonal antibodies as antibacterial therapies: Thinking outside of the box. The Lancet. Infectious Diseases, 21(9), 1201–1202. https://doi.org/10.1016/S1473-3099(21)00062-1

Kolodny, T., Schallmo, M.-P., Gerdts, J., Edden, R. A. E., Bernier, R. A., & Murray, S. O. (2020). Concentrations of Cortical GABA and Glutamate in Young Adults With Autism Spectrum Disorder. Autism Research, 13(7), 1111–1129. https://doi.org/10.1002/aur.2300

Kong, X.-J., Liu, J., Liu, K., Koh, M., Sherman, H., Liu, S., Tian, R., Sukijthamapan, P., Wang, J., Fong, M., Xu, L., Clairmont, C., Jeong, M.-S., Li, A., Lopes, M., Hagan, V., Dutton, T., Chan, S.-T. (Phoebe), Lee, H., … Song, Y. (2021). Probiotic and Oxytocin Combination Therapy in Patients with Autism Spectrum Disorder: A Randomized, Double-Blinded, Placebo-Controlled Pilot Trial. Nutrients, 13(5), 1552. https://doi.org/10.3390/nu13051552

Labus, J. S., Osadchiy, V., Hsiao, E. Y., Tap, J., Derrien, M., Gupta, A., Tillisch, K., Le Nevé, B., Grinsvall, C., Ljungberg, M., Öhman, L., Törnblom, H., Simren, M., & Mayer, E. A. (2019). Evidence for an association of gut microbial Clostridia with brain functional connectivity and gastrointestinal sensorimotor function in patients with irritable bowel syndrome, based on tripartite network analysis. Microbiome, 7(1), 45. https://doi.org/10.1186/s40168-019-0656-z

Lammert, C. R., Frost, E. L., Bolte, A. C., Paysour, M. J., Shaw, M. E., Bellinger, C. E., Weigel, T. K., Zunder, E. R., & Lukens, J. R. (2018). Cutting Edge: Critical Roles for Microbiota-Mediated Regulation of the Immune System in a Prenatal Immune

Activation Model of Autism. The Journal of Immunology, 201(3), 845–850. https://doi.org/10.4049/jimmunol.1701755

Larroya-García, A., Navas-Carrillo, D., & Orenes-Piñero, E. (2019). Impact of gut microbiota on neurological diseases: Diet composition and novel treatments. Critical Reviews in Food Science and Nutrition, 59(19), 3102–3116. https://doi.org/10.1080/10408398.2018.1484340

Lee, E., Cho, J., & Kim, K. Y. (2019). The Association between Autism Spectrum Disorder and Pre- and Postnatal Antibiotic Exposure in Childhood-A Systematic Review with Meta-Analysis. International Journal of Environmental Research and Public Health, 16(20), E4042. https://doi.org/10.3390/ijerph16204042

Leylabadlo, H. E., Ghotaslou, R., Feizabadi, M. M., Farajnia, S., Moaddab, S. Y., Ganbarov, K., Khodadadi, E., Tanomand, A., Sheykhsaran, E., Yousefi, B., & Kafil, H. S. (2020). The critical role of Faecalibacterium prausnitzii in human health: An overview. Microbial Pathogenesis, 149, 104344. https://doi.org/10.1016/j.micpath.2020.104344

Li, Q., Liang, J., Fu, N., Han, Y., & Qin, J. (2021). A Ketogenic Diet and the Treatment of Autism Spectrum Disorder. Frontiers in Pediatrics, 9, 650624. https://doi.org/10.3389/fped.2021.650624

Liu, X., Liu, J., Xiong, X., Yang, T., Hou, N., Liang, X., Chen, J., Cheng, Q., & Li, T. (2016). Correlation between Nutrition and Symptoms: Nutritional Survey of Children with Autism Spectrum Disorder in Chongqing, China. Nutrients, 8(5), 294. https://doi.org/10.3390/nu8050294

Luna, R. A., Oezguen, N., Balderas, M., Venkatachalam, A., Runge, J. K., Versalovic, J., Veenstra-VanderWeele, J., Anderson, G. M., Savidge, T., & Williams, K. C. (2017). Distinct Microbiome-Neuroimmune Signatures Correlate With Functional Abdominal Pain in Children With Autism Spectrum Disorder. Cellular and Molecular Gastroenterology and Hepatology, 3(2), 218–230. https://doi.org/10.1016/j.jcmgh.2016.11.008

Madra, M., Ringel, R., & Margolis, K. G. (2020). Gastrointestinal issues and Autism Spectrum Disorder. Child and Adolescent Psychiatric Clinics of North America, 29(3), 501–513. https://doi.org/10.1016/j.chc.2020.02.005

Marí-Bauset, S., Llopis-González, A., Zazpe, I., Marí-Sanchis, A., & Suárez-Varela, M. M. (2016). Nutritional Impact of a Gluten-Free Casein-Free Diet in Children with Autism Spectrum Disorder. Journal of Autism and Developmental Disorders, 46(2), 673–684. https://doi.org/10.1007/s10803-015-2582-7

Martel, J., Chang, S.-H., Ko, Y.-F., Hwang, T.-L., Young, J. D., & Ojcius, D. M. (2022). Gut barrier disruption and chronic disease. Trends in Endocrinology & Metabolism, 33(4), 247–265. https://doi.org/10.1016/j.tem.2022.01.002

Masini, E., Loi, E., Vega-Benedetti, A. F., Carta, M., Doneddu, G., Fadda, R., & Zavattari, P. (2020). An Overview of the Main Genetic, Epigenetic and Environmental Factors Involved in Autism Spectrum Disorder Focusing on Synaptic Activity. International Journal of Molecular Sciences, 21(21), 8290. https://doi.org/10.3390/ijms21218290

Masuda, F., Nakajima, S., Miyazaki, T., Yoshida, K., Tsugawa, S., Wada, M., Ogyu, K., Croarkin, P. E., Blumberger, D. M., Daskalakis, Z. J., Mimura, M., & Noda, Y. (2019). Motor cortex excitability and inhibitory imbalance in autism spectrum disorder assessed with transcranial magnetic stimulation: A systematic review. Translational Psychiatry, 9(1), 110. https://doi.org/10.1038/s41398-019-0444-3

Matenchuk, B. A., Mandhane, P. J., & Kozyrskyj, A. L. (2020). Sleep, circadian rhythm, and gut microbiota. Sleep Medicine Reviews, 53, 101340. https://doi.org/10.1016/j.smrv.2020.101340

Matta, S. M., Hill-Yardin, E. L., & Crack, P. J. (2019). The influence of neuroinflammation in Autism Spectrum Disorder. Brain, Behavior, and Immunity, 79, 75–90. https://doi.org/10.1016/j.bbi.2019.04.037

Mawe, G. M., & Hoffman, J. M. (2013). Serotonin signalling in the gut—Functions, dysfunctions and therapeutic targets. Nature Reviews. Gastroenterology & Hepatology, 10(8), 473–486. https://doi.org/10.1038/nrgastro.2013.105

McCormick, D. A. (1989). GABA as an inhibitory neurotransmitter in human cerebral cortex. Journal of Neurophysiology, 62(5), 1018–1027. https://doi.org/10.1152/jn.1989.62.5.1018

McCullough, F. S. W., Northrop-Clewes, C. A., & Thurnham, D. I. (1999). The effect of vitamin A on epithelial integrity. Proceedings of the Nutrition Society, 58(2), 289–293. https://doi.org/10.1017/S0029665199000403

Meltzer, A., & Van de Water, J. (2017). The Role of the Immune System in Autism Spectrum Disorder. Neuropsychopharmacology, 42(1), 284–298. https://doi.org/10.1038/npp.2016.158

Mirkovic, B., & Gérardin, P. (2019). Asperger's syndrome: What to consider? L'Encephale, 45(2), 169–174. https://doi.org/10.1016/j.encep.2018.11.005

Mostafa, G. A., & AL-Ayadhi, L. Y. (2012). Reduced serum concentrations of 25-hydroxy vitamin D in children with autism: Relation to autoimmunity. Journal of Neuroinflammation, 9(1), 201. https://doi.org/10.1186/1742-2094-9-201

Nagy, E., Nagy, G., Power, C. A., Badarau, A., & Szijártó, V. (2017). Anti-bacterial Monoclonal Antibodies. In T. S. Lim (Ed.), Recombinant Antibodies for Infectious Diseases (pp. 119–153). Springer International Publishing. https://doi.org/10.1007/978-3-319-72077-7_7

Neumeyer, A. M., O'Rourke, J. A., Massa, A., Lee, H., Lawson, E. A., McDougle, C. J., & Misra, M. (2015). Brief Report: Bone Fractures in Children and Adults with Autism Spectrum Disorders. Journal of Autism and Developmental Disorders, 45(3), 881–887. https://doi.org/10.1007/s10803-014-2228-1

Ng, Q. X., Loke, W., Venkatanarayanan, N., Lim, D. Y., Soh, A. Y. S., & Yeo, W. S. (2019). A Systematic Review of the Role of Prebiotics and Probiotics in Autism Spectrum Disorders. Medicina, 55(5), 129. https://doi.org/10.3390/medicina55050129

Noriega, D. B., & Savelkoul, H. F. J. (2014). Immune dysregulation in autism spectrum disorder. European Journal of Pediatrics, 173(1), 33–43. https://doi.org/10.1007/s00431-013-2183-4

Obrenovich, M. E. M. (2018). Leaky Gut, Leaky Brain? Microorganisms, 6(4), 107. https://doi.org/10.3390/microorganisms6040107

Parekh, P. J., Balart, L. A., & Johnson, D. A. (2015). The Influence of the Gut Microbiome on Obesity, Metabolic Syndrome and Gastrointestinal Disease. Clinical and Translational Gastroenterology, 6, e91. https://doi.org/10.1038/ctg.2015.16

Parracho, H. M. R. T., Gibson, G. R., Knott, F., Bosscher, D., Kleerebezem, M., & McCartney, A. L. (2010). A double-blind, placebo-controlled, crossover-designed probiotic feeding study in children diagnosed with Autism Spectrum Disorders. International Journal of Probiotics and Prebiotics, 5(2), 69–74. Retrieved June 5, 2022, from https://www.proquest.com/openview/37c94a7a51197eda67d4fad3b5a8431f/1?pq-origsite=gscholar&cbl=136102

Pärtty, A., Kalliomäki, M., Wacklin, P., Salminen, S., & Isolauri, E. (2015). A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: A randomized trial. Pediatric Research, 77(6), 823–828. https://doi.org/10.1038/pr.2015.51

Prebiotics and Probiotics in Digestive Health—ClinicalKey. (n.d.). Retrieved June 5, 2022, from https://www-clinicalkey-com.ezproxy.bu.edu/#!/content/playContent/1-s2.0-S154235651831019X?scrollTo=%23bib23

Probiotics. (n.d.). International Scientific Association for Probiotics and Prebiotics (ISAPP). Retrieved June 5, 2022, from https://isappscience.org/for-scientists/resources/probiotics/

Pulikkan, J., Mazumder, A., & Grace, T. (2019). Role of the Gut Microbiome in Autism Spectrum Disorders. Advances in Experimental Medicine and Biology, 1118, 253–269. https://doi.org/10.1007/978-3-030-05542-4_13

Ramirez, P. L., Barnhill, K., Gutierrez, A., Schutte, C., & Hewitson, L. (2013). Improvements in Behavioral Symptoms following Antibiotic Therapy in a 14-Year-Old Male with Autism. Case Reports in Psychiatry, 2013, e239034. https://doi.org/10.1155/2013/239034

Ristori, M. V., Quagliariello, A., Reddel, S., Ianiro, G., Vicari, S., Gasbarrini, A., & Putignani, L. (2019). Autism, Gastrointestinal Symptoms and Modulation of Gut Microbiota by Nutritional Interventions. Nutrients, 11(11), 2812. https://doi.org/10.3390/nu11112812

Robinson-Agramonte, M. de los A., Noris García, E., Fraga Guerra, J., Vega Hurtado, Y., Antonucci, N., Semprún-Hernández, N., Schultz, S., & Siniscalco, D. (2022). Immune Dysregulation in Autism Spectrum Disorder: What Do We Know about It? International Journal of Molecular Sciences, 23(6), 3033. https://doi.org/10.3390/ijms23063033

Rosero, J. A., Killer, J., Sechovcová, H., Mrázek, J., Benada, O., Fliegerová, K., Havlík, J., & Kopečný, J. 2016. (n.d.). Reclassification of Eubacterium rectale (Hauduroy et al. 1937) Prévot 1938 in a new genus Agathobacter gen. Nov. As Agathobacter rectalis comb. Nov., and description of Agathobacter ruminis sp. Nov., isolated from the rumen contents of sheep and cows. International Journal of Systematic and Evolutionary Microbiology, 66(2), 768–773. https://doi.org/10.1099/ijsem.0.000788

Rossignol, D., & Frye, R. (2011). Mitochondrial Dysfunction in Autism Spectrum Disorders: A Systematic Review and Meta-Analysis. Molecular Psychiatry, 17, 290–314. https://doi.org/10.1038/mp.2010.136

Rutayisire, E., Huang, K., Liu, Y., & Tao, F. (2016). The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: A systematic review. BMC Gastroenterology, 16(1), 86. https://doi.org/10.1186/s12876-016-0498-0

Saad, K., Abdel-rahman, A. A., Elserogy, Y. M., Al-Atram, A. A., Cannell, J. J., Bjørklund, G., Abdel-Reheim, M. K., Othman, H. A. K., El-Houfey, A. A., Abd El-Aziz, N. H. R., Abd El-Baseer, K. A., Ahmed, A. E., & Ali, A. M. (2016). Vitamin D status in autism spectrum disorders and the efficacy of vitamin D supplementation in autistic children. Nutritional Neuroscience, 19(8), 346–351. https://doi.org/10.1179/1476830515Y.0000000019

Sanctuary, M. R., Kain, J. N., Chen, S. Y., Kalanetra, K., Lemay, D. G., Rose, D. R., Yang, H. T., Tancredi, D. J., German, J. B., Slupsky, C. M., Ashwood, P., Mills, D. A., Smilowitz, J. T., & Angkustsiri, K. (2019). Pilot study of probiotic/colostrum supplementation on gut function in children with autism and gastrointestinal symptoms. PLoS ONE, 14(1), e0210064. https://doi.org/10.1371/journal.pone.0210064

Shaaban, S. Y., El Gendy, Y. G., Mehanna, N. S., El-Senousy, W. M., El-Feki, H. S. A., Saad, K., & El-Asheer, O. M. (2018). The role of probiotics in children with autism spectrum disorder: A prospective, open-label study. Nutritional Neuroscience, 21(9), 676–681. https://doi.org/10.1080/1028415X.2017.1347746

Singh, K., & Zimmerman, A. W. (2015). Sleep in Autism Spectrum Disorder and Attention Deficit Hyperactivity Disorder. Seminars in Pediatric Neurology, 22(2), 113–125. https://doi.org/10.1016/j.spen.2015.03.006

Sivamaruthi, B. S., Suganthy, N., Kesika, P., & Chaiyasut, C. (2020). The Role of Microbiome, Dietary Supplements, and Probiotics in Autism Spectrum Disorder. International Journal of Environmental Research and Public Health, 17(8), 2647. https://doi.org/10.3390/ijerph17082647

Slykerman, R. F., Kang, J., Van Zyl, N., Barthow, C., Wickens, K., Stanley, T., Coomarasamy, C., Purdie, G., Murphy, R., Crane, J., & Mitchell, E. A. (2018). Effect of early probiotic supplementation on childhood cognition, behaviour and mood a randomised, placebo-controlled trial. Acta Paediatrica (Oslo, Norway: 1992), 107(12), 2172–2178. https://doi.org/10.1111/apa.14590

Sun, L.-J., Li, J.-N., & Nie, Y.-Z. (2020). Gut hormones in microbiota-gut-brain cross-talk. Chinese Medical Journal, 133(7), 826–833. https://doi.org/10.1097/CM9.0000000000000706

Tan, Q., Orsso, C. E., Deehan, E. C., Kung, J. Y., Tun, H. M., Wine, E., Madsen, K. L., Zwaigenbaum, L., & Haqq, A. M. (2021). Probiotics, prebiotics, synbiotics, and fecal microbiota transplantation in the treatment of behavioral symptoms of autism spectrum disorder: A systematic review. Autism Research, 14(9), 1820–1836. https://doi.org/10.1002/aur.2560

Tello, M. (2020, April 16). Vitamin D: What's the "right" level? Harvard Health Blog. https://www.health.harvard.edu/blog/vitamin-d-whats-right-level-2016121910893

Thapar, A., & Rutter, M. (2021). Genetic Advances in Autism. Journal of Autism and Developmental Disorders, 51(12), 4321–4332. https://doi.org/10.1007/s10803-020-04685-z

Tomova, A., Husarova, V., Lakatosova, S., Bakos, J., Vlkova, B., Babinska, K., & Ostatnikova, D. (2015). Gastrointestinal microbiota in children with autism in Slovakia. Physiology & Behavior, 138, 179–187. https://doi.org/10.1016/j.physbeh.2014.10.033

Uthayakumar, D., Sharma, J., Wensing, L., & Shapiro, R. S. (2021). CRISPR-Based Genetic Manipulation of Candida Species: Historical Perspectives and Current Approaches. Frontiers in Genome Editing, 2, 606281. https://doi.org/10.3389/fgeed.2020.606281

Vacca, M., Celano, G., Calabrese, F. M., Portincasa, P., Gobbetti, M., & De Angelis, M. (2020). The Controversial Role of Human Gut Lachnospiraceae. Microorganisms, 8(4), 573. https://doi.org/10.3390/microorganisms8040573

Van Der Zanden, E. P., Boeckxstaens, G. E., & De Jonge, W. J. (2009). The vagus nerve as a modulator of intestinal inflammation. Neurogastroenterology & Motility, 21(1), 6–17. https://doi.org/10.1111/j.1365-2982.2008.01252.x

Vendrik, K. E. W., Ooijevaar, R. E., de Jong, P. R. C., Laman, J. D., van Oosten, B. W., van Hilten, J. J., Ducarmon, Q. R., Keller, J. J., Kuijper, E. J., & Contarino, M. F. (2020). Fecal Microbiota Transplantation in Neurological Disorders. Frontiers in Cellular and Infection Microbiology, 10, 98. https://doi.org/10.3389/fcimb.2020.00098

Vuong, H. E., & Hsiao, E. Y. (2017). Emerging Roles for the Gut Microbiome in Autism Spectrum Disorder. Biological Psychiatry, 81(5), 411–423. https://doi.org/10.1016/j.biopsych.2016.08.024

Wang, H.-X., & Wang, Y.-P. (2016a). Gut Microbiota-brain Axis. Chinese Medical Journal, 129(19), 2373–2380. https://doi.org/10.4103/0366-6999.190667

Wang, H.-X., & Wang, Y.-P. (2016b). Gut Microbiota-brain Axis. Chinese Medical Journal, 129(19), 2373–2380. https://doi.org/10.4103/0366-6999.190667

Wang, J.-W., Kuo, C.-H., Kuo, F.-C., Wang, Y.-K., Hsu, W.-H., Yu, F.-J., Hu, H.-M., Hsu, P.-I., Wang, J.-Y., & Wu, D.-C. (2019). Fecal microbiota transplantation: Review and update. Journal of the Formosan Medical Association = Taiwan Yi Zhi, 118 Suppl 1, S23–S31. https://doi.org/10.1016/j.jfma.2018.08.011

Wang, Y., Li, N., Yang, J.-J., Zhao, D.-M., Chen, B., Zhang, G.-Q., Chen, S., Cao, R.-F., Yu, H., Zhao, C.-Y., Zhao, L., Ge, Y.-S., Liu, Y., Zhang, L.-H., Hu, W., Zhang, L., & Gai, Z.-T. (2020). Probiotics and fructo-oligosaccharide intervention modulate the microbiota-gut brain axis to improve autism spectrum reducing also the hyper-serotonergic state and the dopamine metabolism disorder. Pharmacological Research, 157, 104784. https://doi.org/10.1016/j.phrs.2020.104784

Wang, Y., Wiesnoski, D. H., Helmink, B. A., Gopalakrishnan, V., Choi, K., DuPont, H. L., Jiang, Z.-D., Abu-Sbeih, H., Sanchez, C. A., Chang, C.-C., Parra, E. R., Francisco-Cruz, A., Raju, G. S., Stroehlein, J. R., Campbell, M. T., Gao, J., Subudhi, S. K., Maru, D. M., Blando, J. M., … Jenq, R. R. (2018). Fecal microbiota transplantation for refractory immune checkpoint inhibitor-associated colitis. Nature Medicine, 24(12), 1804–1808. https://doi.org/10.1038/s41591-018-0238-9

Warner, B. B. (2019). The contribution of the gut microbiome to neurodevelopment and neuropsychiatric disorders. Pediatric Research, 85(2), 216–224. https://doi.org/10.1038/s41390-018-0191-9

Whiteley, P., Shattock, P., Knivsberg, A.-M., Seim, A., Reichelt, K., Todd, L., Carr, K., & Hooper, M. (2013). Gluten- and casein-free dietary intervention for autism spectrum conditions. Frontiers in Human Neuroscience, 6. https://www.frontiersin.org/article/10.3389/fnhum.2012.00344

Wu, S., Ding, Y., Wu, F., Li, R., Xie, G., Hou, J., & Mao, P. (2015). Family history of autoimmune diseases is associated with an increased risk of autism in children: A systematic review and meta-analysis. Neuroscience & Biobehavioral Reviews, 55, 322–332. https://doi.org/10.1016/j.neubiorev.2015.05.004

Xiao, L., Yan, J., Yang, T., Zhu, J., Li, T., Wei, H., & Chen, J. (n.d.). Fecal Microbiome Transplantation from Children with Autism Spectrum Disorder Modulates Tryptophan and Serotonergic Synapse Metabolism and Induces Altered Behaviors in Germ-Free Mice. MSystems, 6(2), e01343-20. https://doi.org/10.1128/mSystems.01343-20

Xu, M., Xu, X., Li, J., & Li, F. (2019). Association Between Gut Microbiota and Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. Frontiers in Psychiatry, 10, 473. https://doi.org/10.3389/fpsyt.2019.00473

Yap, C. X., Henders, A. K., Alvares, G. A., Wood, D. L. A., Krause, L., Tyson, G. W., Restuadi, R., Wallace, L., McLaren, T., Hansell, N. K., Cleary, D., Grove, R., Hafekost, C., Harun, A., Holdsworth, H., Jellett, R., Khan, F., Lawson, L. P., Leslie, J., … Gratten, J. (2021). Autism-related dietary preferences mediate

autism-gut microbiome associations. Cell, 184(24), 5916-5931.e17. https://doi.org/10.1016/j.cell.2021.10.015

Zerbo, O., Qian, Y., Yoshida, C., Grether, J. K., Van de Water, J., & Croen, L. A. (2015). Maternal Infection During Pregnancy and Autism Spectrum Disorders. Journal of Autism and Developmental Disorders, 45(12), 4015–4025. https://doi.org/10.1007/s10803-013-2016-3

Zhang, L. S., & Davies, S. S. (2016). Microbial metabolism of dietary components to bioactive metabolites: Opportunities for new therapeutic interventions. Genome Medicine, 8(1), 46. https://doi.org/10.1186/s13073-016-0296-x

Zhao, H., Zhang, H., Liu, S., Luo, W., Jiang, Y., & Gao, J. (2021). Association of Peripheral Blood Levels of Cytokines With Autism Spectrum Disorder: A Meta-Analysis. Frontiers in Psychiatry, 12, 670200. https://doi.org/10.3389/fpsyt.2021.670200

Zoccante, L., Ciceri, M. L., Gozzi, L. A., Gennaro, G. D., & Zerman, N. (2022). The "Connectivome Theory": A New Model to Understand Autism Spectrum Disorders. Frontiers in Psychiatry, 12, 794516. https://doi.org/10.3389/fpsyt.2021.794516

Zurawski, D. V., & McLendon, M. K. (2020). Monoclonal Antibodies as an Antibacterial Approach Against Bacterial Pathogens. Antibiotics, 9(4), 155. https://doi.org/10.3390/antibiotics9040155

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