Chapter 13
Role of the Gut Microbiome in Autism Spectrum Disorders

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Abstract Autism spectrum disorder (ASD) is a severe neurodevelopmental or neuropsychiatric disorder with elusive etiology and obscure pathophysiology. Cognitive inabilities, impaired communication, repetitive behavior pattern, and restricted social interaction and communication lead to a debilitating situation in autism. The pattern of co-occurrence of medical comorbidities is most intriguing in autism, compared to any other neurodevelopmental disorders. They have an elevated comorbidity burden among which most frequently are seizures, psychiatric illness, and gastrointestinal disorders. The gut microbiota is believed to play a pivotal role in human health and disease through involvement in physiological homoeostasis, immunological development, glutathione metabolism, amino acid metabolism, etc., which in a reasonable way explain the role of gut-brain axis in autism. Branded as a neurodevelopmental disorder with psychiatric impairment and often misclassified as a mental disorder, many experts in the field think that a therapeutic solution to autism is unlikely to emerge. As the pathophysiology is still elusive, taking into account of the various symptoms that are concurrent in autism is important. Gastrointestinal problems that are seen associated with most of the autism cases suggest that it is not just a psychiatric disorder as many claim but have a physiological base, and alleviating the gastrointestinal problems could help alleviating the symptoms by bringing out the much needed overall improvement in the affected victims. A gut disorder akin to Crohn's disease is, sometimes, reported in autistic children, an extremely painful gastrointestinal disease which is named as autistic enterocolitis. This disturbed situation hypothesized to be initiated by dysbiosis or microbial imbalance could in turn perturb the coordination of microbiota-gut-brain axis which is important in human mental health as goes the popular dictum: "fix your gut, fix your brain."

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13.1 Introduction

Life will not be possible as we know it today without microbes. Hitherto unknown
and unpredicted, the role of gut microbiome in human health and disease is being
explored eloquently with the help of next-generation techniques and allied improve-
ments. Coined by Joshua Lederberg, the term microbiome signifies the natural com-
munity of microorganisms that factually share our body space and can be categorized
as commensal, symbiotic, or pathogenic and have been all but ignored as determi-
nants of health and disease [1]. Gone are the times when the role of microbes in
human health was just focused on identifying and treating pathogens in patients,
usually with antibiotics. Having colonized by trillions of microorganisms [2], these
microbial communities outnumber the total number of human cells by a factor of 10
and contain 150 times as many genes as those encoded by the human genome [3],
suggesting us to consider human gut microbiota as an organ or superorganism that
coevolved with the human host to achieve a beneficial symbiotic relationship [4].
The term "superorganism" would refer to a collection of individuals which behave
as a single unit with enhanced function [4]. The human host is immensely benefitted
by the gut microbiota, attested by the fact that there is a plethora of interactions hap-
pening between them throughout the development [4] and its disruption could lead
to manifold negative consequences [5]. The major role of these microbiota can be
defined in terms of a metabolic one. A marvelous metabolic capacity equivalent to
that of the liver is envisioned by the combined metabolic capacity of the microbiota,
justifying their portrayal as an additional human organ [6] and revealing the exem-
plary host-microbiota mutualism in the gut. Their supreme role in the maintenance
of host health includes facilitating digestion of otherwise indigestible dietary fibers
into readily absorbable short-chain fatty acids (SCFAs), synthesis of vitamins and
other beneficial metabolites, immune system regulation, detoxification of harmful
substances, and enhanced resistance against colonization by pathogenic microor-
ganisms [5]. The defense against pathogen colonization is achieved by nutrient
competition and production of antimicrobial activities [7]. Their role in fortification
of intestinal epithelial barrier and their ability to induce secretory IgA to limit pen-
etration of bacteria into tissues are not to be overlooked. Researches on germ-free
mice reveal that absence of microbes causes significant immune defects at structure
levels like decreased Peyer's patches, lamina propria, and isolated lymphoid folli-
cles. At cellular levels, defects like decreased intestinal $CD8^+$ T cells and $CD4^+$ T
helper 17 cells and reduced B-cell production of secretory IgA have been reported
[8] (Fig. 13.1).

The microbiome at a specific niche is believed to cause local as well as systemic
effects on host biology [7]. Chronic low-grade intestinal inflammation seen in irri-
table bowel syndrome (IBS) [9] and intense intestinal autoimmunity observed in the
inflammatory bowel disease (IBD) [8] are examples of local effects of the
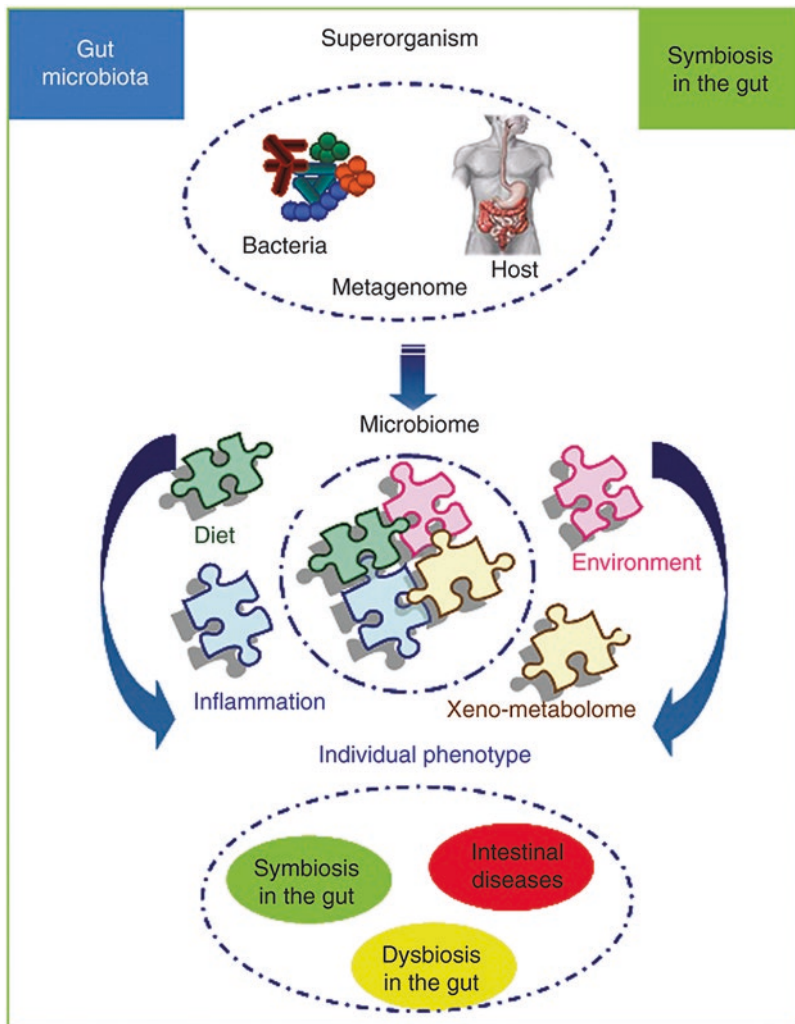
Fig. 13.1 The human gut microbiota as a superorganism. The largest microbiome is accredited as that seen in our gastrointestinal tract. It is influenced by several external factors, such as diet, inflammation stage, environment, and xeno-metabolome. Each microbiome refers an individual phenotype, able to describe symbiosis-, dysbiosis-, and disease-related gut conditions (Adapted from Putignani et al. (2014) Pediatr Res 76:2–10)

microbiome on host biology. The systemic effect of microbiome on host biology is evident from the fact that the gut microbiome contributes to the etiology of experimental disease models affecting remote organ systems. Immune cells stimulated at the intestinal site, like microbe-sensing antigen processing cells (APCs) and adaptive immune cells, are trafficked to distal tissue sites by systemic diffusion [7].

The bidirectional interaction between the gut-intestine tract and brain via the microbiome is crucial for maintaining equilibrium between health and disease. The normal microbiome promotes increased metabolic capacity, immune system maturation, and SCFA production, while dysbiosis of microbiome leads to increased inflammation and overabundance of *Enterobacteriaceae*. This leads to decreased intestinal mucus, immune cell differentiation, gut-associated lymphoid tissue and metabolic capacity, and SCFA production, causing an overall ill health in the host [4]. Recent studies indicate that microorganisms within and among the body habitats exhibit intricate relationships. They play a critical role in driving physical factors such as oxygen, moisture, and pH. They, also, play a role in the host immunological regulation microbial interactions. All these point out the fact that the microbiome plays more crucial role in health and disease than expected so far [10].

The crucial intervention by intestinal communities implicated in diseases like allergies [11], late-onset autism [12], inflammatory bowel disease [13], cancer [14], obesity, and type 2 diabetes [15] reinstates that our understanding of microbial ecology will have a direct bearing on our ability to manage and maintain human health in the future. The major move toward studying changes in composition of the intestinal microbiota in relation to diseases relies primarily on the phylogenetic characterization of the microbiota of diseased individuals in comparison with healthy candidates. Given the substantial interindividual and intraindividual variations in addition to age-related changes in the composition of the intestinal microbiota, it is a difficult task to establish the presence and relative abundance of specific microbial communities in relation with human health status. However, options are realistic to use specific changes in compositional and functional diversity of microbiota as biomarkers for health or specific diseases.

The gut microbiota is a chief contributor in maintaining normal physiology and energy production throughout life. Energy-dependent processes like body temperature regulation, reproduction, and tissue growth are partly influenced by gut microbial energy production [16] further giving evidences for the importance of gut microbiota. The gut microbiome dysbiosis is believed to have a negative impact on gastrointestinal tract-related disorders such as Crohn's disease and ulcerative colitis, systemic diseases like metabolomic disorders, and central nervous system (CNS)-related disorders [17]. This review highlights the importance of the gut microbiome in autism which is a non-immune-mediated CNS disorder in which the role of gut microbiome has been implicated in its exacerbation.

13.2 Plausible Mechanisms Involved in Gut-Brain Axis Through Gut Microbiome Intervention: Bidirectional Communication

Gut-brain axis (GBA) can be defined as a physiological framework in which the gut microbiota communicates with the CNS and vice versa through neural, endocrine, and immune pathways. The communication being bidirectional, the role of the brain

on the microbial content of the gut and how the intestinal microbiota influences the brain and behavior solicit equal attention. Homeostases of several systems like gastrointestinal tract (GI) function, appetite, and weight control are a few major factors that are significantly affected by the GBA. The microbial habitat is determined by GI motility as well as epithelial functions. Since these factors can be regulated by the CNS, any changes induced by the CNS can result in alteration and perturbation of intestinal microbiota leading to several pathological situations [9].

The enteric microbiota can assert their influence on gut homeostasis by means of regulation of bowel motility, modulation of intestine pain, immune responses, and nutrient processing [18]. Mounting evidences indicate that gut microbiome does affect the development and regulation of hypothalamic-pituitary-adrenal axis (HPA) and behavior [19]. Characterization of the bidirectional interactions between the CNS, the enteric nervous system (ENS), and the GI tract has brought about results which convincingly suggest the role of the gut microbiota in these gut-brain interactions. This type of bidirectional communication which mediates the symbiotic and pathogenic relationships between the bacteria and the mammalian host is called "microbial endocrinology" [20] or "interkingdom signaling" [21]. The gut microbiota appears to influence the development of emotional behavior, stress and pain modulation systems, and brain neurotransmitter systems. Some of the recent researches explain the multiple mechanisms involved. Endocrine and neurocrine pathways facilitate gut microbiota-to-brain signaling, and the brain, in turn, alters microbial composition and behavior through the autonomic nervous system with the active involvement of immune and humoral system [22].

The enteric microbiota does assert a vital impact on "gut-brain axis" (GBA), interacting locally with intestinal cells and the ENS and directly with the CNS through neuroendocrine and metabolic pathways (Fig. 13.2). To enumerate the main assistances rendered by bacterial colonization in terms of their absence or presence would include development and maturation of both the ENS and CNS [23], altered expression and turnover of neurotransmitters in both nervous systems [24], alterations of gut sensory-motor functions leading to delayed gastric emptying and intestinal transit [25], and enlarged cecal size [26]. The neuromuscular abnormalities caused via the intervention of microbiota results in downregulation of gene expression of enzymes related to the synthesis and transport of neurotransmitters and muscular contractile proteins [27]. These aberrations have been seen to be normalized once the experimental animals are colonized with specific bacterial species.

It has been observed that germ-free (GF) mice generally show decreased anxiety and an increased stress response. The organism with enhanced levels of ACTH and cortisol demonstrates that microbiota influences stress reactivity and anxiety-like behavior. The microbiota also regulates the set point for HPA activity and modulates the serotoninergic system. GF animals are also known to suffer from memory dysfunction with an altered expression of brain-derived neurotrophic factor (BDNF) [28].

The main principal mechanisms by which the bidirectional brain-gut-microbiota axis works have been deduced. The gut microbiota asserts its role over the brain through its influence on production, expression, and turnover of neurotransmitters
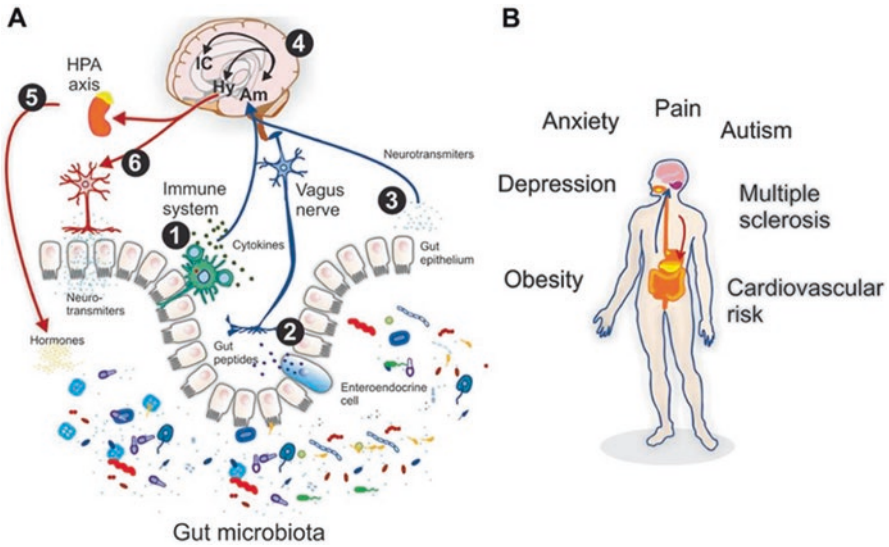
Fig. 13.2 Microbiota-gut-brain (MGB) axis. Pathways either directly or indirectly influence the bidirectional interactions between the gut microbiota and the central nervous system (CNS), involving endocrine, immune, and neural pathways (Courtesy: Augusto et al. (2013) Front Integr Neurosci 7:70)

like serotonin and gamma-aminobutyric acid (GABA) and neurotrophic factor (BDNF), protection of the intestinal barrier and tight junction integrity, modulation of enteric sensory afferents, production of various bacterial metabolites, and mucosal immune regulation, while the brain claims its influence over microbiota through their capacity to bring about alterations in mucous and biofilm production, alteration in motility, alteration of intestinal permeability, and alteration in immune function [28]. The decisive role of the gut microbiota in the production of biologically active, free catecholamines in the gut lumen of mice is established. Catecholamines are crucial in regulation of various types of body functions such as cognitive abilities, mood, and gut motility. The principal catecholamines like norepinephrine (NE) and dopamine (DA) are utilized in the CNS and peripheral nervous systems [29]. A comparative study between specific pathogen-free mice (SPF-M) and germ-free mice (GF-M) showed that substantial levels of free dopamine and norepinephrine were available in the gut lumen of SPF-M. Also the available catecholamine levels were found to be in a biologically inactive, conjugative form in GF-M. When GF-M was introduced with *Clostridium* species or SPF fecal flora, which are known to have abundant β-glucuronidase activity, drastic elevation of free catecholamines was visible indicating the role of gut microbiota and in particular the need of β-glucuronidase activity in these organisms [30]. The role of gut microbiota in regulation of sympathetic nervous system (SNS), a component in bidirectional signaling, has also been reported to take place with the help of short-chain fatty acids and ketones produced by microbiota and their promotion of sympathetic outflow via G

protein-coupled receptor 41 (GPR41) [31]. The effect of short-chain fatty acids on enterochromaffin cells promotes colonic serotonin production [32] and influences memory and learning process [33]. Another plausible mechanism of microbiota-GBA interaction could be through the release of biologically active peptides from enteroendocrine cells which in turn is regulated by the nutrient availability of the microbiota [34].

When microbiota influences the brain in ways mentioned above, the GBA being a bidirectional signaling pathway, it should be an unavoidable occurrence that the brain dictates or influences microbiota composition and function through various other mechanisms. One major mechanism by which the brains assert its impact on the microbial population is exposure to stressors. A pyrosequencing study carried out to assess the effects of a single 2-hour exposure to a social stressor made a substantial alteration on colonic mucosa-associated microbial profiles of C57BL/6 mice, significantly reducing the relative proportions of the two genera and one family of highly abundant intestinal bacteria, including the genus *Lactobacillus* [35]. This can happen either directly via host-enteric microbiota signaling or indirectly via changes in the intestinal milieu. A more direct and evident influence on microbiota is facilitated by secretion of signaling molecules by neurons, immune cells, and enterochromaffin cells, under the regulation of the brain. As there are neurotransmitter receptors on bacteria, binding of neurotransmitters is capable of influencing the function of components of the microbiota that have influence on inflammatory and infection stimuli [21]. An example in this regard can be found in *Pseudomonas fluorescens* which is able to produce GABA and express GABA-binding proteins, and they also increase their necrotic-like activity on eukaryotic glial cells. This particular study also proved that GABA can regulate the lipopolysaccharide (LPS) structure and cytotoxicity in specific strains of *P. fluorescens* [36]. Another interesting dimension is the microbial biofilms in which individual groups of bacteria are found to occupy different microhabitats and metabolic niches in the human gastrointestinal tract. The prominent role of the brain in modulation of gut functions, such as motility; secretion of acids, bicarbonates, and mucus; intestinal fluid handling; and mucosal response, is important for the maintenance of the mucus layer. The individual groups of bacteria exist in different microhabitats, and metabolic niches as biofilms in these areas are also modulated by this brain function [37]. The brain affects the microbiota through various stresses induced mainly by bringing about variation in size and quality of mucus secretion [38], by delaying the recovery of the migrating motor complex pattern and inducing a transient slowing of gastric emptying [39], by increasing the frequency of cecocolonic spike-burst activity through the central release of corticotrophin-releasing factor [40]. This causes regional and global changes in gastrointestinal transit which has a profound effect on the way nutrients are delivered to the enteric microbiota. The composition and biomass of the enteric microbiota get modulated by different types of psychological stressors both in adult [41] and newborn animals [42]. When postnatal stress was induced in animal models by separating the mothers, reduction in lactobacilli was observed, disrupting the integrity of the intestinal microbiota with the appear-

ance of stress-indicative behaviors [42]. The animals in study were also more susceptible to opportunistic infection compared to unstressed control animals.

A microbial connection has been linked to anxiety and stress, the common forms of mood disorders. Germ-free mice exhibit increased motor activity and reduced anxiety, suggesting that gut-associated pathogens can exacerbate anxiety. Decreased neurotransmitter receptors and increased tryptophan metabolism observed in the GF condition tend to suggest that the gut microbiome regulates the set point for the HPA axis [24]. When rodents were infected with the food-borne pathogen *Campylobacter jejuni*, rodents showed perceptible anxiety-like behavior. Increased c-Fos expression as a result of infection with *Campylobacter jejuni* underpins the notion that gut-associated pathogens can intensify anxiety [43]. While *Citrobacter rodentium* followed a mechanism similar to *Campylobacter jejuni* [44], *Trichuris muris* elevated anxiety via immunological and metabolic mechanisms [45]. The anxiolytic properties exhibited by the specific species of the *Lactobacillus* and *Bifidobacterium* genera are almost in contrast to the pathogenic bacteria [46].

13.3   Autism and Gut Microbiome

Autism is a non-immune-mediated CNS disorder in which the role of gut microbiome has been implicated in its exacerbation. Among many non-immune-mediated CNS disorders, autism stands out due to its prevalence and bewildering increase in epidemic levels throughout the world. As per Centers for Disease Control and Prevention data, 1 in 500 to 1 in 166 children has an autism spectrum disorder. Various physiological parameters observed in autism include autoimmune reactions, food reactions, diagnostic connection of upper GI disease, abnormal stools, autistic enterocolitis, leaky gut syndrome, excessive inflammation, aberrant glutathione levels, irregular metal, mineral levels, etc. [47], all supporting the importance of the gut microbiome. The aforementioned parameters are either influenced by microbiota or vice versa, revealing the tantalizing link between autism and gut microbiome.

Seemingly pervasive, autism spectrum disorders (ASD) are characterized by impaired communication, difficulty in social interactions, and stereotyped behavioral patterns [48]. People with autism most frequently tend to display unusual ways of learning, paying attention, and reacting to different sensations. Their imaginative skills are also known to suffer [49]. Coupling genetic predisposition with environmental factors seems to raise the risk involved in autistic children.

Considered as a psychiatric disorder, many physical symptoms common in patients with autism have largely gone unnoticed or even ignored by the medical establishment, and coinciding GI symptoms are a major example. Anecdotal reports as well as various studies indicate that gastrointestinal problems are frequently associated with autism [50] and presumed to have a correlation with autism severity [48]. Various independent studies have verified that the GI dysfunction in these children includes diarrhea, unformed stools, constipation, bloating, flatulence, etc.

[51, 52]. It was Wakefield and colleagues at the Royal Free Hospital in London who described a new variant of inflammatory bowel disease, named as autistic enterocolitis which is characterized by chronic patchy inflammation and lymphonodular hyperplasia in the terminal ileum or in the colon [53]. Intestinal permeability is another grave issue faced by children with autism, suggesting a leaky gut which can lead to neurological disability as these children are forced to absorb neurotoxic molecules across a gut membrane damaged by inflammation [54]. An abnormal level of intestinal permeability has also been documented in independently carried out studies [55, 56]. A leaky gut allows molecules to enter the bloodstream, otherwise kept at bay. Immune activation, tissue damage, and effects on the brain, including damage to brain tissue, are a few corollary problems that could emerge over time. The opioid peptides that are produced from certain diets can disrupt the normal neurotransmitter function in the brain, causing certain typical behavioral patterns observed in children with autism such as decreased socialization, decreased response to pain, abnormal language, and self-abusive or repetitive behaviors. Direct effects on the neuronal structure of the brain tissue and on the immune system by these opiate-like molecules cannot be ruled out. We need to investigate if neurotoxic and cytotoxic molecules produced by microorganisms can contribute to intensify the abovementioned situations in *autistic enterocolitis* and leaky gut and their possibility to assert greater damage by working independently or in tandem with each other.

Studies undertaken at different research labs indicate a link between the gut microbiome and ASD, although a direct causality or indirect consequence of atypical patterns of feeding and nutrition has yet to be proved. Children with ASD tend to show food selectivity with strong preferences for starches, snack, and processed food, while most tend to reject fruits, vegetables, and/or proteins [57, 58]. A chronic imbalance of gut microbiota known as intestinal dysbiosis is suspected in children with autism, and many investigators have found evidence of this imbalance in autistic patients [12]. However, most of the gut microorganisms are beneficial to host, and dozens of species of pathogenic organisms, if allowed to thrive due to the compromised immune system, can cause disease. Apart from causing local effects on gut tissue, abnormal bacteria can have an effect on the brain. The toxins that are produced by the harmful bacteria are not properly metabolized. They can build up in the brain by way of the bloodstream, resulting in confusion, delirium, and even coma. The GI inflammation and abnormal immune functions observed in children with autism may increase the abnormal levels of harmful bowel organisms, and metabolites produced by the harmful bacteria can create havoc intensifying GI inflammation, gut permeability, and abnormal immune functions. Yeasts also seem to play a negative role in vulnerable children, as yeasts are known producers of chemicals that have neurological effects and children with autism indicate elevated levels of chemicals that are found in yeasts [59].

A succession of microbial consortia studies in infants has proven that the microbiome gets enriched as associated with life events and as per the diet being introduced. A healthy microbiome is observed to be capable of assisting in the breakdown of complex plant polysaccharides, promoting digestion and overall host health [60].

Therefore, it could be predicted that deviations in the establishment and maintenance of the gut microbiome could lead to pain and discomfort. This hypothesis has been corroborated by William et al. whose study revealed that children with ASD tend to have less of *Bacteroidetes* which play a prominent role in the digestion of polysaccharides. This study could also underpin the suggestion that children with autism have anomalous carbohydrate digestion capacity and mucosal dysbiosis in the intestines. Metagenomic analysis revealed that they have a decrease in *Bacteroidetes*, an increase in the ratio of *Firmicutes* to *Bacteroidetes*, and an increase in *Betaproteobacteria* [49]. A pyrosequencing study of fecal microflora of children with autism showed significant differences in the *Actinobacteria* and *Proteobacterium* phyla in comparison with healthy controls. The same study also showed higher prevalence of *Desulfovibrio* species and *Bacteroides vulgatus* in stools of severely autistic children [12]. Basic anaerobic culturing techniques to count and isolate microorganisms, followed by polymerase chain reaction (PCR) targeting the 16S rDNA in the isolates cultivated in children with late-onset autism against neurotypical children, showed that the number and type of *Clostridium* and *Ruminococcus* species significantly differed from normal children [12]. A follow-up study by Song et al. using quantitative real-time PCR found that *Clostridium* cluster groups I and XI and *Clostridium bolteae* were significantly higher in children with autism [61]. Another culture-independent study by Parracho et al. using fluorescence in situ hybridization (FISH) reported elevated levels of *Clostridium hystolyticum* in the ASD children compared to typical children [49]. Disruption of gut microbiota might contribute for the over-colonization of neurotoxin-producing bacteria, exacerbating autistic symptoms. *Clostridium tetani* is looked upon as an organism that could induce autism [62]. *Clostridia* are known propionate producers [63], and the property of propionate to bring upon neurological effects in rats [64] has led to the hypothesis that incidence of autism is related to extensive exposure to *Clostridium* spores. A study into investigation of prevalence of four types of beneficial bacteria including *Bifidobacteria*, *Lactobacillus* spp., *E.coli*, and *Enterococcus* revealed that the children with autism had much lower levels of *Bifidobacterium*, slightly lower levels of *Enterococcus*, and much higher levels of *Lactobacillus* [65]. Given that all *Lactobacillus* strains are beneficial, their higher levels seem to be paradoxical and need to be understood. With regard to commensal bacteria which are neither beneficial nor harmful, the same study found that the autism group was more likely to have *Bacillus* spp. and less likely to have *Klebsiella oxytoca*. However, a significant piece of information procured by the same study was the finding of significantly lower levels of short-chain fatty acids observed in autistic children. This was thought to occur due to lower saccharolytic fermentation by beneficial bacteria further substantiating the suspected link between autism and gut microbiome [50].

The finding of distinctive gut microbes associated with ASD was brought about in a small pilot study using a high-throughput sequencing of the 16S rDNA gene by Kang et al. An overall less diverse gut microbiome with a lower abundance of the bacterial genera *Prevotella*, *Coprococcus*, and unclassified *Veillonellaceae* was reported in this study. The said organisms are versatile carbohydrate-degrading and/or fermenting bacteria, and the changes in the spectrum of metabolites produced

from a given diet could be greatly influenced by them [66]. The possibility cannot be ruled out that the differences in microbiota composition in ASD may have negatively influenced the microbial interactions so as to result in a decreased overall diversity and reduced function. Another intriguing observation is that *Prevotella* is a genus highly enriched in populations of agrarian societies, and its depletion in ASD shall be probed if certain environmental factors like industrialization contribute to the prevalence of autism. Individuals with untreated HIV infection are reported to have uniformly high *Prevotella* [67], and a reverse pattern is observed in ASD. The rationale behind this observation can be explained as HIV patients with a suppressed adaptive immune system tend to have a higher number of *Prevotella*, whereas those with ASD have a hyperactive adaptive immune system with less *Prevotella* occurrence.

The metabolites produced by various microorganisms could be performing the same or different functions affecting the system in manifold ways, depending upon their differential abundance. It has been proven that differential abundance of bacteria-produced metabolites has the potential to directly affect neural processes. Increased urinary excretion of an abnormal phenylalanine metabolite of *Clostridia* species, namely, 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA), has been confirmed in urine samples from patients with autism. This tyrosine analogue is thought to be responsible for depletion of catecholamines, and, thus, it is believed to be a chief contributor in the exacerbation of typical autistic symptoms like stereotypical behavior, hyperactivity, and hyper-reactivity in experimental animals [59]. P-Cresol is another microbial metabolite which can initiate damage in cases of autism as they compete with neurotransmitters for enzymes and cofactors needed for sulfonation reactions in the liver [68]. A recent study using a maternal immune activation (MIA) model of ASD in mice showed a significant increase in 4-ethylphenylsulfate (4EPS), a metabolite produced by gut bacteria [69]. Bacterial tag-encoded FLX-titanium amplicon pyrosequencing carried out by De Angelis et al. showed that the highest microbial diversity presented with autism. The same study also identified higher abundance of Caloramator, *Sarcina* and *Clostridium* genera in autistic children. *Alistipes* and *Akkermansia* species were higher in autism along with almost all of the identified *Sutterellaceae* and *Enterobacteriaceae*. Concomitantly, the levels of free amino acids and volatile organic compounds of fecal samples were markedly different in autism [70]. Notably, lower levels of *Prevotella*, *Coprococcus*, and unclassified *Veillonellaceae* were observed by Kang et al. in autistic children in a study carried out using 16 s rRNA gene pyrosequencing analysis from fecal DNA samples [66].

13.3.1   Mineral Elements and Gut Microbiome

Higher average levels of several toxic metals are evident in autism severity. Lead, mercury (Hg), arsenic (As) [71], thallium, tin, and tungsten [72] are among a few of the metals which correlate with autism severity. The role of

environmental pollutants such as these heavy metals in the alteration of physiological functions causing detrimental effects on health has been established. Recognized as neurotoxicants with known effects on neurodevelopment, their role in microbial dysbiosis is inferred adding to the exacerbation of autism symptoms. Although, the exact mechanism has not been deduced, the essential role of the intestinal microbiome in limiting the heavy metal body burden has been established using GF mouse studies [73]. An interesting conclusion of this particular study was that genes relevant for divalent metal transporters and oxidative pathways were expressed with significant differences depending on the microbial status of the animal along with the dose and type of metals present, suggesting the complex host-microbe interplay induced by the environmental pollutants inside the gut. The ability of metals such as Hg and As to exert toxic effects on human health has been well characterized. The volatile derivatives of these metals interact directly with host cells causing irreversible damage and aggravate the diseased state by disturbing the physiological microflora [74]. The critical role of gut microbiota in intestinal homeostasis is characterized by the fact that different types of dysbiosis cause diseases outside and inside the intestine. A study by Breton et al. proved that oral exposure to heavy metals does lead to specific changes in bacterial commensal communities. Their study showed that, relative to the control animals, test animals had notably lower numbers of *Lachnospiraceae* and higher numbers of *Lactobacillaceae* and *Erysipelotrichaceae* [73].

Chronic dietary depletion of elements like iron (Fe) [75] and zinc (Zn) [76] is found to induce significant taxonomic alterations in the gut microbial profile. Understanding the effects of Zn deficiency on the host may help to elucidate the influence of gastrointestinal microbiota on physiology from a novel perspective. The need of Zn, almost in double the amount, in conventionally raised mice against their germ-free counterparts as indicated in studies points out the role of the host microbiota in Zn homeostasis [77]. It has also been seen that optimal levels of Zn administration on various animal models had the benefits of increased concentration of short-chain fatty acids (SCFAs) [78], increased overall bacterial species richness and diversity [79], and favorable change in metabolic activity [80]. Studies have also revealed that gut microbial diversity of Zn-deficient organisms bear a resemblance to that of GI microbiota in pathological states. The mentioned study also suggested that chronic Zn deficiency may reshape the gut microbiome. The metagenome predictive analysis showed that cecal microbiome metabolism was perturbed in Zn-deficient organisms since aberrant pathways involving lipid metabolism, carbohydrate digestion, and mineral absorption were prominent [76].

With practical concern for human health, Fe is also an important trace element to study. Low Fe conditions are seen to cause a decrease in *Roseburia* spp. and an increase in *Lactobacillus* spp., along with a parallel decrease in butyrate and propionate [75]. Thus low Fe conditions may contribute to weaken the barrier effect of microbiota by strong dysbiosis and decreased production of beneficial metabolites.

13.3.2 Glutathione Metabolism and Gut Microbiota

One of the most important molecules, glutathione, is popularly called the mother of all antioxidants. This prototype antioxidant is capable of protecting cells from the harmful effects of oxidative stress and can act as a defensive agent against toxic xenobiotics [81]. Although the role of gut microbiota and dysbiosis is inferred as a cause for many pathological situations, the mechanistic insight into how the specific microbial populations lead to the progression of such disorders has not been studied extensively. It has been assumed that microbiota in the small intestine consumes glycine as well as other amino acids to support its growth and survival, curtailing the availability to the host and thus causing decreased levels of the amino acids and glutathione metabolism [82]. This could underlie the mechanism of how the gut microbiome plays a pivotal role in the exacerbation of certain metabolism-related disorders.

13.4 Conclusions

The realization is that the microbes that live inside and on us outnumber our somatic and germ cells by an estimated tenfold. This has given them the status of a supraorganism with their capacity of providing traits in human beings and has significantly increased the importance of gut microbiome in health and disease. This concept is expected to lead to a paradigm shift in the strategies involved in diagnosis, prognosis, and treatment of a few disorders involving the gut-brain axis in which autism claims a position. Without a cure so far and with limited knowledge on the etiology of the disorder, autism is a topic that needs immediate attention from the researchers. As some of the studies indicate that the gut microbiome modulates the glutathione and certain minerals like zinc, copper, and iron in the experimental organism, studies on the gut microbiome in autism can give some serendipitous insights into the etiology, diagnosis, and treatment of this condition.

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