Autism Spectrum Disorder

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Autism spectrum disorder (ASD) is a serious neurodevelopmental disorder characterized by the impairment of the cognitive function of a child. Studies suggested that the intestinal microbiota has a critical role in the function and regulation of the central nervous system, neuroimmune system, and neuroendocrine system. Any adverse changes in the gut-brain axis may cause serious diseases. Food preferences and dietary patterns are considered as key influencing factors of ASD development. Several recent reviews narrated the importance of dietary composition on controlling or reducing the ASD symptoms. It has been known that consumption of probiotics confers several health benefits by positive amendment of gut microbiota. Influence of probiotic intervention in children with ASD have also been reported and it has been considered as an alternative and complementary therapeutic supplement for ASD. The present manuscript discussed the role of microbiota in the development of ASD.

Autism spectrum disorder   Microbiome   Cognition.

1. Introduction

Autism spectrum disorder (ASD) refers to a group of neurodevelopmental disorders characterized by the impairment of social interaction and communication skills with rigid and repetitive behaviors. Autism affects both children and adults, based on severity and intellectual ability; they may either lead a normal life or suffer a devastating disability requiring institutional care.[1] Distinctive symptoms of ASD are deficits in social behaviors and nonverbal interactions such as the avoidance of eye contact, inability to control emotion or understand the emotions of others, facial expression and body gestures in the first three years of life. Paul Eugen Bleuler is a swiss psychiatrist that coined the term "autism" (Greek word "autos" means "self") for group of symptoms related to schizophrenia, while Hans Asperger and Leo Kanner designed the modern study of autism. An epidemiological survey revealed that ASD is the most prevalent non-immune mediated CNS disorder with an incidence rate of 1 ASD per 500 children aged eight years, with a higher incidence in boys (23.6 per 1000) when compared to girls (5.3 per 1000).[2] ASD-affected individuals exhibit unusual ways of learning and reactions to sensation. ASD is a multifactorial disorder caused by the interaction of both genetic and non-genetic risk factors.

2. Etiological Factors Leading to Autism

Mounting evidence revealed that de novo mutations, copy number variations, rare and common variants are major genetic factors leading to ASD. Around 50% of ASDs are hereditary caused due to defects in the gene and chromosomal abnormalities leading to disruption in the neuronal connection, brain growth, and synaptic morphology.[3][4][5][6] Siblings born in families with ASD have a 50% enhanced risk of ASD with a reoccurrence rate

of 5%–8%. In monozygotic twins, the concordance rate is 90%, while in dizygotic twins the rate of incidence is 10%.[7] Genetic studies revealed that a mutation in the single gene involved in synaptogenesis alters the developmental pathways of neuronal and axonal structures. The fragile X syndrome, tuberous sclerosis, hyperexcitability of neocortical circuits and abnormal neural synchronization were considered as probable disorders leading to ASD.[8][9] In-depth genomic studies revealed that the chromosomes 2q, 7q, 15q, and 16p have genes susceptible for ASD that have not yet been completely studied.[10] Inborn metabolic errors such as phenylketonuria, creatine deficiency syndromes, adenylosuccinate lyase deficiency, and metabolic purine disorders account for 5% of ASD incidence. Recent reports revealed that the gene ENGRAILED 2 mainly involved in cerebellar developmental patterning, GABA system genes, and serotonin transporter genes, has been considered to be associated with ASD.[11]

Non-predisposed factors such as exposure to environmental factors and pharmaceutical drugs, an autoimmune disorder, microbial infection and diet during the prenatal and postnatal periods cause gut dysbiosis and immune dysregulation together contributing to ASD (Figure 1). Recent research revealed that the severity of ASD depends on the complex interaction of genetic susceptibility and environmental factors, so unraveling this relationship will help in identifying a treatment strategy for ASD.[12]
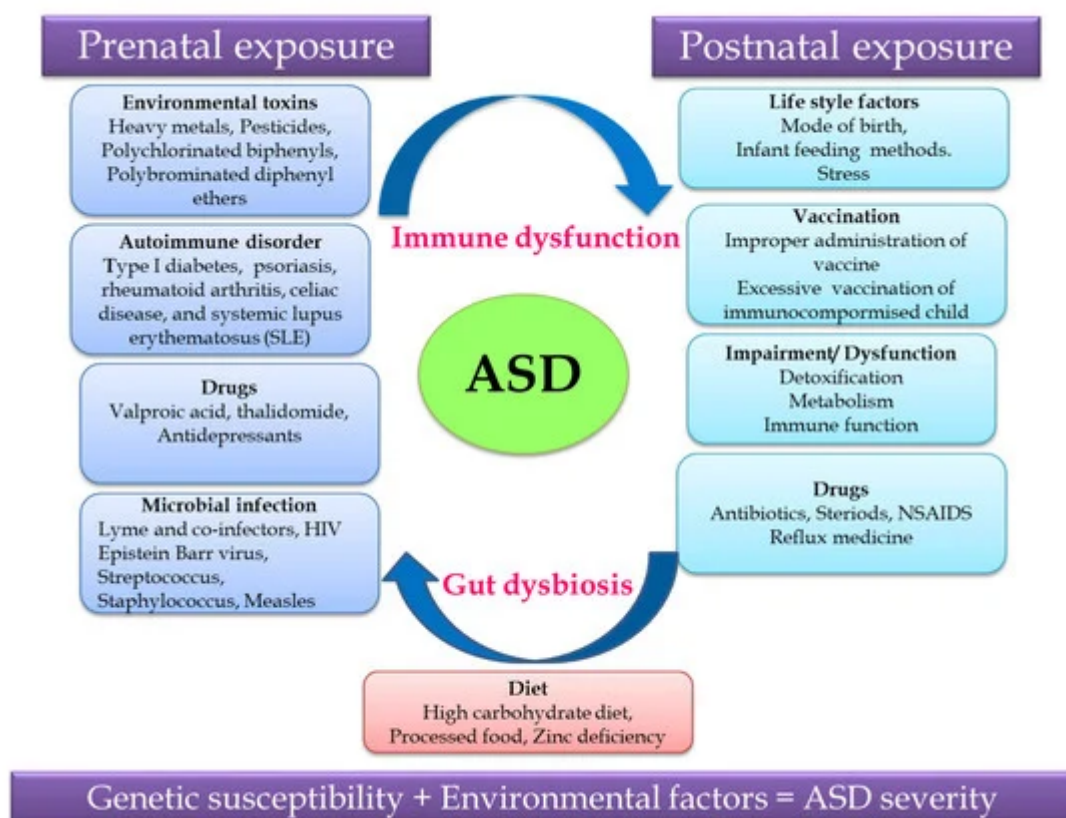

Figure 1. Putative autism spectrum disorder (ASD)-related and environmental factors contributing to ASD.

3. The link between Gut Microbiome and ASD

3.1. Role of Gut Microbiota in Human Nutrition and Health

Gut-brain cross-talk involves a complex communication system involved in the proper maintenance of GI homeostasis, which is termed as the gut-brain axis (GBA). The GBA is a bidirectional communication network between the central nervous system (CNS) and the enteric nervous system linking the emotional and cognitive centers of the brain with peripheral intestinal functions via neuro–immuno–endocrine mediators.[13] The bidirectional communication network of the GBA involves the brain and spinal cord of the CNS, autonomous nervous system (ANS), the enteric nervous system (ENS) and the hypothalamic pituitary adrenal axis (HPA). Sympathetic and parasympathetic limbs of ANS transmit the afferent signals from the lumen to the CNS through the enteric, spinal and vagal pathways and efferent signals from the CNS to the intestinal wall.[14] The HPA axis is the core stress efferent axis, which adapts the organism to various stresses and is part of the limbic system involved in memory and emotional response. On exposure to stresses such as environmental factors and pro-inflammatory cytokines, the HPA axis activates the release of the corticotrophin release factor (CRF) from the hypothalamus, stimulating the secretion of the adrenocorticotrophic hormone (ACTH) from the pituitary gland, which in turn activates the adrenal gland to release the stress hormone cortisol affecting various organs including the brain. Hence, both neuronal and hormonal interactions play a vital role in influencing the activities of intestinal functional effector cells, such as immune cells, epithelial cells, enteric neurons, smooth muscle cells, interstitial cells of cajal and enterochromaffin cells which in turn are under the control of gut microbiota via brain–gut reciprocal communication.[15] Moreover, the epithelial cell lining of the GI tract and its motility, which is controlled by the CNS, influences the composition of the gut microbiome. Hence, any dysregulation in the CNS alters the intestinal microbiota leading to pathological consequences and gut microbial dysbiosis which affects the development and regulation of the hypothalamic-pituitary-adrenal axis (HPA) and behavior. This bidirectional relationship of the gut microbiome with the host brain axis is termed as microbial endocrinology or inter-kingdom signaling.[16]

Gut microbiota interacts with the brain through endocrine and neurocrine pathways, while the brain influences the microbial composition through the autonomic nervous system with the active involvement of the immune and humoral systems. Gut microbiome modulates the brain by influencing the production of neurotransmitters such as serotonin, gamma aminobutyric acid (GABA) and the brain-derived neurotrophic factor (BDNF) via short-chain fatty acid, tryptophan metabolites, secondary bile acids, and ketones thereby influencing memory and learning processes. These molecules transmit signals by interacting with the farnesoid receptor (FXR) and G protein-coupled bile acid receptor (TGR5) in the enteroendocrine cells (EEC) releasing fibroblast growth factor (FGF19), which readily crosses the blood-brain barrier and regulates the secretion of neuropeptide Y in the hypothalamus, thereby regulating the glucose metabolism via the release of glucagon-like peptide (GLP-1).[17][18][19] The release of serotonin (5 hydroxy tryptamine-5HT) by enterochromaffin cells (ECC), triggered by the stimuli from efferent neurons of the CNS based on the availability of the dietary tryptophan level, which in turn is controlled by the gut microbiome, represents the bidirectional gut microbiome–brain axis.[20] Certain microbially-derived molecules escape the intestinal barrier, reach the brain directly by crossing the blood-brain barrier via systemic circulation and propagate the signal on interaction with the FXR and TGR5 expressed in brain neurons.[21]

In addition, the secretion of the biologically active peptide by enteroendocrine cells, mainly involved in GBA interaction, is controlled by the nutritional level of the microbiota. SCFA acts as the major signaling molecule mediating the gut microbiome–brain communication, via EEC and ECC. The brain influences the microbial population through several stresses: by altering the size and quality of the mucus secretion, by slowing the recovery of the migratory motor complex pattern, by the induction a transient delaying of gastric emptying, and by enhancing the frequency of cecocolonic spike burst activity, which affect the GI transit modulating the nutrient supply to enteric microbiota. Different psychological stressors in adults and newborns modulate the composition and biomass of enteric microbiota.[22] (Figure 2).
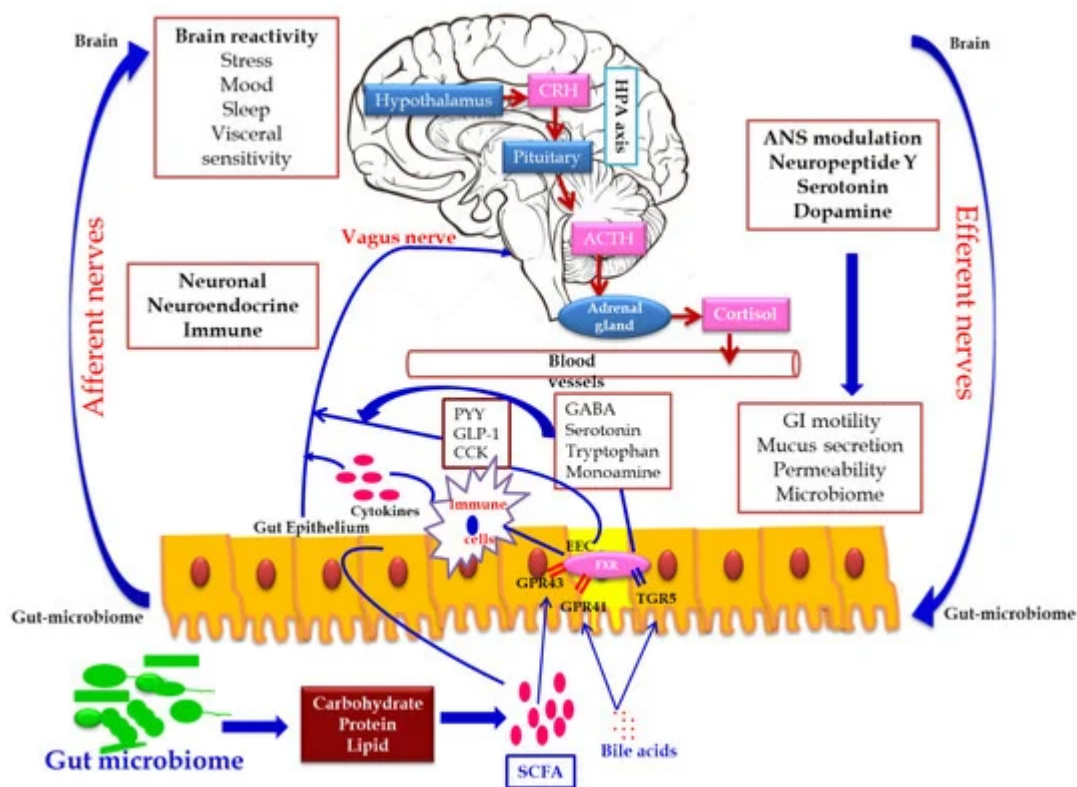

Figure 2. Gut microbiome–brain axis: bidirectional signaling pathways illustrating the relationship between the gut microbiome, intestinal barrier and the brain. Gut microbiota communicates with the brain through the neuro–endocrine–immune network either indirectly via the gut-derived molecules acting on afferent vagal nerve endings, or directly via the microbe-generated signals. The brain's structural connections (the multiple interconnected structural networks of the central nervous system) regulates the gut microbiota via the autonomic nervous system. Disturbance in the bidirectional interaction response gain is due to psychosocial or gut-derived stress manifests to brain–gut disorders.

3.2. The link between Gut Microbiome and ASD

Gut microbiota, which is non-genetic and inheritable, has a great impact on immune, metabolic and neuronal developments. As gut microbiota is a notable contributor for human health, gut microbial dysbiosis leads to negative consequences such as GI-tract-related disorders such as Crohn's disease and ulcerative colitis, systemic

diseases such as metabolomic disorders and CNS-related disorders.[23] Noticeable evidence illustrated that ASD patients in addition to psychiatric disorders were found to be associated with an extremely painful GI disease termed as autistic enterocolitis or other GI discomforts such as constipation, diarrhea and bloating. This hypothesis leads to the fact that microbial imbalance affects the co-ordination of the microbiota-gut–brain axis in human health leading to several neurological disorders which turned the focus of the researcher towards gut microbiota.

Wakefield and colleagues reported the incidence of a new variant inflammatory bowel disease (autistic enterocolitis), which is characterized by chronic patchy inflammation and lymph nodular hyperplasia in the ileum or colon of individuals with ASD.[24] Scientific evidences also illustrated the relationship between ASD patients and gut microbiome, which has a direct/indirect influence over the feeding pattern and nutrition.[25] The investigation has shown that children with autism suffer from intestinal dysbiosis characterized by the imbalance between beneficial microbes and pathogenic microbes residing in the gut. Neurotoxic and cytotoxic molecules, such as opioid peptides, produced by these pathogenic bacteria enter the bloodstream due to leaky gut, thereby activating the immune mechanism causing tissue damage and GI inflammation. In addition, these toxic molecules affect the neurotransmitter function in the brain, leading to abnormalities in behavioral patterns such as decreased socialization, decreased response to pain, abnormal language, and self-abusive or repetitive behaviors, resulting in confusion, delirium, and even coma.[14] In ASD-vulnerable children, yeast also produces abundant chemicals leading to neurological effects.

The metagenomic analysis of ASD-gut-microbiome showed mucosal dysbiosis with a low level of *Bacteriodetes* and an increased ratio of *Firmicutes* to *Bacteriodetes*. Pyrosequencing of fetal microflora in the fecal stools of autistic children showed a high prevalence of *Desulfovibrio* species and *Bacteroides vulgatus* when compared to healthy volunteers.[26] The 16S r DNA sequencing of gut microbiome, isolated from late onset autism patients, showed a high incidence of *Clostridium* and *Ruminococcus* species, while a real-time PCR analysis showed a rich source of *Clostridium* cluster groups I and XI and *Clostridium bolteae*.[27] Culture-independent fluorescence in situ hybridization studies revealed the elevated level of *Clostridium hystolyticum* in the ASD children compared to healthy children. [28] The accumulation of neurotoxin-producing bacteria such as Clostridia worsens the autistic symptom. Another study on the gut microbial composition of autistic children revealed a low level of *Bifidobacterium* and *Enterococcus* and an increased level of *Lactobacillus* strains, despite being beneficial, which is quite paradoxical. Commensal bacteria such as *Bacillus* spp. and *Klebsiella oxytoca*, that are neither harmful nor beneficial, were reported to be present in autistic children.[29] A pilot study by Kang et al. [30] reported the presence of low-level carbohydrate degrading/fermenting bacteria such as *Prevotella, Coprococcus* and *Veillonellaceae* in ASD, substantiating the link between gut microbiome and ASD. Pyrosequencing results showed the altered gut microbial diversity in autistic children with a relatively high abundance of *Caloramator*, *Sarcina* and *Clostridium* genera*, Alistipes* and *Akkermansia* species, *Sutterellaceae* and *Enterobacteriaceae* and low level of *Prevotella*, *Coprococcus* and unclassified *Veillonellaceae*, concomitantly associated with the altered level of free amino acids and volatile organic compounds in fecal samples compared to the control samples.[31] The above reports illustrate altered gut microbiota in patients suffering from autism, when compared to healthy volunteers, which shows the direct/indirect relationship of gut microbiome with autism.

3.3. Microbial Metabolites Interrelated with ASD

The metabolites produced by gut microbiome affect the neural process based on their level. Metabolomic studies in urine, fecal and serum samples of ASD patients using LC-MS and GC-MS revealed an enhanced level of microbial metabolites, such as increased levels of SCFAs, para-cresol and ammonia, which affect the neural process.[32] [33] SCFA is the double edged sword that plays a vital role in human health and disease. SCFA is considered as a major trigger factor for ASD. Propionic acid, the widely used preservative in the food industry, is also one of the SCFAs produced by ASD-associated bacteria, such as *Clostridium*, *Bacteroides* and *Desulfovibrio.* Experimental studies with rodents treated with propionic acid exhibited ASD-associated symptoms, such as impaired and restricted social behavior and cognition, together with an enhanced neuro-inflammatory response, which might be due to alteration in mitochondrial function or the epigenetic modulation of ASD-associated genes.[34] The elevated level of another microbial metabolite para-cresol (*p*-cresol) and its conjugate *p*-cresylsulfate were observed in the urinary samples of children affected by autism. The increased level of *p*-cresol, derived either from the environment or gut microbiome, aggravates the ASD severity by inhibiting the neurotransmitters associated with enzymes and cofactors required for sulfonation reaction in the liver.[35]

Urinary metabolomic research in ASD children revealed the presence of an abnormal level of common microbial metabolites such as dimethylamine, hippuric and phenylacetylglutamine and altered tryptophan, when compared to healthy children.[36][37] The increased level of tyrosine analogue 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA), reported in the urine samples of autistic patients, might be responsible for the catecholamine depletion, worsening autistic symptoms such as stereotypical behavior, hyperactivity and hyper-reactivity.[38] Serum metabolomic studies depicted the presence of 11 metabolites in abnormal level among which, the level of sphingosine 1-phosphate and docosahexaenoic acid were consistent in all models.[39] The abnormal gut microbiome-associated metabolites in ASD patients revealed an altered metabolism leading to the aberrant increase in metabolites, worsening the symptoms of ASD.

3.4. Gut Microbiome-Associated Immune Deregulation

Several studies in human and animal models of ASD revealed the presence of an enhanced level of pro-inflammatory cytokines, brain-specific auto-antibodies in the cerebrospinal fluid and serum, illustrating an elevated immune response substantiating the fact that immune dysregulation acts as a key factor contributing to the pathophysiology of ASD.[40][41] Further autopsy of the brain specimens of ASD patients showed the presence of activated microglial cells, together with an increased level of cytokine, such as interferon (IFN)-γ, IL-1β, IL-6, IL-12p40, tumor necrosis factor (TNF)-α and chemokine C-C motif ligand (CCL)-2.[42][43] Elevated cytokines and chemokines were related to aberrant stereotypical behavior and cognitive impairment. In the newborn infants, the immune system development and homeostasis are regulated by the maternal gut microbiome colonizing the fetal gut. Multiple evidences revealed that the immune dysregulation in ASD is associated with gut microbiota[44] Certain species of gut microbiota regulate the T lymphocyte differentiation, while few microbes, such as *Bacteroides fragilis* colonization, restrict T helper cell response eliciting an autoimmune disorder.[45] Although several studies reported

that impairment of the immune system in ASD is linked with gut microbiome, the mechanism behind it is not clearly known.

3.5. Maternal Risk Factors Regulating Gut Microbiome

Epidemiological and experimental studies revealed a strong linkage between maternal infection and the development of ASD in the offspring. The gut microbial composition of a newborn infant varies with respect to the mode of delivery primarily colonized by the maternal microbiota. Hence, any imbalance in maternal microbiome with respect to environmental stress or genetic risk will be transferred to the offspring at the time of birth.[46] [47] Scientific evidences revealed that a maternal high-fat diet and exposure to stress during the gestation period increases the risk of neurodevelopment and behavioral disorders in offspring.[48] Maternal immune activation studies using animal models exposed to poly (I:C) during the prenatal stage revealed a change in gut microbiome, leading to lifelong neuropathology and altered behaviors in the offspring.[49][50][51] Based on the reports, it is clear that maternal risk factors increase the incidence of ASD in the offspring by multiple pathways, such as the altered modulation of the placenta, epigenetic modification and immune dysregulation.

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