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Review Article

An Update on Targeting the Interlinked Epigenetic and Gut Microbiome Alterations in Autism through Diet and Pro/Prebiotics

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ABSTRACT

Autism and Autism Spectrum Disorders (ASD) have complex etiologies as both genetic and environmental factors such as maternal infection, gut microbiome alterations, contaminants/toxins are involved in their pathogenesis and progression mainly through epigenetic modifications. Owing to the importance of gut microbiome in controlling the gut barrier permeability, reduced integrity of the gastrointestinal barrier and leakage of hazardous substances into the blood stream in early life may lead to Blood-Brain Barrier (BBB) instability, increasing the risk of ASD via different mechanisms, including inflammation and epigenetic alterations. In the present review, we first discuss the potential role of microbiota dysbiosis, leaky gut and the microbiota–gut–brain axis dysfunction in ASD pathogenesis, along with dietary and other environmental factors which during pregnancy play critical roles in accelerating or preventing ASD in offspring by changing the epigenetic landscape. Then, we will highlight treatment strategies that target gut microbial composition and the interlinked epigenetic alterations for alleviating cognitive and behavioral deficits in autistic and ASD patients.

INTRODUCTION

Autism and Autism Spectrum Disorders (ASD) are complicated neurodevelopmental disorders with broad phenotypes which are mainly recognized in children around the age of three years of old. The prevalence of ASD is 1:44 children, with a male to female ratio of > 4:1 [1]. Autism and ASD are caused by the interplay between genetics and environmental factors and in general exhibit co-morbid conditions, like a distinct gut microbial composition, gastrointestinal abnormalities, epilepsy, abnormal behavior and mental retardation among others [2]. ASD is characterized by reduced verbal and social interactions, emotional dysregulation, repetitive patterns of behaviors, and limited interests and social activities [3]. According to previous studies, while genetic susceptibility to autism and ASD is significant (up to 50–80 % heritability), 60 to 65% of autism cases are associated with prenatal, natal, or postnatal environmental risk factors. There is a large number of known risk factors for autism, including exposure to environmental contaminants, toxins, drug use during pregnancy, immune system activation at specific time frames during pregnancy, maternal infection, nutritional shortage/surplus, antibiotic intake and gestational

diabetes which generally affect the epigenetic landscape [4]. These environmental risk factors also play critical roles in shaping the infant intestinal microbiome [5].

The human Gastrointestinal (GI) tract has been known to be a dynamic repository of about ~10^{14} micro-organisms collectively containing about 10^{17} active genes [6], while the total number of gut microbial genes is almost 150-fold higher than the total number of human genes [7]. A well-balanced gut microbial composition is required to maintain homeostasis and hence normal brain functions since about 40% of all human metabolites, particularly neuroactive substances, are produced by the gut microbiome [8,9]. The Central Nervous System (CNS) and Enteric Nervous System (ENS) are influenced by any imbalance in the community and quantity of gut microbiome, in particular during certain times of a child's development. While it has been shown that GI symptoms intensify abnormal behavior in ASD [10], and nutritional changes affect microbial composition in patients with ASD [11], it has been proposed that microbiota dysbiosis of the GI system and its metabolites may induce or accelerate ASD [12]. However, the interplays between the brain and gut microbiome in ASD are complex, as interaction between different environmental factors and the genetic architecture involves epigenetic mechanisms as well, which are under the influence of diverse products of the gut microbiome.

Epigenetic mechanisms like DNA methylation and histone modifications are capable of acting at the interface of genes and environment and play critical roles in human brain development [13]. In recent years, it has been well documented that the perturbation of these epigenetic mechanisms and their underlying molecular processes are linked to ASD pathogenesis [14]. Additionally several lines of evidence indicates that environmental factors, which are involved in brain dysfunction and ASD pathogenesis induce gut microbiome alterations, and affect the gut–brain axis via epigenetic mechanisms [5]. Considering that akin to genetic mutations, the acquired epigenetic modifications are heritable [15,16], and similarly, the infants gut microbiome is predominantly established through exposure to the familial microbiome (essentially inherited), it becomes plausible to suggest that a significant proportion of the estimated 50–80 % of autism "heritability" actually originates from environmental factors rather than genetics. This rationale warrants a more thorough investigation into the roles of epigenetic and microbiota alterations in the pathogenesis and the treatment of autism as well as other major mental diseases.

In this review, we present the latest discoveries concerning epigenetic and microbiome aberrations in ASD. Subsequently, we delve into important roles of the increased intestinal permeability and leaky gut in ASD pathogenesis. In addition, we will focus on microbiome-gut-brain axis for its role in ASD involving epigenetic mechanisms. Lastly, we will explore the influence of dietary factors and other environmental risk factors in accelerating or delaying the onset of ASD via epigenetic mechanisms and introduce nutritional interventions that may target the microbial side to improve impaired social recognition and interaction and cognitive deficits via epigenetic mechanisms in ASD subjects.

EPIGENETIC ALTERATIONS IN AUTISM AND ASD

Epigenetic, at the top of genetic, predominantly involves molecular events that mediate formation of complexes at regulatory regions of DNA or histone proteins to affect gene expression without altering the primary DNA sequences [17]. Sequence-specific proteins and various enzymes or regulatory RNAs trigger epigenetic alterations. In post-replication events, the addition of a methyl group (CH_3-) from the methyl donor S-adenosyl-l-methionine to the cytosine or adenine DNA nucleotides, especially at the C5 position of CpG or CpA dinucleotides is catalyzed by the DNA methyltransferase enzymes (e.g., DNMT1, DNMT3A, DNMT3B) to modulate the gene transcription in different tissues/cells (Figure 1). There are at least two principal mechanisms for silencing genes by DNA methylation: I) suppressing the binding of transcription factors to their recognition elements following methylation of the critical sites [18,19], and II) recruitment of methylated DNA binding proteins to the gene regulatory regions which, in turn results in condenses chromatin by introducing histone modifications [20,21]. Histone modifications, mediated by acetylation, methylation, phosphorylation, and other modifications of different amino acids residues of the histone tails are among other epigenetic mechanisms involved in gene expression regulation and the etiology of ASD [22,23].

Disruption of histone-modifying enzymes, especially histone methylation enzymes, are also linked to the pathophysiology of ASD [24].

MicroRNAs (miRNAs), which are short (18-24 nucleotides) non-coding RNA molecules capable of regulating target genes in a tissue-specific manner represent another category of epigenetic modifiers [25]. Notably, miRNA-mediated post-transcriptional regulation of gene expression is one of the main functions of miRNAs in neuronal plasticity and neuronal development [26].

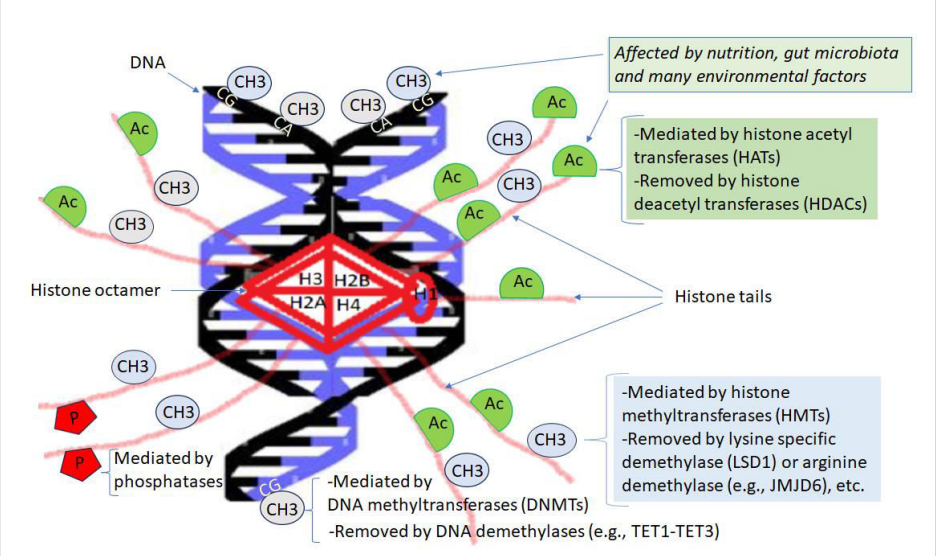

Figure 1: DNA with a negative electric charge wrapped around histone core proteins with positive electric charge. Four pairs of histone core proteins (H2A, H2B, H3 and H4) assembled together and Histone-1 (H1) as the linker histone fastens the associated DNA to the nucleosome at entry and exit sites. Different amino acids of histone tails are subjects to diverse modifications such as Acetylation (Ac), methylation (CH3) and Phosphorylation (P) mediated or removed by different enzymes which affect the status of DNA-histone binding and facilitate or inhibit accessibility of transcription factors to the associated DNA. DNA is also subject to methylation at the C5 position of CG or CA dinucleotides mediated by a number of enzymes (e.g., DNMT1, DNMT3A, DNMT3B) which affect transcription. Both DNA methylation and histone modifications are under the influence of nutrition, gut microbiota, and many other environmental factors.

Accumulating evidence suggests that environmental factors in interaction with epigenetic mechanisms play a powerful role in the pathogenesis of ASD [27]. For example, in post-mortem brain studies it has been well documented that initiation and progression of ASD is associated with various epigenetic aberrations such as DNA methylation [27,28], histone modifications [22], and miRNAs alterations [29,30]. There is also a report indicating that an epigenetic delay in the course of regular age-associated DNA methylation leads to initiation of ASD in early developmental stages [31]. Altered miRNAs expression has been shown in saliva and lymphoblastoid cell lines derived from ASD patients as well [32,33]. The most recent findings on epigenetic aberrations associated with ASD are summarized in Table 1.

LEAKY GUT AND ITS ROLE IN ASD VIA EPIGENETIC CHANGES

The principal determinants of GI barrier function are tight junction proteins such as zonula occludens-1 (ZO1), occludin (OCLN), and claudin 1 (CLDN1) [49]. Leaky gut is defined as an increase in the permeability of the intestinal mucosa which provides the opportunity for small molecules, bacterial toxins, the toxic digestive tract metabolites, and bacteria to enter into the blood circulation [50]. While blood-based transcriptome analysis found ASD affected genes are highly related to immune responses [51], it has been reported that leaky gut plays a crucial role in the pathophysiology of autism since it allows translocation of pro-inflammatory factors, chemokines, and pathogens or xenobiotics (neurotoxins, pesticides, heavy metals, and drugs) into the blood stream [52]. In fact, experimental evidence supports the notion that disruptions in

barrier integrity pave the way for initiation of inflammation in the brain by facilitating the translocation of intestinal components like Lipopolysaccharide (LPS), pro-inflammatory molecules and gram-negative bacteria from the gut lumen to the mesenteric lymph and blood circulation. The BBB's permeability is subsequently altered by low-grade systemic inflammatory responses caused by the translocation of pro-inflammatory molecules across the intestinal barrier. There are a number of alterations in intestinal permeability markers in autistic subjects addressing the disruption of tight junctions and increasing intestinal permeability, including higher zonulin levels in serum and increased diamine oxidase (DAO) activity [53,54].

Table 1: Recent epigenetic alterations identified in autism, ASD, and animal models of ASD.

Epigenetic alterations	Type of study/ tissue	Main findings	Ref.
DNA Methylation	Clinical study (South African children)/blood/brain	Identification of differentially methylated CpG sites between ASD and controls that mapped to 898 genes relevant to mitochondrial metabolism and protein ubiquitination.	[34]
DNA Methylation	Clinical study (children with ASD)/ blood	Higher NCAM1 methylation levels in ASD children than healthy children	[35]
DNA Methylation	Clinical study (children with ASD)/ blood	DNA hypomethylation and elevating inflammatory mediators such as CCR2 and MCP-1 in the neutrophils of ASD subjects	[36]
DNA methylation	Experimental study (mice model)/brain	Increasing the Mecp2 promoter methylation in the hippocampus	[37]
DNA methylation	Clinical study (Mexican population cohort with autism)/ buccal epithelium cells	A differentially methylated region (DMR) over the 5'UTR region of ZFP57 and one of its targets, RASGRF2 in ASD patients	[38]
DNA methylation	Clinical study (93 ASD and 52 controls)/ Buccal cells from patients	Hypermethylation of PGC-1α , the transcriptional regulator of mitochondrial biogenesis, at eight CpG sites of gene promoter in ASD cohort of South Africans	[39]
DNA methylation	Clinical study (five pairs of ASD-discordant monozygotic twins and four pairs of ASD-concordant monozygotic twins)/ blood	Association between abnormal methylation of SH2B1 and ASD	[40]
DNA methylation	Clinical study (14 autism cases, 7 males, 7 females)/placenta	Identification of 9655 CpGs differential methylation in autism compared to control	[41]
DNA methylation	Experimental study (cerebral organoids generated from induced pluripotent stem cells (iPSCs) from adults with a diagnosis of ASD)/ human cerebral organoids	Higher methylation levels across the majority of CpG sites within GAD1 region in ASD compared to controls	[42]
DNA methylation	Clinical study (paternal sperm with or without ASD)/ sperm	A highly significant set of 805 DMRs in paternal sperm as a biomarker for ASD susceptibility in offspring	[43]
Histone methylation/demethylation	Human and Experimental study (mouse model)/brain	Decreasing histone lysine 4 dimethylation (H3K4me2) in the prefrontal cortex of autistic patients and mutant mice model of autism	[44]
Histone methylation/demethylation	Experimental study (mouse model)/brain	Increasing histone methyltransferases EHMT1 and EHMT2, as well as histone lysine 9 dimethylation in the PFC of Shank3-deficient mice	[24]
Histone acetylation	Experimental study (valproic acid, VPA)-exposed rats)/brain	Reducing histone H3K9 acetylation in the hippocampus of VPA group compared to the control	[45]
Protein acetylation	Experimental study (mice model)/brain	Increasing acetylation of FoxO1 using SIRT2 gene deletion and consequently enhancing neuroinflammation in the hippocampus	[46]
microRNAs (miRNAs)	Clinical study in ASD patients/blood	Up-regulation of miR34c-5p, miR92a-2-5p, miR-145-5p and miR199a-5p and down-regulation of miR27a-3p, miR19-b-1-5p and miR193a-5p in ASD patients	[47]
miRNAs	Clinical study in children with ASD/saliva	Differential expression of miRNAs patterns within the ASD cohort	[32]
miRNAs	Clinical study in ASD patients/blood	Differential expression of miR-500a-5p and miR-197-5p in ASD patients	[48]
miRNA	Clinical study/saliva of ASD patients	Many dysregulated miRNAs (e.g., increased miR-1246 and miR-199b-5p, & decreased miR-96-5p and miR-149-5p)	[30]

One study in mice showed that "leaky gut" is capable to develop ASD through the activation of "lipopolysaccharide-mediated toll-like receptor 4 (TLR4)–myeloid differentiation factor 88 (MyD88)–nuclear factor kappa B [NF-κB] signaling pathway" and their downstream inflammatory cytokines in the cerebral cortex [55]. Dysbiosis is one of the most important players in generating leaky gut and subsequent absorption of xenobiotics. Mycotoxin-producing molds and neurotoxin-producing bacteria contaminate food and infect the intestinal tract, which in turn causing leaky gut, immunosuppressive activity, and generation of neurotoxins involved in ASD [56]. Ochratoxin A (OTA) is a microbial toxin that is produced by strains of *Aspergillus* and *Penicillium* during gut dysbiosis and confers susceptibility to ASD via epigenetic mechanism, possibly through dysregulation of microRNAs [57]. It has been found that intestinal dysbiosis and increased mucosal permeability in the upper and lower intestines result in reduced concentrations of vitamin B6, folic acid (vitamin B9) and vitamin B12 in autistic patients and subsequently leads to alterations in protein and DNA methylation levels [58]. Autistic children also exhibit noticeable reduction in protein and DNA methylation levels, which is associated with increased concentration of 5-methyltetrahydrofolate and therefore a lower availability of methyl group as well as significant reduction in urinary methionine and S-adenosyl-L-methionine (SAM) concentrations, the major methyl donor [58]. In addition, it has been reported that dysregulated non-coding RNAs (particularly miRNAs and piRNAs) as transcriptional modulators are involved in intestinal permeability, altering microbiome composition, and inflammation in autism [59]. As the function and integrity of the gut epithelium barrier can be regulated by the gut microbiome and its metabolic products, alterations in gut microbial diversity may affect the gut barrier integrity, intestinal permeability, and consequently prevent ASD [60]. For example, transient hyperglycemia in maternal diabetes can result in persistent epigenetic alterations and expression suppression of tight junction proteins associated with altered gut microbiota compositions, increased intestinal permeability and oxidative stress, inflammation, which subsequently triggers autism-like behavior in mouse offspring [61].

MICROBIOME-GUT-BRAIN AXIS AND EPIGENETIC ALTERATIONS IN ASD

There is a relationship between the CNS and the gut microbiome via metabolic, immune, endocrine, and neural pathways [62,63]. A complex bidirectional system named the "microbiome-gut-brain axis" mediates communication between the GI tract and the CNS. Microbiome-gut-brain axis have been shown to play a crucial role in a large number of physiological processes like metabolic homeostasis, immune response, and brain development and its disruption has been linked to ASD pathogenesis [64]. Microbiome-gut-brain axis includes efferent and afferent signals. The enteroendocrine system, gut products, metabolites, cytokines, Vagus nerve and neuroactive molecules play an important role in triggering afferent signals from the GI tract to the brain. Efferent signals originates in the brain and transmitted to the gastrointestinal tract and are involved in epithelial permeability, gastrointestinal motility, neuroendocrine and autonomic regulation [65].

Two interrelated mechanisms have been proposed for the communication between the GI tract and the CNS, both of which are associated with ASD pathogenesis, epigenetics and redox signaling [66]. The gut bacterial-derived metabolites can act as epigenetic agents and contribute to gene regulation and expression. In fact, several lines of evidence (Table 2) indicates that epigenetic changes in the gut and potentially in the brain of patients with ASD can be induced by the gut microbiota and its fermentation products [67]. In addition to their epigenetic effects, gut bacterial-derived metabolites (e.g., short-chain fatty acids, SCFAs) play a crucial role in host signaling via facilitating and even substituting host Reactive Oxygen Species (ROS) production [68]. ROS are considered as second messengers which exert oxidative activity on proteins for influencing immune and other signaling processes [69]. The epigenetic mechanisms and ROS are thought to have interactive effects in brain development [70]. ROS not only play an important role in cellular redox alterations and signaling pathways but also affect redox-sensitive transcription factors, histone/protein deacetylation, and chromatin remodeling [66].

A variety of alterations in the composition of the gut microbiota and its metabolites (especially SCFAs) have been reported in patients with ASD [71]. For example, a higher level of fecal valeric acid and lower levels of fecal acetic acid and butyrate have been found in ASD subjects [72]. In addition, autistic patients exhibit an increased abundance of gut valeric acid associated bacteria (*Acidobacteria*) and decreased abundances of key butyrate-producing taxa (*Ruminococcaceae, Eubacterium, Lachnospiraceae* and *Erysipelotrichaceae*). Owing to their activity as histone deacetylase inhibitors, microbiome–derived SCFAs play a critical role in numerous physiological processes such as maturation of microglia in the CNS, producing neurotransmitters, promoting the differentiation of T cells, and immune homeostasis [73,74]. Changes in the composition of the gut microbiota and its metabolites also heavily affect central neurotransmitter metabolism via related pathways of the gut-brain axis. For example, a significant increase in betaine level, but decreased levels of butyric acid, acetic acid, isobutyric acid, valeric acid, and isovaleric acid along with reduced levels of several neurotransmitter related molecules (e.g., threonine, 5-hydroxyindoleac etic acid, kynurenine, betaine aldehyde chloride, and tryptophan) were observed in the prefrontal cortex of valproic acid model rats versus the control rats [75]. The composition change of the gut microbiome can induce ASD-like symptoms, as well. For instance, Canonaco et al. examined whether Fecal Microbiota Transplant (FMT) from autistic children to wild-type mice confers the colonization of ASD-like microbiota and autistic behaviors [76]. They found a significant reduction ($p < 0.001$) in *Actinobacteria and Candidatus S.* and increased populations of *Tenericutes* in the gastrointestinal region of recipient mice associated with autistic behaviors and increased expression of pro-inflammatory factors (e.g., IL-1β, IL-6, COX-1 and TNF-α) in small intestine and brain compared to the control mice. They also found that these molecular alterations are due to DNA hypomethylation. As summarized in Table 2, a large amount of evidence in recent years connect gut microbiota alterations to various types of epigenetic anomalies associated with ASD.

Table 2: Recent experimental or clinical evidence linking gut microbiome to epigenetic alterations.

Type of study	Changes in gut microbiota or its products	Key Findings	Epigenetic changes	Ref.
Experimental study (fecal microbiota transplant via gavage from autistic children to mice)	Increasing abundance of *Tenericutes* and decreasing abundance of *Actinobacteria* and *Candidatus S.*	A significant decrease in the brain DNA methylation and key role of gut microbiota in ASD.	DNA methylation	[76]
Experimental study (mouse model)	Increased intestinal permeability and altered microbiota compositions	Gene suppression of tight junction proteins via epigenetic changes and consequently triggering autism-like behavior	DNA methylation	[61]
Clinical study (Sixty children with idiopathic ASD)	Intestinal dysbiosis and altered microbiota compositions	Reducing protein and DNA methylation in autistic children	Protein and DNA methylation	[58]
Experimental study (mice model)	Changes in the abundance of taxa of several bacterial genera, like *Lactobacillus*	Elevated levels of 5-hydroxymethylcytosine (5-hmC), in the hypothalamus	DNA methylation	[77]
Clinical study (Chinese children with autism)	Decreases in key butyrate-producing taxa (*Ruminococcaceae, Eubacterium, Lachnospiraceae* and *Erysipelotrichaceae*) and lower levels of fecal acetic acid and butyrate	Altered levels of short chain fatty acids	Histone acetylation	[72]
Experimental study (mouse model)	Gut dysbiosis and altered gut microbiota	Dysregulation of HDAC1-mediated epigenetic machinery and hence hyperactive microglia in the brain	Histone acetylation	[78]
Clinical study (120 children diagnosed with ASD)	Altered gut microbiota and short chain fatty acids	Lower levels of melatonin and 3-hydroxybutyric acid as a histone deacetylase inhibitor	Histone acetylation	[79]
Experimental study (rat model)	Altered gut microbiota and short chain fatty acids	Decreasing butyric acid metabolism as histone deacetylase inhibitor	Histone acetylation	[80]
Clinical study (36 children with ASD)	Altered gut microbiota and short chain fatty acids	Decreasing the abundance of genes linked to production of butyric acid in the ASD metagenomes	Histone acetylation	[8]
Experimental study (rat model)	Gut microbiota dysbiosis and altered short chain fatty acids (SCFA) in valproic acid model rats	Decreasing acetic acid, butyric acid, and isobutyric acid	Histone acetylation	[75]
Clinical study (Saliva of 53 children with ASD)	Increase in the abundance of *Weeksellaceae, Aggregatibacter, Rothia, Ralstonia, Actinobacillus, Pasteurellaceae,* and *Filifactor*, but decreases in *Tannerella, Moryella* and *TM7-3*	Dysregulation of miRNAs and microbiome in the saliva of children with ASD and their association with cognitive impairments	miRNAs	[81]

MATERNAL DIET, ASD, AND THE ROLES OF ENVIRONMENTAL RISK FACTORS VIA EPIGENETIC MECHANISMS

As illustrated in Figure 2, a growing body of evidence has shown that environmental factors like nutrients, toxins, contaminants, drugs, infections and many other players can affect the gut microbiome composition, which in turn affect epigenetic landscape and consequently change gene expression patterns involved in ASD pathogenesis [82,83]. For example, the escalating use of antibiotics may give rise to disruption of the GI microbiome and subsequently the development of neurobehavioral symptoms similar to ASD [84]. It has been shown that treatment of the newborn mice with a single antibiotic (ampicillin or vancomycin) could induce dysbiosis and subsequently hippocampal dysfunction and ASD-like behavior by remodeling serum metabolome (elevating the serum 4-methylphenol, a small aromatic metabolite produced by gut bacteria) possibly through epigenetic changes [85]. Recent investigations have also suggested a relationship between maternal gut microbiota alterations and neurodevelopment in offspring. In reality, during the first year of human life, the impact of the maternal prenatal gut microbiome on children's neurodevelopment surpasses the effects of children's own gut microbiome [86]. This underscores the crucial role of the maternal prenatal gut microbiome in neurodevelopment and/or the onset of ASD in offspring.

As maternal infection or immune activation alter expression and epigenetic property of autism associated genes in brain [87,88], there are various other risk factors during pregnancy, such as GI disorders, stress, and obesity which have the potential to disrupt the balance of gut microbiota, and alter the newborn BBB permeability and, subsequently, contribute to the development of ASD in offspring [89]. A recent study in a mouse model of ASD has demonstrated the importance of environmental factors such as diet (fish oil) on gut microbiota dysbiosis and hence improvement of ASD phenotype [90]. In another interesting example, a maternal high-fat diet could contribute to a predisposition for ASD-like phenotypes in male adolescent offspring by elevating cortical global DNA methylation levels and the expression of miR-423 and miR-494 [91]. Furthermore, exposure to Valproic Acid (VPA) during pregnancy is considered an environmental factor to induce ASD in offspring via glycolysis-mediated histone acetylation of neuron specific transcription factors [92]. VPA is also capable of impairing mitochondrial functions and elevation of glycolysis which in turn result in increases in H3 (histone-3) and H3K9 acetylation (H3K9ac), and H3K9ac binding to the promoters of two transcription factors (Ngn2 and Mash1), which determine the fate of excitatory neurons [92].

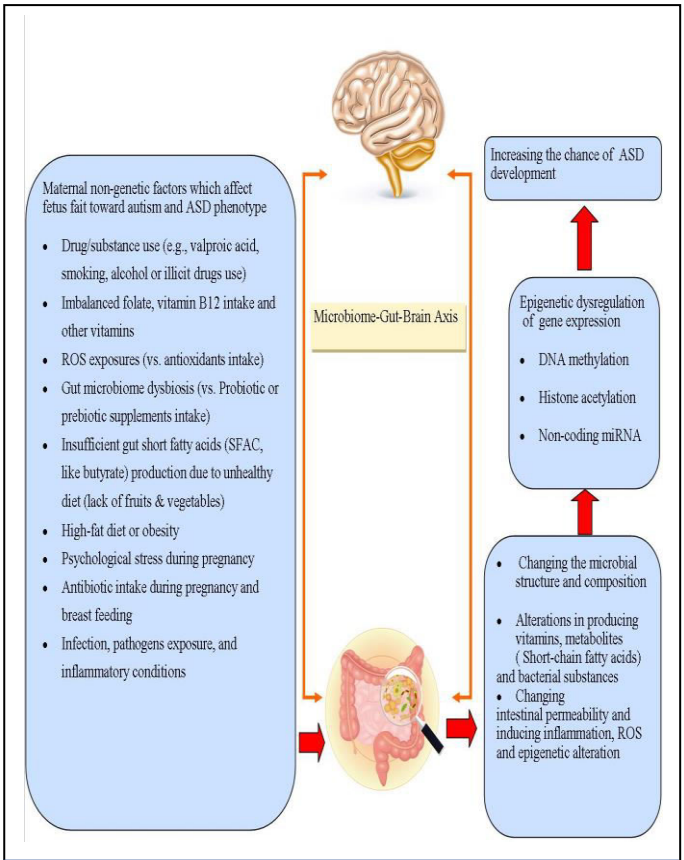

Figure 2: There are many maternal risk factors involved in autism pathogenesis which through affecting the offspring gut microbiome composition alter the epigenetic landscape (as summarized in Table 2) increasing the chance of autism or ASD development in the offspring.

On the other hand, specific changes in diet or medication use were shown to prevent maternal diabetes-mediated autism-like behaviors in male offspring of diabetic dams by altering gut microbiota compositions and intestine permeability. For example, Yao et al. examined the effects of treatment with superoxide dismutase mimetics (MnTBAP, or SR1078, an agonist of retinoic acid-related orphan receptor alpha, RORA which is decreased in autism), in male offspring of diabetic

dams. They evaluated H3 methylation on the RORA promoter and found that the treatment group exhibits improved maternal diabetes-mediated GI symptoms and reduced oxidative stress and inflammation in the brain by increasing H3K9me3 (tri methylation of lysine 9 of H3) compared to the control subjects [93]. According to Cristiano and et al. studies maternal treatment with sodium butyrate, a histone deacetylases inhibitor, could also rescue ASD-like symptoms in offspring of BTBR mouse model of ASD by attenuating long-term synaptic plasticity deficits and hampering the cerebellar cortex hypertrophy and the Purkinje cells firing [94]. However, it is intriguing to explore why VPA (another histone deacetylases inhibitor) induces ASD-like symptoms, but sodium butyrate may exert beneficial effects.

IMMUNE SYSTEM IN ASD AND ITS ASSOCIATION WITH EPIGENETIC MECHANISMS

Nearly a decade ago, experimental evidence led to hypothesized that endogenous histone deacetylase inhibitors produced by the gut microbiome are capable of minimizing inflammation, oxidative stress, and normalizing the aberrant expression of brain genes which in turn reduce synaptic and social deficits pertinent to autism [95]. Other lines of evidence indicated that gut microbiota dysbiosis can affect gut neurotransmitters (e.g., serotonin) production, immune system, the BBB permeability, and brain epigenetic alterations involving microglia [96,97]. As gut microbiota is one of the major sources of compounds which affect histone acetylation, a recent study unraveled that dysregulation of HDAC1-mediated epigenetic machinery during embryogenesis alters both aorta-gonad-mesonephros and yolk sac progenitors which in turn reduces the AP-1 complex expression and microglia development [78]. While the impairment of microglial maturation and development leads to the dysregulation of the brain immune system, mostly associated with microglia hyperactivity, several lines of evidence indicate that microbiota-derived metabolites play an important role in initiation of microglia inflammatory responses via epigenetic mechanisms [98]. For instance, it has been shown that microbiota-derived acetate (a SCFA, like butyrate) is capable of triggering intestinal innate immunity through the Tip60 histone acetyltransferase complex, inducing chromatin remodeling [99]. In another study, it was shown that microbiota-derived acetate contribute to microglia maturation, modulation of microglial phagocytosis and neurodegeneration, involving epigenetic mechanisms [100]. Taking into account the therapeutic implications of these findings, Yan et al. found that histone deacetylase inhibitor MS-275 could enhance N-methyl-D-aspartate receptors (NMDAR) and synaptic functions and improve autistic social preference in a Shank3-deficient mouse model of autism by increasing histone acetylation in the prefrontal cortex [101]. Nevertheless, VPA with similar mechanisms of action may induce ASD phenotype, likely by affecting other classes of the mammals HDACs, a subject that calls for more studies in this era.

THERAPEUTIC APPROACHES FOR ASD BY REBALANCING THE MICROBIOME AND CHANGING EPIGENETIC STATUS

One of the promising strategies for prevention or treatment of autism is rebalancing the maternal and offspring microbiome inside their bodies, and thereby modulating disease-associated epigenetic and gene expression alterations. This can be achieved by changing diet, lifestyle or by supplementation with beneficial bacteria or their metabolites. Here are some examples of therapeutic approaches to achieve these objectives.

The ketogenic diet in treatment of autism involving microbiome and epigenetic modulations

The ketogenic diet has been known to be an appropriate-protein, high-fat, and low-carbohydrate diet that is capable of mimicking the fasting state (or caloric restriction) of the body with beneficial effects for treatment of autism by modulating the gut microbiome, improving mitochondrial function and morphology, and regulating neurotransmitters, through epigenetic mechanisms [102-104].

The ketogenic diet has been found to be an endogenous inhibitor of class I HDACs, increasing the level of histone acetylation in Prefrontal Cortex (PFC) neurons through the major product of β-hydroxybutyrate. Experimental studies in BTBR mice demonstrated that ketogenic diet could ameliorate ASD-like conditions by remodeling gut-brain axis (increasing relative abundances of putatively beneficial microbiota, *Akkermansia* and *Blautia,* and reducing *Lactobacillus* in BTBR

mice feces), attenuating pro-inflammatory cytokines, and oxidative stress [105]. In another study, Qin et al. found reduced histone acetylation in Shank 3 deletion mouse model [106]. A 4-week treatment with a ketogenic diet resulted in a prolonged rescue of social preference deficits by promoting the transcription and histone acetylation of Grin2a and Grin2b and restoring the reduced NMDAR synaptic function in PFC neurons. It has also been shown that β-oxidation of beta-hydroxybutyrate (βHB) produced by the ketogenic diet was responsible for increasing NAD^+ levels, promoting mitochondrial elongation and, consequently, activation of SIRT deacetylases which in turn could improve locomotor behavior in the shank3+/- zebrafish model of ASD [107].

Gut microbiome-derived metabolites with epigenetic activity in the treatment of autism

SCFAs are a group of compounds produced by the gut microbiome which have extensive effects on gut, brain, and behavior associated with ASD [108]. As the expression of monocarboxylate transporters facilitate the entrance of SCFAs into the brain tissue, and SCFAs interact with G protein-coupled receptors (GPCRs) or HDACs, they also affect psychological functions through vague nerve signaling, hormonal and immune pathways [109,110]. The Hypothalamic–Pituitary–Adrenal (HPA) axis is a part of the microbiota–brain axis that includes the endocrine system and the CNS, adjusting the balance of hormones in response to stress. Stress affects hypothalamus, stimulating the pituitary gland to secrete hormones which in turn stimulate the secretion of cortisol from the adrenal glands. HPA activity in response to stressors can be aggravated by microbiota deficiency, indicating crucial role of the gut microbiome in the HPA axis regulation [111].

It has been reported that SCFAs are promising candidates to hamper social deficits in prenatal Lipopolysaccharide (LPS)-exposed rat model of ASD by altering the HPA axis function via epigenetic mechanisms. For example, Chen and coworkers examined whether sodium butyrate is capable of improving ASD-like symptoms and alleviating social deficit through epigenetic regulation of the HPA axis in offspring [112]. In their study, as higher cortisol levels and lower SCFA concentrations were seen in children with ASD along with reduced histone acetylation activity, decrease in SCFA-producing bacteria, and impaired Corticotropin-Releasing Hormone Receptor 2 (CRHR2) expression in prenatal LPS-exposed rat model of ASD. They found the normalization of corticosterone and CRHR2 expression in vivo, as well as increased histone acetylation at the CRHR2 promoter in vitro, following treatment with sodium butyrate. In another study, it has been found that treatment with sodium phenylbutyrate could improve cognitive impairment and core ASD symptoms such as sociability deficit and repetitive behaviors by enhancing histone acetylation in the hippocampus, cerebral cortex, and striatum in the BTBR and the VPA mouse models of ASD [113]. Previously, Kratzman et al. also reported that the treatment by sodium butyrate could ameliorate social deficits in the BTBR mouse model by regulating the inhibitory pathway transcripts and down-regulation of the activity-related transcriptome in the PFC [114].

Probiotic, prebiotic, and symbiotic treatment for ASD via epigenetic alterations

Probiotics are live microorganisms that account for a range of positive health benefits like immunomodulatory capabilities and neuroprotective effects which are achieved in part through epigenetic modifications [115]. Therefore, the administration of pro-, sym- and prebiotics is regarded as a promising strategy either to increase the abundance of microorganisms and metabolites with beneficial effects on CNS-driven behavior or to decrease the abundance of harmful microorganisms and related metabolites with detrimental effects on behaviors [116-119]. It has been reported that consumption of multispecies probiotics containing *Limosilactobacillus reuteri, Levilactobacillus brevis,* and *Bacillus amyloliquefaciens* increases the population of potentially beneficial bacteria (*Ruminococcaceae*, *Catenibacterium*, *Catonella*, *Acidaminococcus*, and *Olsenella*) and reduces the abundance of pathogenic bacteria like *Chlamydia* and *Escherichia* [119].

Bifidobacterium and *Lactobacillus* are well-studied probiotic bacterium and their efficiency for treatment of neurodevelopmental disorder such as ASD are heavily depend on several factors such as the host characteristics, dosing patterns, dose, and the underlying luminal microbial environment [120]. Recent studies have reported that beneficial effects of probiotic bacteria against ASD are associated with

alerting gut microbial composition, reducing potent biomarkers of leaky gut (occludin and zonulin), and attenuating oxidative stress [121]. The protective effect of probiotics against ASD is linked to epigenetic alterations, as well. For example, it was shown that probiotic treatment by *L. helveticus CCFM1076* could contribute to alleviating autistic-like behavioral symptoms in VPA-treated rats by reducing *Turicibacter* abundance and restoring butyric acid level, as a histone deacetylases inhibitor [122]. Moreover, it has been shown that prenatal probiotic exposure (*Lactobacillus Reuteri*) in mice could confer protective effects in offspring by changing DNA methylation profile of some genes such as the Dlg2, Shank3, and Agap3 [123].

In addition to changing the balance of intestinal microbiome composition, neuroprotective effects of probiotics, prebiotics, and synbiotics against ASD are associated with altering the concentrations of bacteria producing metabolites such as SCFAs. For instance, Sivieri et al. investigated the effects of probiotics (*Limosilactobacillus* (*L.*) *reuteri* + *Bifidobacterium* (*B.*) *longum*), prebiotic (Galacto-Oligosaccharide (GOS)), and synbiotic (*L. reuteri* + *B. longum* + GOS) on gut microbiota composition and metabolism of ASD children using an in vitro (under simulated gastrointestinal conditions) fermentation model (SHIME®) [124]. In addition to positive modulation of the gut microbiota, prebiotic and synbiotic were capable of increasing acetic, propionic and butyric acids (as histone deacetylases inhibitors) in the ascending, transverse, and descending colons.

CONCLUSION AND FUTURE PROSPECTS

The findings of this review support the idea that epigenetic abnormalities caused by environmental factors are associated with altered gut microbiome, and its metabolites playing critical roles in increasing the risk of ASD. A well-balanced gut microbial composition promotes brain function and behavior by maintaining the tight junctions of intestinal epithelial cells, generating the gut regulatory neurotransmitters and removal of toxins and waste. However, microbiome dysbiosis disrupts normal function of the gut-brain axis and increases risk of ASD by enhancing neuroinflammation and oxidative stress via alerting genes epigenetic landscape and thus expression. Although the imbalances of beneficial microbes have been found in ASD subjects, the detailed mechanism of their impacts remains widely unknown, thus necessitating efforts to address the unmet challenges in this era. For instance, there are very large variations of GI symptoms in ASD patients (from 9–91%), which mainly attributed to various study populations, small sample size, lack of consensus in clinicians about GI symptomology, and various methodological methods (e.g., time period for reporting and data source). Therefore, more studies with larger cohorts and consensus in clinicians regarding GI symptomology will help investigators to precisely confirm the relationship between GI problems and ASD as well as the gut microbiome, inflammation and epigenetic alterations in autism. Additionally, potential sub-types of gut microbiome in ASD subjects, geographical differences among study population, differences in methods and technologies for determining microbial composition, and insufficient statistical control for examining multiple-hypotheses may lead to remarkable discrepancies in altered gut microbiome composition and epigenetic alterations in ASD cases versus neurotypical individuals. These can be minimized by well-characterized multinational studies using the same methods of biological analysis.

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