REVIEW ARTICLE

THE BIO-MARKERS BEYOND THE BRAIN: A REVIEW OF GUT DERIVED SIGNATURE IN AUTISM

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ABSTRACT:

In the realm of neurodevelopmental disorders, notably autism spectrum disorder (ASD), the gut-brain axis is attracting significant scholarly interest. Autism spectrum disorders (ASD) are characterized by a unique set of neurodevelopmental traits that are significantly influenced by social and cognitive disorders. These traits can include challenges in social interaction, communication difficulties, and a tendency toward repetitive behaviors or restricted interests. Current scientific understanding posits that ASD results from a combination of genetic predisposition. However, despite advances in research, the exact causes of ASD continue to elude definitive identification, indicating a complex interplay of factors that requires further exploration. The characteristics of ASD are intricately linked to the temporal variations in the composition of microbiomes. This ongoing quest for knowledge is crucial for developing effective interventions and support systems for individuals with ASD and their families. Comorbid conditions, particularly gastrointestinal disorders, are frequently observed among individuals of various racial backgrounds. Extensive research indicates that the gut-brain axis plays a crucial role in the pathophysiology of ASD, highlighting the significant correlation between the severity of neurological impairment and the presence of gastrointestinal disorders. Some behaviors linked to Autism Spectrum Disorder (ASD) have decreased in intensity, correlating with the repair of the gastrointestinal epithelial barrier. It is still uncertain if gastrointestinal microorganisms play a role in ASD or if they are viable targets for therapies. The gut-microbiota-brain axis is a reciprocal communication system connecting the gut, its microbes, and the central nervous system.

KEWORDS: Autism Spectrum Disorder, gut-brain axis, gut microbiota, microbiomes.

INTRODUCTION:

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition marked by repetitive behaviors, restricted interests, and difficulties in social interactions[1]. Epidemiological data suggest that the prevalence of ASD and typical autism ranges from 5 to 20 cases per 10,000 individuals, with variations reported between 18.7 and 60 cases per 10,000[2]. Research has indicated that multiple factors, such as genetic predispositions, immunological responses, inflammatory processes, environmental influences, and more recently, gut microbiota, may play significant roles in the development and prognosis of this disorder[3]. Individuals with autism spectrum disorders (ASD) often experience a range of associated conditions, including intellectual disabilities, gastrointestinal (GI) disorders, and irregularities in nutrition and sleep patterns. These GI disorders can present with a variety of symptoms, such as vomiting, excessive gas, diarrhea, constipation, and abdominal discomfort[4]. Research indicates that the prevalence of these gastrointestinal issues among individuals with ASD can range dramatically from 9% to as high as 90%, a figure that starkly contrasts with the rates observed in neurotypical individuals. This significant disparity suggests that the gastrointestinal and neuropsychiatric symptoms frequently reported in autistic populations may be intricately linked to alterations in gut microbiota, highlighting the importance of understanding the gut-brain connection in these individuals[5].

Following the administration of antimicrobial therapies, including metronidazole or vancomycin, children diagnosed with regressive autism have exhibited notable and swift enhancements in their autistic behaviors. This observation has led researchers to propose that alterations in the gut microbiota may contribute to the neurological underpinnings of these conditions[6]. The relationship between the brain and the gastrointestinal tract is facilitated by intricate networks referred to as the gut-brain axis, which enables bidirectional communication between the gastrointestinal system and the central nervous system. Key elements of this system encompass the immune response, enteric bacteria, the enteric nervous system, and the autonomic nervous system[7].

A crucial area of research related to Autism Spectrum Disorder (ASD), especially in younger populations, is the interaction between the brain, gut, and microbiome. Within the human body, there exists a vast population of trillions of microbial cells, which harbor a genetic repertoire that is approximately one hundred times greater than that of the human genome[8]. Recent corrections have revised the previously accepted ratios of 10:1 and 100:1, determining that the

ratio of bacterial cells to human cells is actually 1:1[9]. Research studies involving both animal models and human participants have revealed a relationship between intestinal bacteria and the central nervous system, which may influence behaviors related to autism spectrum disorders. Nonetheless, the existing evidence is still insufficient to conclusively establish a causal relationship between gut microbiota and ASD[10]. Among the four principal phyla found in the gastrointestinal tract, Bacteroides and Firmicutes dominate, comprising more than 90% of the bacterial population in a healthy adult. Additionally, the phylum Proteobacteria includes genera such as Enterobacter, Bacteroidetes, and Prevotella. Microorganisms have the potential to influence neuronal function through the action of ASD, which may lead to irregular serotonin activity, reduced systemic inflammation, and changes in metabolic profiles[11].

Clostridium tetani is recognized for producing the neurotoxin P-cresol, which is linked to anxiety-related behaviors, along with other detrimental metabolites such as phenolic compounds and indole derivatives. Additionally, Clostridium bartlettii produces trans-3-indole acrylic acid (IAA) and participates in glycine binding to form indolyl-3-acryloyl (IAG) glycine, which has been identified as a potential urinary diagnostic biomarker for ASD[12].

The microbiota present in individuals with Autism Spectrum Disorder (ASD) can affect neuronal function through various mechanisms, including diminished systemic inflammation, an impaired serotonergic system, and/or alterations in metabolic profiles[13]. Intestinal metabolites, which can be either intermediary or final products of bacterial metabolism, play a significant role in this process. In addition to short-chain fatty acids (SCFAs) and aromatic amino acids obtained from dietary sources such as fruits and fiber, endogenous metabolites are produced from cholesterol and bile acids[14]. There is a connection between these metabolites and a range of disorders, including neuropsychiatric, neurodegenerative, and neurodevelopmental issues like Parkinson's disease (PD), Alzheimer's disease, and autism spectrum disorder (ASD)[15]. The predominant short-chain fatty acids (SCFAs) that are produced in notable quantities include butyric acid, propionic acid, and acetate, whereas other byproducts are synthesized in only small traces[16]. An examination of the pathophysiological aspects of gastrointestinal and irritable bowel syndrome found that participants with the variety had diminished levels of fecal acid, butyric acid, and propionic acid, alongside heightened levels of valeric acid[17].

In experiments with mice, ACFA showed that the removal of proinflammatory cytokines, specifically IL-1β, TNF-α, and IL-6 mRNA levels, along with the stimulation of microglia, led to the prevention of neuroinflammation[18].

IMMUNE EFFECT:

The proposition that immunorestriction is the principal factor influencing ASD has been validated by a wealth of research indicating that proinflammatory cytokines and certain brain-related autopolypeptides are found in elevated amounts in serum and cerebrospinal fluid in models of human disorders[19]. These heightened levels of cytokines and chemokines are correlated with cognitive deficits and inadequate stereotypical behaviors. Moreover, a significant number of studies have pointed to a relationship between microbiota and intestinal ASD, particularly in the context of immune dysfunction[20].

DYSBOSIS:

Children with Autism Spectrum Disorder (ASD) present oxidative disturbances in their gut microbiota. This condition is characterized by a high occurrence of specific bacterial groups, including Bacteroides, Parabacteroides, Clostridium, and Faecalibacterium, alongside lower levels of Bifidobacterium and Coprococcus. Such an imbalance may affect the severity and symptoms of ASD, indicating that probiotics or prebiotics could play a role in regulating behaviors associated with autism[21]. Furthermore, psychological and physiological stress can alter acidity by modifying mucus secretion patterns, which may lead to a temporary decrease in gastric emptying, changes in intestinal permeability, and the activation of local immune responses. These factors can have a significant impact on the distribution of intestinal bacteria[22].

A potential link may exist between gut health, cytokine-related symptoms, and those associated with TSA. This suggests that research has clarified the relationship between the intestinal microbiota and increased levels of pro-inflammatory cytokines in ASD[23]. Dysbiosis within microbiomes can primarily lead to inflammatory disorders and various psychological conditions. The activation of the immune system triggers gastrointestinal inflammation and raises pro-inflammatory cytokine levels in the bloodstream, which in turn activate astrocytes and microglial cells. When these cells cross the blood-brain barrier (BBB), they provoke inflammation in the central nervous system[24].

In addition to its inflammatory effects, IL-6 reveals a significant negative association with socialization and full-scale intelligence quotient (FSIQ) in individuals with autism spectrum disorder (ASD), indicating a potential relationship between inflammatory processes and behavioral outcomes[25]. Cytokines might mediate the connection between behavioral difficulties, the stability of rigid social ties, and inflammation. Moreover, changes in the expression of intestinal tight junction proteins and the integrity of the intestinal barrier have been linked to increased levels of IL-6[26]. The diminished levels of anti-inflammatory cytokines, including IL-10 and IL-1Ra, indicate that those with Autism Spectrum Disorder (ASD) could demonstrate a discrepancy between pro-inflammatory and anti-inflammatory activities [27].

DIETARY INTERVENTIONS:

The link between nutrition and autism spectrum disorder (ASD), a complex developmental disorder, provides a fresh perspective. Evidence suggests that dietary factors may influence the emergence of ASD and could also assist in reducing its symptoms[28]. Moreover, the use of exclusionary diets, particularly gluten-free and casein-free (GFCF) diets, has been effective in alleviating ASD symptoms. A randomized clinical trial found that following a gluten-free diet led to improvements in gastrointestinal symptoms and behavioral difficulties associated with ASD[29]. Probiotics offer a flexible option compared to strict dietary regulations. Their ability to produce digestive enzymes and antioxidants plays a crucial role in protecting the intestines from infections, regulating immune responses, reducing variations in immune function, restoring or preserving intestinal barriers, strengthening or stabilizing dense compounds, and promoting mucin production[30].

LEAKY GUT:

The notion of "leaky gut" has attracted significant scholarly attention because of its links to a range of gastrointestinal (GI) and non-GI conditions, such as type 2 diabetes, asthma, irritable bowel syndrome, and specific neurological disorders[31]. Consequently, strategies designed to enhance "gut integrity," including the use of probiotics, dietary changes, and various therapeutic methods, have also gained increased focus as possible interventions for numerous health concerns[32]. Autism Spectrum Disorder (ASD) has been found to correlate with a non-uniform distribution of gut microbiota and notable structural modifications in organs that are pertinent to the disorder. These changes are believed to impact the functionality of the intestinal barrier, a concept often described as intestinal permeability. This suggests that the balance of

microbial populations and the physical state of the gut may play a crucial role in the pathophysiology of ASD[33].

BIOMARKERS:

1. **Lippopollysaccharides (LPS):**

An imbalance in microbiota, known as dysbiosis, triggers the release of lipopolysaccharides (LPS), which in turn generate and spread powerful pro-inflammatory agents found in endotoxins. This phenomenon can impact the central nervous system, particularly increasing activity in the tonsils, which are vital for managing emotions and behavior[34]. It is hypothesized that this may lead to a decline in mobility and negatively influence both emotional and cognitive capabilities. This research demonstrates that lipopolysaccharide (LPS) activates microorganisms, resulting in endothelial cell (EC) damage and compromising the blood-brain barrier (BBB). Research conducted in vitro has focused on the connections between endocannabinoids (EC), lipotesic acid (LTA), and lipopolysaccharide (LPS), which are crucial components of the cell wall of gram-positive bacteria[35].

The outcomes indicate that the endothelial barrier is effectively shielded from the effects of these toxins. The expression of mRNA for inflammatory mediators associated with alpha tumors, specifically TNF-α and beta-interleukin-1 (IL-1β), has demonstrated a notable increase[36]. In contrast, the levels of ZO-1, occludin, and junctional adhesion molecules (JAM) have experienced a reduction. Additionally, the levels of lipopolysaccharide have decreased after the introduction of metformin[37]. Additionally, the expression of key components associated with 4D88-NF corn, specifically TLR4 and MyD88, is reduced, leading to a decrease in the inflammatory cytokines related to these components in the cerebral cortex. As a result, the intestinal fluid may trigger the TLR4-MyD88-NF-Ukraine pathway, potentially playing a role in the development of autism in reaction to lipopolysaccharides[38].

2. **Short acting fatty acids(SCFAs):**

The term short-chain fatty acids (SCFAs) refers to neuroactive compounds such as Butyrate, Propionate, and Acetate, which are primarily formed in the colon through the fermentation of resistant starch and dietary fiber by bacteria. There is a growing recognition of the colon's critical functions in supplying energy and nutrition, as well as its role in sustaining the balance of regulatory T cells (Treg)[39]. A further component impacting this situation is the information system concerning short-chain fatty acids (SCFAs), which affects the entire body, including both the colon and the brain. Notably, there is a correlation between higher SCFA levels and

obesity, while lower levels are associated with Parkinson's disease (PD) and alterations in neuropeptide[40]. These factors affect the operation and conduct of the brain. Studies have shown a significant rise in PPA and AA, along with a similar increase in PPA and BA. Furthermore, individuals with Autism Spectrum Disorder (ASD) exhibited higher levels of AA, PPA, BA, and isobutyric acid when compared to control groups. Additionally, individuals diagnosed with propionic acidemia frequently present with characteristics of ASD[41].

3. **Zonulin:**

Zone phosphorus is acknowledged as an important indicator of intestinal permeability and is often associated with a range of chronic illnesses, such as diabetes, celiac disease, inflammatory bowel disease, obesity, and conditions related to the ASD spectrum[42]. The mucosal layer of the gastrointestinal tract contains secretory immunoglobulin A (SIGA), which serves as a crucial defense mechanism, protecting the intestinal epithelium from internal toxins and pathogens. Individuals with ADHD demonstrated distinct social functioning, particularly those with increased blood zone levels in comparison to control subjects[43].

Furthermore, children from certain racial groups may experience a more severe disruption of the intestinal epithelial barrier relative to their peers. This situation may provide insight into the various symptoms and clinical presentations associated with the disorder[44].

EFFECT OF BIRTH AND MOTHER HEALTH:

Investigations using germ-free (GF) mice have revealed that the microbiota may play a role in maintaining the integrity of the blood-brain barrier (BBB). Notably, adult GF mice that were implanted with pathogen-free fecal matter demonstrated higher concentrations of tight junction proteins (TJ) and a marked decrease in the permeability of the blood-brain barrier (BBB)[45].

DISSCUSSION:

Autism disorders represent a group of neurological developmental issues characterized by repetitive behaviors and social communication difficulties, mainly observed in children. The estimated global prevalence is around 23 cases per 1,000 people. These disorders frequently coexist with a range of physical, psychological, and neurological comorbidities, which can exacerbate the disparities in life expectancy and overall quality of life for individuals with autism[46]. A link has been established between the nervous system and the gastrointestinal system, especially concerning how gut microbiota affects the pathophysiology of various neurological and psychiatric conditions. Evidence shows that microbial metabolites from the

intestines can impact the enteric nervous system through both direct and indirect mechanisms[47].

Children with ASD are at a fourfold higher risk for gastrointestinal issues compared to their neurotypical counterparts, and the severity of these gastrointestinal conditions is related to behavioral symptoms [48]. Modifications in the microbiota axis cause disruptions in the communication between chemical signals and cytokines, leading to dysbiosis of the gut microbiota. Consequently, individuals affected by this condition show elevated neuroactive compound levels in their brains [49].

A range of neuroimaging studies has demonstrated a notable relationship between gut microbiota and the functional dynamics of the islets and tonsils. MRI research involving emotional facial tasks revealed that the intake of probiotic-rich fermented dairy products is associated with diminished activity in extensive functional networks, including brain regions responsible for emotional regulation, sensory perception, attention, and integrative processing in healthy females[50].

CONCLUSION:

In conclusion, evidence suggests that the gut microbiome is connected to the behavioral and gastrointestinal symptoms that are typical of Autism Spectrum Disorder (ASD). Additionally, a range of functional metrics, along with specific microbial taxa such as reduced butyrate levels and the presence of Proteobacteria and Steltella, indicate significant microbial characteristics associated with ASD. An overview of the expanding research indicates a significant link between Autism Spectrum Disorder (ASD) and the gut-brain axis. Several factors are associated with ASD, including stress, inappropriate antibiotic use, the influence of early microbial colonization on neonatal health, initial dysbiosis of the microbiota during pregnancy, and the delivery method. These factors can lead to the colonization of harmful microbes and dysbiosis in the gut microbiome, which subsequently affects central nervous system function through the generation of neurotoxins. The detection of specific harmful bacteria, such as Clostridium, commonly present in the colons of children, points to an elevated risk for ASD. Only a few of the many pre-symptomatic biomarkers currently under investigation have diagnostic accuracy estimates based on initial research findings. Similarly, while various diagnostic biomarkers hold promise, studies concerning them remain in the early stages. For instance, there is no existing research to evaluate the diagnostic capability of ABC-CT, which is a key initiative aimed at developing a diagnostic biomarker. Overall, autism spectrum

disorder (ASD) indicates the presence of potential biomarkers; however, the supporting evidence is still in a formative stage.

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