Gut Microbiota and Fecal Metabolome Perturbation in Children with Autism Spectrum Disorder

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ABSTRACT

The brain-intestinal axis concept describes the communication between the intestinal microbiota as an ecosystem of a number of dynamic microorganisms and the brain. The composition of the microbial community of the human gut is important for human health by influencing the total metabolomic profile. In children with autism spectrum disorder (ASD), the composition of the fecal microbiota and their metabolic products has a different configuration of the healthy child. An imbalance in the metabolite derived from the microbiota in children with ASD affect brain development and social behavior. In this article, we review recent discoveries about intestinal metabolites derived from microbiota based on high-yield molecular studies in children with ASD as part of the “intestinal brain axis”.

KEYWORDS:
Autism spectrum disorder (ASD), Intestinal microbiota, Fecal metabolites, ASD children

INTRODUCTION

The development of intestinal microflora begins at birth and subsequently the colonization of bacterial composition in the human colon can be reached to more than 70 genera with about 95% belonging to four main phyla: Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria.1 The arrangement of gut microbiota in the first years of life is an important occasion in the development of the immune responses and in the production of vitamins.2,3 Low microbial diversity in the early stages of life leads to the onset of allergic diseases and defects in innate immune responses in older age.4 In the same way, the study of germ-free mice has shown that there must be a continuous association between the immune system and intestinal microbiota metabolites to keep the normal population of microglial in their brains.5 Among the non-communicable diseases, neuropsychiatric disorders are increasing worldwide.6 Intestinal dysbiosis is known as a risk factor for a series of neurodevelopmental disorders and psychological diseases such as autism spectrum disorder (ASD) and schizophrenia.7,8 Individuals with ASD show a wide range of characterizing repetitive behaviors and difficulties in social communication. Children with ASD show gastrointestinal (GI) symptoms such as abdominal pain, gaseousness, diarrhea, constipation, and flatulence, which are associated with the...
severity of ASD. Although the exact aetiology and pathology of ASD are not yet clear, the involvement of the microbiota and its metabolites in the pathophysiology of ASD received considerable attention. Genetic, environmental, and biological factors play a fundamental role in behavioral development during the mother’s pregnancy and immediately after birth. Short chain fatty acids (SCFAs) produced by the intestinal microflora act as histone deacetylase inhibitors (HDAC), which modulate cellular gene expression related to neurotransmission systems. It has been shown that the treatment of stress-induced mice with beneficial bacteria such as Lactobacillus rhamnosus and Lactobacillus reuteri improved the poor social and cognitive behaviors by reducing stress-induced corticosterone and restored oxytocin. However, functional analysis such as metagenomics, metatranscriptomics, and metabolomics should follow studies of microbial composition. Metabolomics has the advantage of providing information on the final products of microbial functions, although most metabolomics studies focus on urine and blood metabolites.

Metabolomics of fecal samples may provide clues to the intestinal microbial metabolism. In this article we recap on some of the recent studies on intestinal microbiota metabolites in early childhood with ASD.

Colonization of intestinal microbiota in early life

A typical human intestinal microbiota contains an unimaginable complexity of hundreds of phylotypes, with a great compositional diversity among individuals. Infants are born without gut microbiota, however, soon after birth, the gut is hosting with bacteria coming from the mother and the adjacent environment commonly belong to two main groups of anaerobic bacteria of Firmicutes and Bacteroidetes phyla. The dominant bacteria of the intestine are strictly anaerobic and highly sensitive to oxygen, and therefore the creation of extremely resistant spores during colonization allows them to spread in the host and in environmental diffusion. During the first year of life in response to the alteration of diet and environmental exposures, the composition of the microbiota contributes significant changes.

Gut microbiota in the presence of ASD

Recent findings show that the intestinal microbiota by involving metabolic pathways and employing immune system are responsible for ASD symptoms in children. GI symptoms, including constipation and diarrhea, are common in children with ASD, which are connected to behavioral terms, such as anxiety, self-injury, and aggression. Dysbiosis, changes in the proportion of primary bacterial phyla, is the manifestation of children with ASD. The intestinal microbiota of children with ASD are less different with the lowest level of Firmicutes. Using the next-generation sequencing technology, it has been shown that potentially useful phylotypes related to Prevotella, Coprococcus, and unclassified Veillonellaceae in autism children with GI symptoms are failed. The amounts of Faecalibacterium with
anti-inflammatory effect as well as ability to SCFAs synthesis and Roseburia intestinalis and Roseburia faecis with ability to starch degradation and carbohydrate fermentation to synthesize the SCFAs, are also defined at the lowest level in the fecal sample of children with ASD.29,30 The relative abundance of Haemophilus parainfluenzae was also lower in the feces of children with ASD. Furthermore Bifidobacterium with beneficial effect was reduced in children with ASD.31,32 Bacteria with lower frequency are presented in table 1.

In contrast, an increase in the quantities of Lactobacillus, Clostridium, Bacteroidetes, Desulfovibrio, Caloramator, and Sarcina was detected in fecal samples of children with ASD and in a strong association with the severity of autism. The genus Lactobacillus, as probiotics, Prevotella with beneficial effect, and the harmful bacteria Sutterella, Enterococcus and Streptococcus thermophilus are abundant in ASD.33,34 Moreover, The genus of bacteria belongs to Bacteroidetes phyla such as Bacteroides, Barnesiella, Odoribacter, Parabacteroides, Prevotella, and Alistipes are more widespread. However, the Parasutterella genus of Proteobacteria phyla in addition to Proteobacteria is one of the most abundant phyla in the fecal samples of children with ASD.35,36 Bacterial species with higher frequency are shown in table 2. The Clostridaceae species (Clostridium histolyticum cluster II and I) with ability to produce metabolic by-products such as phenols, p-cresol, and indole derivatives with toxic effects and Akkermansia muciniphila as a mucin degrading bacteria are present at life-threatening number in children with ASD.37,38

Candida (in particular Candida Albicans) is more abundant in the fecal samples of children with ASD, which results in carbohydrates and mineral absorption but releasing higher toxins.8,33 Candida produces ammonia and toxins that persuade autistic behaviors. The presence of Candida, that aggravates dysbiosis, is induced by alterations of bacterial microbiota in individuals with ASD.39

Fecal metabolomic profile in children with ASD

Almost all metabolomics studies in the context of autism have been conducted on urine and blood metabolites.15,16,24 The study of Persico and colleagues showed urinary p-cresol and sulfate conjugate derivative of p-cresol regularly are present in autistic children and in contribution to worsening severity of autism.40 The other metabolites of intestinal microbiota such as SCFA, phenolic derivatives, and free amino acids (FAA) have the emotional impact on the behaviors of individuals with ASD.41 Higher urinary volatile organic compounds (VOCs) related to the intestinal microbiota in children with ASD have been also reported by Gevi and colleagues.15 Nonetheless, few studies have focused on fecal metabolites related to intestinal microbiota in children with ASD. The association of fecal p-cresol and phenolic substances containing p-cresol in children with autism has been reported in previous studies along with increased levels of p-cresol in the blood and urine.15,16,33,40 P-cresol that binds to human

| Genus/species             | Characteristics                                      | Bacteria Classification                                      | Function                                                                 |
|---------------------------|------------------------------------------------------|--------------------------------------------------------------|--------------------------------------------------------------------------|
| Prevotella, Coprococcus   | Gram-negative bacteria                                | Bacteroidetes; Bacteroidetes; Bacteroidales; Prevotellaceae; Prevotella | Oral and vaginal flora, its anaerobic qualities allows it to grow successfully in the human intestines |
| Bifidobacterium           | Gram-positive, non-motile, often branched anaerobic bacteria | Actinobacteria; Actinobacteria; Actinobacteridae; Bifidobacteriales; Bifidobacteriaceae; Bifidobacterium | Inhabitants of the gastrointestinal tract, vagina, and mouth - Some bifidobacteria are used as probiotics |
| Faecalibacterium          | anaerobic, gram-positive, slightly curved rod-shaped and motile by means of multiple subterminal flagella | Firmicutes; Clostridia; Clostridiales; Faecalibacterium | Commensal bacteria of gut microbiota - produce butyrate and other SCFAs |
| Roseburia intestinalis    |                                                       | Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Roseburia; R. intestinalis | a saccharolytic, butyrate-producing bacterium first isolated from human faeces |

Table 1: Gut microbiota with lower quantity in children with ASD
The gut microbiome plays a crucial role in health and disease, including in autism spectrum disorder (ASD). The gut microbiota is a diverse ecosystem of microorganisms that can influence both the host's health and the development of ASD. For instance, Clostridium difficile, a Gram-positive anaerobic bacterium, can cause infections and inflammation in the gut. Other bacteria like Akkermansia muciniphila and Bacteroides fragilis can play a role in the gut-brain axis, contributing to the development of ASD symptoms. The table below summarizes the changes in the microbiome in children with ASD:

| Genus/species       | Characteristics                                           | Bacteria Classification                                      | Function                                                                 |
|---------------------|-----------------------------------------------------------|--------------------------------------------------------------|--------------------------------------------------------------------------|
| Lactobacillus       | Gram-positive, facultative anaerobic or microaerophilic, rod-shaped, non-spoore-forming bacteria | Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus | Produce lactic acid, probiotic                                           |
| Clostridum           | Motile, gram-positive, Strict anaerobe, oval-shaped bacterium                                        | Firmicutes; Clostridia; Clostridiales; C. histolyticum       | Secretes potent exotoxins, pathogenesis of ulcerative colitis           |
| Akkermansia muciniphila | Gram-negative, strictly anaerobic, non-motile, non-spoore-forming, oval-shaped bacterium             | Verrucomicrobia; Verrucomicrobia; Akkermansia; A. muciniphila | Mucin-degrading bacterium                                                |
| Bacteroides         | Gram-negative, non-spoore-forming, anaerobic or aerobic, and rod-shaped bacteria                      | Bacteroides; Bacteroidiales; Bacteroidaceae; Bacteroides     | Abundant organism in the faeces of warm-blooded                         |
| Desulfovibrio       | Gram-negative sulfate-reducing bacteria aerotolerant                                                | Proteobacteria; Desulfovibrionales; Desulfovibrio; Desulfovibrio | Sulfate-reducing bacteria                                               |
| Caloramator         | Gram-positive cocci bacteria                              | Firmicutes; Clostridia; Clostridiales; Caloramator          | Produced p-cresol                                                        |
| Sarcina             | Gram-positive cocci bacteria                              | Firmicutes; Clostridia; Clostridiales; Sarcina              | A synthesizer of microbial cellulose                                     |
| Enterococcus        | Gram-positive cocci (diplococci or short chains)                                                    | Firmicutes; Bacilli; Lactobacillales; Enterococcaceae; Enterococcus | Common commensal organisms                                               |
| Streptococcus        | Gram-positive bacterium, and a fermentative facultative anaerobe                                       | Firmicutes; Bacilli; Lactobacillales; Streptococcaceae; Streptococcus; S. thermophilus | Lactic acid bacterium                                                    |
| Proteobacteria      | Gram-negative bacteria                                     | Phylum: Proteobacteria Include a wide variety of pathogens, such as Escherichia, Salmonella, Vibrio, Helicobacter, Yersinia, Legionella, | Produce phenol from tyrosine                                             |
| Candida              | Genus of yeasts                                           | Fungi; Ascomycota; Saccharomyces; Saccharomycescetes; Candida | Commensals or endosymbionts invade and cause disease when mucosal barriers or the immune system is disrupted |
| Ruminococcus        | Gram-positive anaerobic gut microbes                      | Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Ruminococcus | Found in significant numbers in the intestines of humans                |
| Sutterella spp.     | Gram-negative rod bacteria                                | Proteobacteria. Proteobacteria. Burkholderiales; Sutterellaceae; Sutterella | Previously been isolated from canine feces                              |

Table 2: Microbiota in the intestines of humans that are increased in children with ASD

Serum albumin is produced from tyrosine or dietary toluene by some bacteria in the intestine. It has deleterious impact on the colon epithelial cell through DNA damage and is inversely associated with the presence of resistant starch in the diet. Increased intestinal permeability allows further communication by discharging microbiota metabolite and toxins from the intestine to the brain. For example, the main component of the cell wall of gram-negative bacteria, lipopolysaccharide (LPS), and other markers of immune-inflammatory activation in the serum may contribute to the pathophysiology of inflammation with a negative effect on social interaction in autism. Kang and co-workers in their recent study showed that the metabolite, isopropanol (or propan-2-ol) was the significant metabolite of stool that considerably increased in children with ASD. Isopropanol is a neurotoxic organic solvent that irritates mucosal surfaces to induce GI complications. Alcohol dehydrogenase metabolizes isopropanol to acetone, propylene glycol, and acetate to be converted to glucose or other intermediary products. The predominant mechanism of action of isopropanol is major depression in the brain stem that subsequently affect central nervous system and lead to respiratory depression. Greater isopropanol levels in feces of children with ASD explain the function of species of lactic acid bacteria such as Lactobacillus and Bacteroides.
as *Lactobacillus brevis*, and *Clostridium*. It was also observed that while neurotransmitters-glutamate was the highest in children with autism, the trends of gamma-aminobutyrate (GABA) concentrations were lower in feces of children with ASD. Large amounts of FAA from the hydrolysis of proteins and peptides that are created by proteolytic bacteria (*e.g.*, *Clostridium* and *Bacteroides*) found in the fecal samples of children with ASD.

Different types of SFCAs, as principal products of non-digestible carbohydrate fermentation by gut microbiota or by presence in the diet, show the increasing importance in healthy individuals. Commensal bacteria, such as *Clostridia* and *Bifidobacteria* produce SCFAs in the cecum and the colon that could bring into the hepatic, portal, and peripheral blood. De Angelis and colleagues and Adams and co-workers reported that total SCFAs with the exception of propionic and acetic acids reduced in the feces of children with ASD, however, Wang and others showed increased levels of SCFAs and ammonia in the fecal samples of children with ASD. In the study of Wang and colleagues when concentrations of fecal acetic, butyric, isobutyric, valeric, isovaleric, and caproic acids were assessed, all but caproic acid had a meaningfully higher level in children with ASD. Consistent with them, Adams and others presented children with ASD had significantly lower levels of SCFAs. The low level of SCFAs that was reported by Adams and others could possibly because of either lower production by beneficial bacteria or more absorption into the body as a result of increased gut permeability. SCFAs such as butyrate have significant benefits for human by developing the neurotransmitters and regulating the catecholaminergic biosynthesis by influencing the transcription of the tyrosine hydroxylase gene during life. SCFAs also modulate the expression

| Metabolite                        | Description                                                                 | Structure |
|-----------------------------------|-----------------------------------------------------------------------------|-----------|
| p-Cresol (4-methylphenol)         | The end-product of protein breakdown—metabolites of tyrosine, phenylalanine, which are converted to 4-hydroxyphenylacetic acid by intestinal bacteria | ![Chemical structure](image1) |
| p-Cresol sulfate                  | A microbial metabolite that is found in urine and likely derives from secondary metabolism of p-cresol and is often considered to be a uremic toxin | ![Chemical structure](image2) |
| Isopropanol (Isopropyl alcohol or 2-propanol) | Small amounts of this alcohol are produced naturally by gut microbial flora. | ![Chemical structure](image3) |
| Glutamate                         | It is found naturally primarily in the cell walls of certain bacteria and in certain foods | ![Chemical structure](image4) |
| Gamma-aminobutyrate (GABA)       | An inhibitory neurotransmitter establish in the nervous systems of many species—Low plasma GABA has been reported in some depressed patients—Aspartic acid and glutamic acid probably inhibit GABA effects | ![Chemical structure](image5) |
| Butyric acid                      | End-product of a fermentation process by obligate anaerobic bacteria. Formed in the human colon by bacterial fermentation of carbohydrates (dietary fiber), and suppresses colorectal cancer (CRC) | ![Chemical structure](image6) |
| Propionic acid                    | An end-product of the microbial digestion of carbohydrates                  | ![Chemical structure](image7) |

| Table 3: A list of key gut microbiota metabolite in children with ASD | Description                                                                 | Structure |
|---------------------------------------------------------------------|----------------------------------------------------------------------------|-----------|
| p-Cresol (4-methylphenol)                                           | The end-product of protein breakdown—metabolites of tyrosine, phenylalanine, which are converted to 4-hydroxyphenylacetic acid by intestinal bacteria | ![Chemical structure](image1) |
| p-Cresol sulfate                                                    | A microbial metabolite that is found in urine and likely derives from secondary metabolism of p-cresol and is often considered to be a uremic toxin | ![Chemical structure](image2) |
| Isopropanol (Isopropyl alcohol or 2-propanol)                      | Small amounts of this alcohol are produced naturally by gut microbial flora. | ![Chemical structure](image3) |
| Glutamate                                                          | It is found naturally primarily in the cell walls of certain bacteria and in certain foods | ![Chemical structure](image4) |
| Gamma-aminobutyrate (GABA)                                         | An inhibitory neurotransmitter establish in the nervous systems of many species—Low plasma GABA has been reported in some depressed patients—Aspartic acid and glutamic acid probably inhibit GABA effects | ![Chemical structure](image5) |
| Butyric acid                                                       | End-product of a fermentation process by obligate anaerobic bacteria. Formed in the human colon by bacterial fermentation of carbohydrates (dietary fiber), and suppresses colorectal cancer (CRC) | ![Chemical structure](image6) |
| Propionic acid                                                     | An end-product of the microbial digestion of carbohydrates                  | ![Chemical structure](image7) |
of the host gene through inhibitor activity of histone deacetylase and epigenetic mechanism. Butyric acid acts as a potent inhibitor of histone deacetylase (HDAC) in the regulation of neurotransmitters dopamine, norepinephrine, and epinephrine. It also modulates the inflammatory and oxidative states of the intestinal mucosa. However animal studies have shown that propionic acid causes some autistic behaviors and compromised social manners. 

Similar effects of propionic acid to behavioral and electrographic derivation have been identified in humans. Propionic acid is mainly produced by Clostridia, Bacteroidetes, and Desulfovibrio, however Bifidobacterium, Faecalibacterium, Ruminococcus, and Eubacterium are positively correlated with butyric acids. Table 3 summarizes the intestinal metabolites that are reported in fecal sample of children with ASD.

CONCLUSION

In this review, we summarized the recent findings on faecal microbiota and related metabolites in children with ASD. It has been shown that Clostridiaceae species exist at the highest level in such children, which as principal bacterial groups, synthesize important organic neurotoxic metabolites such as phenols, p-cresol, and isopropanol. The application of high-yielding methods to recognize the fecal microbiota and the metabolites will support the control of an imbalance of the gut-brain axis in children with ASD and the implementation of new therapeutic strategies.

ETHICAL APPROVAL

There is nothing to be declared.

CONFLICT OF INTEREST

The author declares no conflict of interest related to this work.

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