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Gut microbiota on gender bias in autism spectrum disorder

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Abstract: Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder. Its three core symptoms are social communication disorder, communication disorder, narrow interest and stereotyped repetitive behavior. The proportion of male and female autistic patients is 4:1. Many researchers have studied this phenomenon, but the mechanism is still unclear. This review mainly discusses the related mechanism from the perspective of gut microbiota and introduces the influence of gut microbiota on the difference of ASD between men and women, as well as how gut microbiota may affect the gender dimorphism of ASD through metabolite of microbiota, immunity, and genetics, which provide some useful information for those who are interested in this research and find more gender-specific treatment for autistic men and women.

Keywords: autism spectrum disorder; gender bias; genetics; gut microbiota; immunity; metabolite.

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Introduction

Autism is a complex neurodevelopmental disorder that occurs during the first few years of life (Estes et al. 2015). Autism and related disorders, collectively called autism spectrum disorder (ASD), are characterized by the deficits in social communication and interaction and the presence of restrictive, repetitive patterns of behavior (Baio et al. 2018). Until now, there is no treatment to alleviate core symptoms or cure ASD. Male and female patients usually have different symptoms. For example, male superiority is in visual spatial tasks and female superiority is in language skills in ASD (Park et al. 2012). Gender bias is evident in high-functioning autistic patients as well (Margari et al. 2019). In the United States, one out of 68 children is affected, four times more boys than girls are affected (Li et al. 2018). ASD is a susceptible disease for men (Guerra et al. 2019). Boys with ASD exhibit less greater communication deficits but more restrictive, repetitive and stereotyped behavior than girls (Knutsen et al. 2019). Women may have a protective mechanism that makes them less susceptible to autism (Kopec et al. 2018). Interestingly, autistic girls have higher levels of social motivation and qualitatively different friendship experiences than autistic boy, indicating that girls on the autism spectrum in particular may struggle with identifying and dealing with conflict in their social lives (Sedgewick et al. 2016).

A comprehensive literature search has implicated that many factors contribute to the development of ASD, including nutrition factors, immune factors genetic factors, environmental factors, and even family culture–influenced factors (Kreiser and White 2014; Sealey et al. 2016; Zurita et al. 2020). Gut microbiota, as an environmental factor, is a diverse, host-specific, and symbiotic bacterial environment for mammalian survival and exerts a surprising yet powerful influence on the brain and behavior, which have been studied by many researchers to the etiology of autism (Fattorusso et al. 2019; Ristori et al. 2019). Large amounts of evidence demonstrate that stronger prevalence of gastrointestinal disorders and disturbances in patients with ASD, such as increased permeability/inflammation of the intestinal tract, alters microbiota composition and its metabolism (van Sadelhoff et al. 2019; Vuong and Hsiao 2017). For instances, children with autism

have four times as many gastrointestinal symptoms as normal people, such as constipation, diarrhea, bloating, abdominal pain, reflux, vomiting, gaseousness, foul smelling stools, and food allergies (Srikantha and Mohajeri 2019). The imbalance of gut microbiota is one of the causes of ASD (Ribeiro et al. 2019). In turn, in patients with ASD, the composition of the microbiota can be altered as well. For example, children with ASD have higher levels of actinomycetes, Proteobacteria, Erysipelotrichi, Gammaproteobacteria and bacilli compared with healthy children (Plaza-Diaz et al. 2019). In addition, increased genus *Bacteroides* has been found in female patients while *Escherichia* predominates in male patients with intestinal infections (Singh and Manning 2016). Therefore, in the case of intestinal pathology, the enteric bacteria in the human body all show gender duality.

Given the role of intestinal flora in occurrence and development of ASD and its mechanism, in this review, we try to select the intestinal flora as the key point to explore the possible mechanism of ASD, especially in explaining the reasons for the sex-special. We tend to pick up recent literatures to discuss the relationship between gut microbiota and immunity, as well as genetics in preclinical and clinical studies (Tables 1 and 2), and put them in the context of known or putative sex-specific mechanisms.

Gut microbiota affect the difference between men and women with ASD depending on gut metabolites

The gut is colonized with microbial communities composed of bacteria, fungi, and viruses, collectively known as the gut microbiota, and the microbial genome of these microbes is termed the microbiome (Vemuri et al. 2019). The total mass of bacteria in the human body is 0.2 kg, the same as the number of human cells (Sender et al. 2016). Especially, about 100 trillion bacteria that constitute the human gut microbiota contain at least 1800 genera and 40,000 species of bacteria, which provide 100 times the number of genes than those in the human genome (Forsythe and Kunze 2013). Gut microbiota plays a key role in bidirectional gut–brain axis (GBA), a communication that integrates the gut and central nervous system (CNS) activities, to influence brain function and behaviors (Borrelli et al. 2016). It has been recognized as a key regulator of social behavior and as an important regulator of prefrontal cortex myelin, a key brain region that drives complex cognitive behavior, including age-dependent cognitive decline and neuropsychiatric disorders (Bastiaanssen et al. 2019; Roman et al. 2018). In turn, the CNS can have a potent influence on microbial community composition. The communication between CNS effectors and bacteria may depend on the presence of neurotransmitter receptors on bacteria, while the binding sites for intestinal neurotransmitters produced by the host are present on bacteria and can affect the function of composition of gut microbiota, contributing to increased predisposition to inflammatory and infection stimuli (Hughes and Sperandio 2008). These results demonstrate that interaction between microbiota and GBA should be bidirectional, namely through signaling from gut microbiota to the brain and from the brain to gut microbiota by means of neural, endocrine, immune, and humoral links (Carabotti et al. 2015). On the contrary, the disruption in the microbiota–gut–brain (MGB) axis results in the pathogenesis of neuropsychiatric disorders, associated with cognitive dysfunction, such as ASD, anxiety, and depression (Petra et al. 2015). Germ-free mouse exhibits deficient social behavior, while treatment with a single bacterial species *Lactobacillus reuteri* can restore the impaired behavior (Buffington et al. 2016).

In human beings, intestinal flora is one of the most densely populated microbial ecosystems, some diseases maybe related to dysbiosis, such as depression, schizophrenia, Parkinson disease, and Alzheimer disease (AD), as well as ASD (Filosa et al. 2018). Gut dysbiosis seems to be one of the major causes of the imbalance of male to female ratio of ASD (Sylvia et al. 2017). There are three main uses of gut dysbiosis: as general changes in the microbiota composition; as an imbalance in composition; and as changes to specific lineages in that composition, which normally suffere from infection, chemicals, and stress (Hooks and O'Malley 2017; Molina-Torres et al. 2019; Yang et al. 2018).

Preclinical study

The metabolites of microbes might be the cause of autism, such as short-chain fatty acids (SCFAs), propionic acid (PPA), acetic acid (AA), and butyric acid (BA). Since SCFAs like PPA and BA, which are present in diet and are fermentation products of many gastrointestinal bacteria, induced extensive changes in gene expression, including neurotransmitter system, neuronal cell adhesion molecule, inflammation, oxidative stress, lipid metabolism, and mitochondrial function, they are playing more and more important roles in host health but also may be environmental contributors in neurodevelopmental disorders including ASD (Alam et al. 2017; Forssberg 2019; Nankova et al. 2014). Some specific neurotoxicity substances are

Table 1: Preclinical studies.

Number	ASD model	The abundance of microbial composition (↑increase; ↓decrease)		Reference
		Male	Female	
1	VPA-induced rats	Bacteroidetes↑ Bacteroidia↑ α-Proteobacteria↑ Coriobacteriia ↓	Actinobacteria↑; *Allobaculum*↑ *Bifidobacterium*↑; *Odoribacter*↑ *Staphylococcus*↑; *Candidatus*↑ Arthromitus↑	(Liu et al. 2018)
2	VPA-induced mice	*Erysipelotrichales*↑ *Alistipes*↑; *Mollicutes*↑*Enterorhabdus*↑ *Lactobacillales*↑ Firmicutes↑ *Bacteriodales*↓ Deltaproteobacteria↓	Firmicutes↑ *Bacteriodales*↓ Deltaproteobacteria↓	(de Theije et al. 2014)
3	Shank3 KO mice	*Veillonella*↑ *Lactobacillus*↓; *Prevotella*↓	*Veillonella*↓ *Lactobacillus*↓; *Prevotella*↓	(Tabouy et al. 2018)
4	BTBR mice	*Lactobacillus*↑ Bacteroides↑ Parabacteroides↑ *Coprobacillus*↑ *Dehalobacterium*↓ *Ruminococcus9*↓ *Desulfovibrio*↓	Parabacteroides↑; *Sutterella*↑ Bacteroides↑; *Coprobacillus*↑ U. Desulfovibrionaceae↑ U. Enterobacteriaceae↑; *Prevotella*↑ *Akkermansia*↑ Dehalobacterium↓; *Oscillospir*↓ *AF12* (Rikenellaceae)↓; *U. F16* (TM7) ↓	(Coretti et al. 2017)

VPA, valproic acid; ASD, autism spectrum disorder; BTBR, Black and Tan BRachyury.

Table 2: Clinical studies.

Number	Patient description	Age (year)	The abundance of microbial composition (↑increase;↓decrease)		Reference
			Male	Female	
1	Healthy people	20–50; >60	*Bacteroides–Prevotella* group↑	N/A	(Mueller et al. 2006)
2	Patients with enteric infections	0–69; >70	*Escherichia*↑	*Bacteroides*↑	(Singh and Manning 2016)

more likely to infect men with mental disorders such as autism (Mezzelani et al. 2015). PPA, as a metabolite of *Bacteroides* and *Parabacteroides* genera, is more likely to induce pathological changes of ASD than AA and BA through intraventricular injection in rats (MacFabe et al. 2007; MacFabe 2015). Prenatal and postnatal treatment with PPA results in the change of behavior in a sex-specific manner. For one thing, prenatal and postnatal treatment with PPA promotes anxiety-like behavior in female rats (Foley et al. 2014b). For another thing, prenatal exposure of PPA increases locomotor activity in male offspring compared to prenatal PPA females (Foley et al. 2014a). Prenatal and postnatal administration of PPA significantly reduces prepulse inhibition (PPI) in female offspring but elevates PPI in male offspring, indicating that female offspring are more susceptible to PPA-induced alterations in behavior, and gut-derived illnesses are more prevalent in females relative to males (Foley et al. 2015). Meanwhile, another bacterial metabolite, such as butyrate, is able to improve repetitive behavior in mice through rebalancing the transcription of excitatory and inhibitory genes, which may have beneficial effects on the recovery of ASD-related behaviors (Coretti et al. 2017; Kratsman et al. 2016). Conversely, prenatal exposure to valproic acid (VPA) induces a rearrangement of early microbial colonization in a murine model of autism, leading to an increase of butyrate levels in the gastrointestinal tract of male offspring, which may interfere directly with gene expression in intestinal cells (de Theije et al. 2014). The discrepancy in these studies may arise from the complex pathogenesis of ASD or different ASD models. These results open new avenues in the scientific trajectory of managing ASD. Microbiome manipulation such as specific metabolites in gut microbiota is considered a novel strategy of treating ASD.

Lipopolysaccharide (LPS), as one of the microbiome components, is usually utilized to establish models of autism.

Prenatal LPS treatment results in hypersensitivity to acoustic startle in males but not females and does not alter PPI (Foley et al. 2015). Another factor, such as a specific chemical or drug, may involve in the development of autism (Mandy and Lai 2016). VPA is one antiseizure drug and has been reported to dramatically increase the risk of ASD in offspring (Wang et al. 2018). VPA exposure in the ASD rat model also triggers the differences in gut bacterial dysbiosis between the sexes, which are similar to those in human autism (Liu et al. 2018). Antibiotic treatment alters the gut microbial communities in a sex-dependent manner, and this disruption is correlated to social behavior and ASD (Vemuri et al. 2019).

Clinical study

Patients with ASD had elevated abundance of proteobacteria rather than healthy people. *Bifidobacterium, Blautia*, *Dialister*, *Prevotella*, *Veillonella*, and *Turicibacter* have been found to consistently decline, while *Lactobacillus, Bacteroides*, *Desulfovibrio*, and *Clostridium* have been shown to increase in autistic children compared with healthy people (Liu et al. 2019). Although the consideration of sex difference with autism is often neglected, there is a strong correlation between gender and intestinal microflora composition. A cross-sectional study on intestinal microbiota composition in different European populations has proved that males exhibit much higher levels of the *Bacteroides–Prevotella* group than females, independent of country and age (Mueller et al. 2006). The result reflects the dominant role of gender in the composition of intestinal flora to ASD. Despite the intense interest in ASD, the precise mechanisms underlying the metabolites in the intestinal flora leading to ASD with gender bias is still not illustrated yet. Currently, most of studies are carried out in animals but not in the clinic, which require further exploration.

Gut microbiota affect the difference between men and women with ASD depending on immunity

The complex reciprocity between the host immune system and microbial community is necessary for gut homeostasis and host intestinal immune system (Palm et al. 2015). For one thing, the microbiota plays a fundamental role in the induction, development, and function of the host immune system. In return, the host immune system has evolved multiple means to maintain its symbiotic relationship with the microbiota (Belkaid and Harrison 2017). For another thing, microbial colonization maybe required for the formation of lymphoid tissues and subsequent development of immune system (Gensollen et al. 2016). Recent study has indicated that gut microbiota and immune system are implicated in the pathogenesis of neurodevelopmental, psychiatric, and neurodegenerative disorders, such as ASD, depression, and AD (Fung et al. 2017).

Preclinical study

One of the most prominent rodent models to study ASD is maternal immune activation, induced by the injection of a specific agent, such as polyinosinic:polycytidylic acid (poly I:C) or LPS into pregnant dams leading to the disruption in the integrity of intestinal barrier and inflammation response, which finally results in the autistic-like behaviors of the offspring, associated with dysbiosis of the microbiota (Foley et al. 2014a; Hsiao et al. 2013; Hughes et al. 2018). In a mouse model of ASD, Black and Tan BRachyury (BTBR) mice present increased gut permeability, changed cytokines pattern, and distinct inflammatory cell infiltration in the colon tissue. However, enhanced expressions of IL-6 and CD11c have been found only in male BTBR mice (Coretti et al. 2017). Similarly, increased IL-17a and TNF-α levels have been found in male *shank3* mutant mice but decreased in female mice, whereas IL-6 is the opposite (Tabouy et al. 2018).

Microglia, as the resident histiocyte-type cell and the key innate immune effector of the CNS, are implicated in many psychiatric and neurological disorders with sex bias, such as ASD. Especially, the numbers or the density of microglia has been found to be increased in the postmortem brain of autistic patients (Nelson et al. 2019). Host microflora plays a crucial role in the homeostasis of microglia because of its potential to change the properties of microglia and subsequently influence innate immune responses, through SCFAs, a product of microbial fermentation (Erny et al. 2015, 2017; Mosher and Wyss-Coray 2015). The reduction in the expression of fractalkine microglial receptor (CX3CR1) followed by the prenatal LPS treatment leads to elevated numbers of spines in the granule cells of the dentate gyrus, associated with the process of pruning and shaping neural circuits, only in the male progeny of the LPS challenged dams (Fernandez de Cossio et al. 2017). The transcriptomic and chromatin accessibility analyses further reveal that the microbiome influences prenatal and adult microglia in a sex-specific manner (Thion et al. 2018). In addition, composition of VPA-exposed males is associated with increased levels of ileal neutrophil infiltration but inversely associated with

intestinal levels of serotonin and social behavior scores, indicating that autism-like behavior and its intestinal phenotype are associated with altered microbial colonization and activity in a murine model for ASD, with preponderance in male offspring (de Theije et al. 2014). These results demonstrate that microbiota may influence the progress of ASD through immunity, whose mechanism of action is still not fully achieved yet.

Clinical study

Children with ASD frequently reveal various gastrointestinal (GI) symptoms that may resolve with an elimination diet along with apparent improvement of some of the behavioral symptoms. Evidence suggests that ASD may be accompanied by aberrant (inflammatory) innate immune responses, which may predispose children with ASD to sensitization to common dietary proteins, leading to GI inflammation and aggravation of some behavioral symptoms (Jyonouchi et al. 2002). The abundance of *Faecalibacterium*, which results in systemic immunity dysfunction, has been shown to be high in autistic children compared to healthy people (Strati et al. 2017). However, the role of intestinal microflora in ASD with gender bias via immunity needs further exploration.

The interaction of gut microbiota and genetics affects the difference between men and women with ASD

Recently, copy number variations and *de novo* mutations have been recognized as a strong source of genetic causality, associated with the occurrence of ASD (Ronemus et al. 2014). *FMR1*, *MECP2*, *NLGN3*, and *NLGN4X* are ASD-related genes, which reside on the X chromosome. In most dominant X-linked syndromes, the penetrance and severity index of the phenotype are high in males, while the severity index is low in females (Ferri et al. 2018). Genes on X or Y chromosomes not only determine the traits of men and women but also link to sex differences in some psychiatric diseases, including schizophrenia, ASD, attention deficit hyperactivity disorder, and mood disorders (Zhang et al. 2017). Men are the only ones who inherit Y-chromosome–linked genes, but both men and women can inherit X-chromosome–linked genes (Zhang et al. 2017). X-chromosome inactivation is responsible for sex chromosome dosage compensation in females (XX) (Shvetsova et al. 2019) that is the reason why males have worse symptoms than females. Males are more susceptible and have worse symptoms than females in ASD. Complex interaction between genetic, epigenetic, and environmental factors contributes to the development of ASD (Autism Genome Project C. et al. 2007). Altered composition and function of intestinal microbiota mediate the interaction between genes and environmental risk factors, which substantially contributes to the development of ASD (Yang et al. 2018).

Preclinical study

Multiple genes have been involved in the pathogenesis of autism so that different animal models using transgenic or mutant mice have been utilized to study the mechanism underlying the sex bias induced by gut microbiota in ASD. *Bacteroides, Parabacteroides, Sutterella, Dehalobacterium*, and *Oscillospira* genera act as key drivers of sex-specific gut microbiota profiles associated with pathological traits in BTBR transgenic mice (Coretti et al. 2017). In another mouse model of ASD, both relative abundance of *Veillonella* and *Lactobacillus* is decreased in *Shank3* KO female mice. Interestingly, the relative abundance of *Veillonella* is increased, but *Lactobacillus* is decreased in *Shank3* KO male mice, whose aberrant social behavior can be relieved with treatment with *L. reuteri* (Sungur et al. 2017).

Epigenetics refers to the heritable changes in gene expression without changing the underlying DNA sequence. Specific environmental factors associated with intestinal microbiome, including food, drugs, have been shown to exert some control in epigenetic determination, which play a role in determining the predisposition to ASD in a sex-specific manner (Barua et al. 2016). In utero administration of VPA to pregnant rodents induced increase in H3K4me3 at exons 1 and 4, which contributes to sex differences in ASDs by protecting females from the adverse effects of genetic variants or environmental factors, leading to less autistic-like behaviors than those in male mice (Konopko et al. 2017). In addition, LPS-induced maternal immune activation causes male-specific deficits in certain social responses in the contactin-associated protein-like 2 (Cntnap2) mouse model for ASD, suggesting that the interaction of genetic and environmental factors causes sex-specific effects that may help us to explain the occurrence of ASD with gender bias (Schaafsma et al. 2017).

Clinical study

It has been reported that neurodevelopmental concerns in male children and young adults aged between 3 and 25

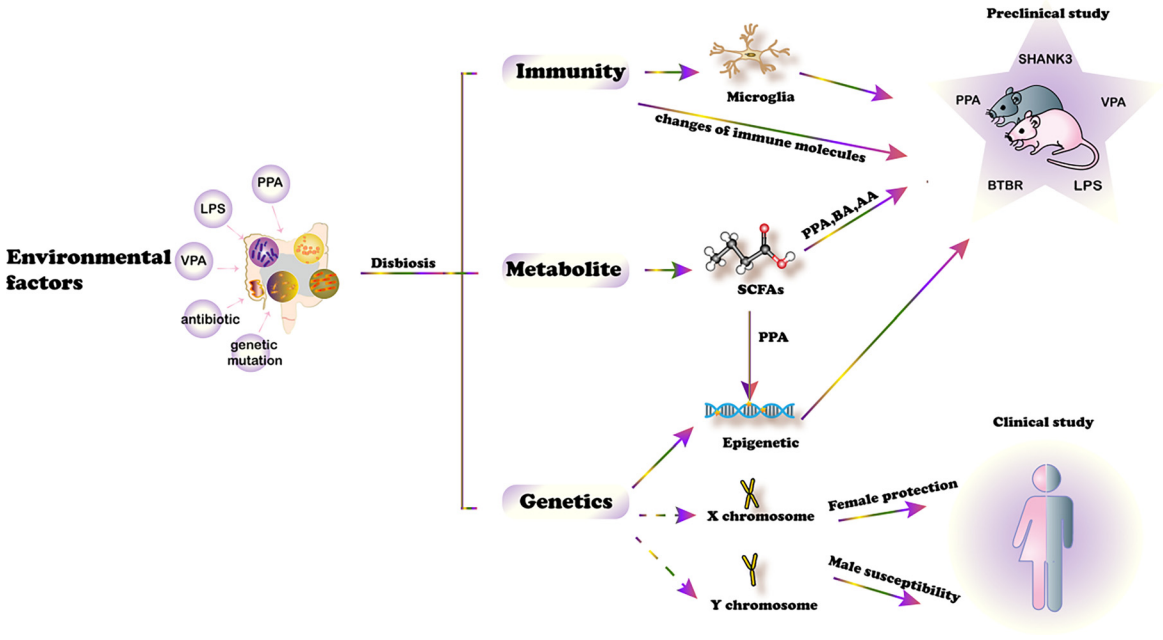

Figure 1: Schematic diagram showing that gut microbiota influences gender bias in ASD through different mechanisms, including immunity, metabolites, and genetics as determined by preclinical and clinical studies. Solid arrow indicates validated mechanisms, and dotted arrow indicates possible mechanisms that need to be further explored. ASD, autism spectrum disorder.

years with sex chromosome aneuploidy (SCA) (XXY/Klinefelter syndrome, XYY, XXYY) include symptoms seen in ASD, such as language impairments and social difficulties. ASD rates in males with SCA are higher than those reported for the general population (Tartaglia et al. 2017). Therefore, studies of SCA and Y-chromosome genes may provide insight into male predominance in idiopathic ASD. Mutation of specific genes, such as *MECP2*, linked with X-chromosome results in ASD with sex bias mediated by the gene locus such as young adult female patients of Rett syndrome, accompanied by the changes of composition in gut flora (Borghi et al. 2017). In addition, a hemizygous *SHANK1* deletion that segregates in a four-generation family in which male carriers, but not female carriers, have ASD with higher functioning (Sato et al. 2012). However, there is still no any clinical evidence on the treatment of patients with ASD and the joint effect of intestinal flora and genetics, which need to be further explored.

Conclusion

Alterations of the MGB axis have been invoked in the pathogenesis of ASD. Social interaction deficits have been successfully revered associated with ASD in mice through a bacterial-based therapy (Sgritta et al. 2019; Sharon et al. 2019). An open-label study and a two-year follow-up suggest that microbiota transplant therapy is relatively safe and effective in significantly reducing gastrointestinal disorders and autism symptoms, changing the gut microbiome structure, and increasing gut microbial diversity in children with ASD (Adams et al. 2019). Probiotic supplementation has beneficial effects on children with ASD for not only specific GI symptoms but also the core deficits of the disorder, cognitive and language development, and brain function and connectivity (Santocchi et al. 2016). These studies indicate widespread potential for the development of noninvasive therapies for ASD and suggest a future in which the microbiome plays a major role in the pathophysiology and the treatment of neurodevelopmental disorders. Nevertheless, the observed behavioral or symptom changes in mice or patients raise the question regarding the mechanisms of action of microbiota involved in the disease and possible therapeutic targets. Consequently, the correlation between changes in distinct populations of gut microbiota and its metabolites and the behavioral changes related to ASD warrant further investigations into the MGB axis aiming at in-depth examination of mechanisms leading to the pathology of ASD. We present here the possible role of gut microbiota in ASD with sex differences through gut metabolites, immunity, and heredity to the gender bias of autistic patients in both preclinical and clinical studies (Figure 1). We hope this discussion can serve as a platform on which to evaluate how gut microbiota contribute to the gender bias in autistic patients and provide a new idea for the accurate medical therapy for ASD.

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