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## TITLE

GUT-BRAIN AXIS IN THE EXECUTIVE FUNCTION OF AUSTISM SPECTRUM DISORDER

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GUT-BRAIN AXIS IN AUTISM SPECTRUM DISORDER

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**ABSTRACT**

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterised by impaired communication and social interactions, and repetitive behavioural patterns. These patterns are thought to be dysfunction symptoms in executive processing, which impact other cognitive functions as attention or cognitive flexibility. In the last years, several studies have shown that certain intestinal bacteria may play a role in shaping cognitive networks encompassing emotional and social domains. Furthermore, the existence of a microbiota-gut-brain axis is known, establishing several mechanisms by which microbiota may modulate brain development, function and behaviour including immune, endocrine and neural pathways. Since aetiology of ASD is largely unknown, some studies have shown that intestinal bacteria may be involved in its pathogenesis. The aim of this review is to focus on the role of gut-brain axis in ASD and, specifically, on the role of gut-brain axis on the executive functions. Firstly, we summarise the relation between the gastrointestinal and cognitive symptoms of ASD patients. In addition, we highlight the evidence that support and emphasise gut microbiota implication, and the putative mechanism underlying the gut-microbiota in this population. Finally, we present evidence from preclinical and clinical studies regarding microbiota modulation and their effects on cognitive symptoms, specifically in that related to the executive function. In conclusion, microbiota manipulation could be a positive intervention in the improvement of ASD symptoms. However, more research evaluating the role of microbiota in ASD cognitive symptoms is needed.

**KEYWORDS**

Gut-microbiota, autism, executive function, diet, microbiome, cognition, microbiota, probiotic, faecal transplantation.

# 1. AUTISM SPECTRUM DISORDERS (ASD): GASTROINTESTINAL AND COGNITIVE SYMPTOMS.

Autism spectrum disorder (ASD) is a neurodevelopmental condition that is characterised by stereotyped behaviour and a deficiency in the individual's ability to socialise, communicate, and use imagination (Lázaro et al., 2016). Estimates of the prevalence of the disorder have been moving towards an apparent increase in rates. Nowadays it is estimated that 1 in 68 children have ASD (World Health Organization (WHO), 2017). Nevertheless, it is still not known as to why autism incidence increased rapidly during the 1990s and is still increasing in the 2000s. There are many possible explanations for this apparent increase in prevalence, including greater awareness, the expansion of diagnostic criteria, better diagnostic tools and better communication (WHO, 2017).

Despite its increasing prevalence, ASD aetiology is still unknown. However, this disorder is associated with neural development, and recent evidence supports the hypothesis of a complex and highly heterogeneous genetic aetiology, and points to a combined effect between the environment and various different genes (Lázaro, 2016). In addition, there is an intriguing commonality among patients that is not well understood: the predominance of ASD in males, given that ASD presents a 4:1 male bias, pointing to a female protective factor, although more research is required in this sense (Werling, 2016; Kopec et al., 2018).

This disorder shows a significant comorbidity with other conditions. The most commonly associated impairments are epilepsy, sleep disturbances or anxiety and depression. Intellectual disability, sensory dysfunctions and attention deficits, as well as gastrointestinal (GI) problems and immune deficiency, have been reported in recent studies and have acquired great relevance in order to find an explanation for autism (Lyall et al., 2017).

Other common symptoms in ASD are cognitive symptoms: some attention difficulties in children with ASD have been noticed, mainly having trouble shifting attention. In addition, some children with ASD have difficulties with selective attention, indicating which environmental information should be focused on, particularly when exposed to a stimulating sensory environment (Hazen et al., 2014). Alongside these difficulties, executive function deficits in children with ASD have been widely documented. There is converging evidence to suggest that children with ASD have poor planning and

flexibility. However, behavioural regulation executive skills have been ambiguous, such as inhibitory deficits and research on working memory (Freeman et al., 2017). Beyond these problems, Martos-Pérez and Paula-Pérez (2011) noticed that ASD individuals are not available to ascribe a mental or emotional state to other people, or find a meaning for their actions. However, more research is needed in order to understand the role of these functions in ASD.

Children with ASD are regularly affected by GI problems. Even a higher risk of problem behaviours has been reported in individuals with ASD and GI symptoms (Grossi et al., 2016). The most comorbid GI symptoms in ASD are constipation, bloating, and diarrhoea. Other studies (Celia et al., 2016) have observed the presence of reflux, colitis/ inflammatory diseases, food intolerances, or symptoms of irritable bowel syndrome as well as loose stools, undigested food, or abdominal distension. A recent review of GI symptoms in ASD (Holingue et al., 2018) has determined a prevalence range for constipation of 4.3–45.5%, for diarrhoea of 2.3–75.6%, and for any or more than one symptom of 4.2–96.8%. The range prevalence of GI symptoms shows a possible link between the gut microbiota and the ASD.

Beyond this, several studies (Adams et al., 2011; Louis, 2012) have reported that autistic individuals exhibit a change in the stability, diversity, composition, and/or metabolism of gut bacteria. Both gut bacteria and microbial metabolites changes reported in ASD patients will be detailed in the second section of this review.

After birth, the gut is rapidly colonised by bacteria achieving concentrations up to $10^{12}$ in the colon (Ohland & Jobin, 2015). This composition depends on different factors: type of delivery (vaginal or caesarean section), diet (breast milk or formula), or consumption of antibiotics, among others. The microbiota is considered stable and similar to adult microbiota around the child's 2$^{nd}$ year of life. An adult's microbiota is composed of 30 species of *Bifidobacterium*, 52 species of *Lactobacillus*, and others, such as *Streptococcus* and *Enterococcus* – ( Wallace et al., 2011; Salazar et al., 2014).

In the last years, the gut microbiota has been the focus for many researchers and studies, given that a healthy gut flora is largely responsible for overall health of the host. Normal gut microbiota has several and specific functions in the host's nutrient metabolism, xenobiotic and drug metabolism, maintenance of structural integrity of the gut mucosal barrier, immunomodulation, and protection against pathogens (Jandhyala et al., 2015).

Even, the gut microbiota has demonstrated the capacity of modulate emotion, motivation and higher cognitive functions (Carabotti et al., 2015), this modulation occurs through the Gut-Brain Axis (GBA). The GBA is a bidirectional communication network between the gut and brain, in which the communication occurs via three different pathways: neural, endocrine and immunological (Mayer et al., 2015).

Although the aetiology of ASD is unclear and there are different hypotheses regarding it, ASD has been related to some epigenetic conditions. Both pre-natal (such as maternal obesity or maternal inflammation) and post-natal factors (such as delivery, stress, diet or antibiotic treatment) are associated to a modification of gut microbiota composition, perturbing microbiota-gut-brain axis (Osokine & Erlebacher, 2017; Cristiano et al., 2018). In this review, we are focusing on the role of the GBA in ASD and, specifically, on the role of the GBA in the executive functions.

## 2. THE ROLE OF MICROBIOTA-GUT-BRAIN AXIS IN ASD

As previously detailed, there is extensive evidence that supports changes or alterations in the gut composition in ASD patients compared to the control group. Therefore, in this section we detail, as far as we know, the research that supports and emphasises the gut microbiota implication, as well as our intention to explain the putative mechanism underlying the gut-microbiota in this clinical population. There are several pathways that support the relation between the gut and ASD: para-cresol (p-cresol), amines produced by degrading-protein bacteria (Macfabe, 2012), an interrelation between *Clostridium* bacteria colonisation of the intestinal tract and autism (Argou-Cardozo & Zeidán-Chuliá, 2018), overproduction of short-chain fatty acid (SCFA) (Macfabe, 2012; Wang et al., 2012), microbiota-related alterations in bile acid and tryptophan metabolism (Golubeva et al., 2017), through serotonin as the nexus for the gut-brain axis in ASD (Israelyan & Margolis, 2018), among others.

*Bacterial changes in ASD*

Firstly, children with ASD appear to have more bacterial diversity and possess lower overall abundance of potentially beneficial taxa, such as *Bifidobacteria* and *Akkermansia* (Sanctuary et al., 2018), among other imbalances. For example, there is an observation of a significant increase in the *Firmicutes*/*Bacteroidetes* ratio in autistic subjects relative to normal subjects, or two times more abundant *Candida* in autistic individuals than in normal individuals (Q. Li et al., 2017).

The specific species of microbes altered in ASD versus controls vary from studies (Table 1). Different studies in humans have highlighted an increase of *Phylum Bacteroidetes* and *Proteobacteria*, and a decrease of *Phylum Firmicutes* and *Actinobacteria* in ASD children in comparison to non-autistic children. These studies have also observed a reduction in *Prevotella, Coprococcus, Enterococcus, Lactobacillus, Streptococcus, Lactococcus, Staphylococcus,* and *Bifidobacterium*, and an increase in *Rominnococcus, Sutterella, Desulfovibrio, Prevotella, Pseudomonas, Aeromonas, Enterobacterias* and *Clostridium* in ASD children. Several studies agree that the genus *Clostridium* increases in autistic children, causing higher propionic acid (PPA) levels and interactions with beneficial bacteria (such as *Bifidobacterium*) (Larroya-García et al., 2018).

*INSERT TABLE 1 AROUND HERE*

All these changes are occasionally reported as dysbiosis or dysbiosis intestinal. However, the use of term dysbiosis could be inadequate in ASD as it is not clearly established as a cause or an effect, although perceived as a real phenomenon. In this way, according to Hooks and O'Malley (2017) the term dysbiosis must be considered when establishing microbiota causality in disease, and, at this moment, it is unclear if these modifications cause particular ASD symptoms or they are a consequence. Mayer et al. (2014) point out that it is likely that GI symptoms in ASD patients are caused by multiple factors: central sensory augmentation, altered modulation of GI function (motility, secretion, and epithelial permeability) by the autonomic nervous system, altered regulation of motility and secretion by the enteric nervous system, or a combination of these factors. Thus, research with larger samples taken at different time points to asseverate dysbiosis in ASD is required.

*Microbial metabolites changes in ASD*

Moreover, bacteria can ferment dietary proteins through the putrefaction process. As a result of this process, gut health can be determined by products of microbial putrefaction. There are different microbial metabolites that have been associated with reduced viability of colonic epithelial cells in vitro, increased intestinal permeability, DNA damage and the inhibition of cellular respiration in colonocytes (Sanctuary, 2018).

A relationship between the degree of myelination and the intestinal bacterial composition has been postulated, thus, a functional microbiota provides adequate myelination in the prefrontal cortex (Hoban et al., 2016). In this way, changes in the gut microbiome were

shown to alter the composition of the microbial metabolome affecting highly permeable bioactive compounds, such as p-cresol, which could impair oligodendrocyte differentiation *in vitro* (Ntranos & Casaccia, 2018). Dysregulated myelination in the prefrontal cortex is then able to affect behavioural responses in mice, shifting them towards social isolation. In addition, the reduced social interactions could then limit microbial exchange (reviewed in Ntranos and Casaccia (2018).

Although there are several microbial metabolites, literature has mainly focused on p-cresol, an aromatic molecule from the environment or synthetized by intestinal bacteria from tyrosine and toluene (Yokoyama & Carlson, 1981; Selmer & Andrei, 2001; Cafaro et al., 2005). Usually, environmental exposure to p-cresol occurs through the skin, as well as the gastrointestinal and respiratory systems. The distribution of cresol is practically throughout body, found in blood, the kidneys, lungs and brain among others (Gadaskina & Filov, 1971; Green, 1975). This fact might indicate effects of p-cresol in the central nervous system (CNS). Additionally, it has been suggested that the urinary p-cresol contributes to the impairment of autism and gut function (Persico & Napolioni, 2013). It has also been proposed as a biomarker of autism liability in small children (Altieri et al., 2011). In this sense, high levels of urinary p-cresol in young ASD children are known, which are influenced by increased intestinal transit time and chronic constipation (Gabriele et al., 2016). Both p-cresol and gut-microbiota seem to be related by gender. The liability of p-cresol as a biomarker is especially useful in females and more severely affected males (Altieri, 2011). Nonetheless, the gut microbiota seemed to differ in a sex-specific manner (Suzuki et al., 2017), which is possibly due to the possible interaction between the microbiome, immune system and sex hormones (Kopec, 2018).

Furthermore, ASD children have higher faecal p-cresol and possibly lower GABA concentrations (Kang et al., 2018), and ASD have altered metabolite profiles in faeces when compared with neurotypical children and warrant further investigation of metabolites in larger cohorts. Other authors suggest an increase in GABAergic inhibitory neurons due an overexpression FOX/G1 gene (Mariani et al., 2015).

On the other hand, p-cresol and 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (a multiple *Clostridia* species metabolite) inhibit a crucial enzyme that converts dopamine to norepinephrine, the dopamine-beta-hydroxylase. The presence of p-cresol in the CNS would lead an excess of dopamine and its metabolites like homovanillic acid (DeWolf et

al., 1988; Southan et al., 1990; Keşli et al., 2014; Xiong et al., 2016). In fact, severity in autism symptoms has been related to urinary homovanillic acid levels and alterations in the brain catecholamines production (Garnier et al., 1986; Martineau et al., 1992). Binding the capacity of serotonin to its receptor was also studied, leading to a decrease in the serotonin transporter in the medial frontal cortex and low serotonin transporter synthesis altering serotoninergic terminals (Makkonen et al., 2008). Moreover, mutations in the SLC6A4 gene, which encodes the serotonin transporter, have been identified in ASD individuals (Adamsen et al., 2014). Also, the concentration of other amino acids, such as glutamate, has been altered in ASD (Lussu et al., 2017). Moreover, the severity of symptoms is correlated with the decrease of glutamate levels in the basal ganglia and anterior cingulate cortex (Horder et al., 2013; Tebartz van Elst et al., 2014), structures related with executive function (Lanciego et al., 2012; Bledsoe et al., 2013). All data suggest that monoaminergic systems are affected in ASD patients.

Concerning other microbial metabolites, a recent study has shown the existence of high concentrations of isopropanol in faeces (Kang, 2018), however the origin of the increased isopropanol is unknown and requires more research, given that isopropanol has not been investigated previously in children with ASD. Though, this finding is remarkable, considering that isopropanol is a neurotoxic organic solvent that contributes to the CNS and respiratory depression.

There is growing evidence that other compounds derivate from fermentation of enteric bacteria as SCFAs, which are present in ASD. One of them is PPA, synthetised by *Clostridia, Bacteriodetes* and *Desulfovibrio.* Intestinal absorption and transport of PPA to the blood and brain were reported many years ago (Cummings et al., 1987; Karuri et al., 1993). After administration of PPA, hippocampal expression of several genes was altered in rats. Thus, GFAP, the gene encoding intermediate filaments of astrocytes, and TNF-α were increased and OCT4, the undifferentiated embryonic stem cell-related gene, was decreased (Choi et al., 2018). Moreover, rats treated with PPA showed deficits in social behaviours and cognition (Shultz et al., 2009; MacFabe et al., 2011), using supra-physiological concentration. The underlying mechanisms of influences by PPA in ASD were considered through the altered mitochondrial activity. For instance, acyl-carnitine and all proteins of complex I of the electron transport chain were augmented, suggesting that these increments in mitochondrial respiratory function would explain the greater sensitivity in oxidative stress (Frye et al., 2013; Rose et al., 2014, 2017). These negative

effects depend on concentrations and exposition time to PPA. An increase in reactive oxygen species has been shown in the mitochondrial membrane in lymphoblastic cell lines using a high concentration without exceeding intestinal absorption concentration of PPA in humans. Interestingly, it has been reported that some ASD individuals may use PPA as an energy source, suggesting that they develop adaptive mechanisms against mitochondrial alterations in the presence of high levels of PPA (Frye et al., 2016).

Likewise, the immune system and inflammatory processes might be the cause GI symptoms appearing in ASD according to research. Thus, lipopolysaccharides (LPS) produced by intestinal microbiota are able to stimulate pro-inflammatory cytokines and cytokines production such as IL-6, TNF-α, IL-8 and IFN-γ among others, which were increased in the cortex of the brain and in the blood in individuals with ASD in comparison to non-ASD individuals (Croonenberghs et al., 2002; Ashwood & Wakefield, 2006; X. Li et al., 2009).

All the above mentioned, alongside evidence indicating changes in the composition of the commensal microbiome, can conclude that complex behaviours can be altered, including anxiety-like behaviour, emotional or depressive behaviour, and locomotor activity, among others, pointing to the possible relationship between the GBA and the development of ASD (Hsiao et al., 2013; Hsiao, 2014).

## 3. GUT-BRAIN AXIS: THE ROLE OF MICROBIOTA IN EXECUTIVE FUNCTION

Currently there is a growing interest in the role of microbiota in cognitive behaviour. In this section, we summarise the evidence from both preclinical and clinical studies regarding microbiota modulation and the effects on cognitive symptoms, specifically in that related to the executive function.

As previously detailed, executive dysfunction is very frequent in ASD children, as they often present difficulties with planning, and cognitive flexibility. In addition, this population also shows more deficits in shifting attention, sustained or selective attention, response inhibition, and working memory. Nonetheless, ASD can present an executive dysfunction in monitoring, a poorer performance on preparatory processing, a deficit in verbal fluency, and an inadequate function concerning concept formation, when the performance is compared to the control group (Hill, 2004; Margari et al., 2016).

According to Shaheen (2014), executive functions are often associated with the maturation of the prefrontal cortex. In fact, young children with rudimentary neurodevelopment of the prefrontal cortex acquire ways to inhibit impulses and regulate behaviour from a very early age. However, it is increasingly recognised that there are multiple areas of the brain involved in executive functions (e.g., dorsolateral prefrontal cortex, anterior cingulate cortex, orbitofrontal cortex, medial prefrontal cortex), and that each of these brain regions have extensive functional connections to other regions of the brain (subcortical areas and brain stem) (Miller et al., 2015).

On the other hand, a relation between obesity and cognitive impairment through the GBA has been found, although evidence in this sense is highly limited. Dietary patterns in ASD based on involving excessive consumption of processed snacks and calorie-dense foods may also explain emerging evidence of a higher incidence of obesity (McElhanon et al., 2014). According to Miller et al., (2015) and Solas et al. (2017), this type of diet influences gut microbiota, producing bacteria modifications and inflammation, and this could affect cognitive functions, although the precise mechanisms that underlie the connections between obesity and the risk of cognitive impairment are still largely unknown.

Some clinical studies have related obesity with the risk of developing mild cognitive impairment related to executive function deficits (Solas, 2017). In fact, a recent review has focused on the relation between hormonal alteration in obesity (Ghrelin, Glucagon-like Peptide 1) and their association with cognition and executive function. Thus, ghrelin activates hippocampal regions relevant for learning and memory or the Glucagon-like Peptide 1 also signals multiple brain regions, including the hypothalamus and the prefrontal cortex. In addition, adipose tissue has shown the capacity to produce substances such as leptin, IL-6, TNF-α, and such substances are associated with executive functions (Miller, 2015).

In light of the above, we could infer that if the executive function is related to obesity and the putative mechanism involved, the gut-microbiota have a relevant aspect in the modulation in the executive function, given that obesity appears to be related to an altered gut-microbiota (Castaner et al., 2018). Since findings indicate that the presence of commensal bacteria in the gut is critical for normal brain development, it raises the possibility that bacteria may influence brain plasticity and cognition later in life (Solas, 2017).

Other data showing the relationship between the GBA and executive function are provided by Labus et al. (2017), who observed a moderate correlation for the *Clostridia* in patients diagnosed with irritable bowel syndrome with several sensory integration regions including the thalamus, basal ganglia (caudate nucleus, putamen, pallidum, nucleus accumbens), and the superior part of the precentral gyrus (motor cortex). Similar correlations were found for the anterior insula and ventral prefrontal regions. It is interesting to note that the *Clostridia* are an abundant strain in ASD patients (Kim et al., 2018).

The microbiome–gut–behaviour axis, as part of the GBA, is being increasingly recognised as a modulator of social behaviour, and as an important regulator of prefrontal cortex myelination, a key brain region for driving complex cognitive behaviour (Ntranos, 2018).

Therefore, manipulations of microbiota with probiotics or diet could produce changes in cognition and executive functions through the GBA. This hypothesis is supported, among others, by the data of Ong et al. (2018), who utilised diffusion tensor imaging to show that overall changes in white matter structural integrity occurred in a diet-dependent manner, changing the microbiome. In fact, results of a clinical study performed on healthy women who intake fermented milk products with probiotics for 4 weeks showed a positive correlation to task-induced periaqueductal gray activity with cortical modulatory regions (medial and dorsolateral prefrontal cortex) (Tillisch et al., 2013). In this sense, Hoban et al. (2016) assume the potential therapeutic target of probiotics for psychiatric disorders involving dynamic myelination in the prefrontal cortex.

In addition, another specific target through a dietary intervention could be interventions that modify the microbial metabolites implicated in ASD. In that sense, dietary interventions could modify the p-cresol level, the metabolite mainly implicated in the microbiome GBA. For example, a daily consumption of 40 g of dark chocolate during a period of 2 weeks reduces the urinary excretion of the stress hormone cortisol, implicated in the GBA, and catecholamines and partially normalised stress-related differences in energy metabolism and gut microbial activities (such as, p-cresol sulphate) (Martin et al., 2009). Likewise, the consumption of 57 g of pistachios on a daily basis in prediabetic patients for 4 months decreased the p-cresol sulphate level in comparison to the control group (Hernández-Alonso et al., 2017). Moreover, the consumption of wheat bran extract (10 g/daily) during a period of 2 weeks decreases the urinary p-cresol excretion

and faecal *bifidobacteria* levels were significantly increased after a daily intake of 10 g, so then the gut health could be improved (François et al., 2012). In addition, probiotics and symbiotics have shown a reduction in the p-cresol level, the daily administration of a symbiotic formulation during 4 weeks reduced the plasma p-cresol level in CKD patients (Guida et al., 2014). Another study performed on a similar population who received a symbiotic therapy over 6 weeks (4-week washout) has shown a decrease in serum PCS and the stool microbiome was favourably modified (Rossi et al., 2016). In addition, the symbiotics administration during 30 days have shown to decrease the plasma p-cresol level in kidney transplant patients (Guida et al., 2017).

Other data showing the effects of dietary interventions in ASD are provided by BTBR mice, that is, one of the most widely used model of ASD (Needham et al., 2018). Social behaviour deficits in BTBR mice are associated to marked GI distress, evidenced by changes in intestinal permeability, transit and the enteric nervous system morphology, as well as altered microbiota composition (Golubeva, 2017). In that sense, dietary supplementation of a precursor tryptophan enhanced social interaction preference in BTBR (Zhang et al., 2015). Moreover, a ketogenic diet produced microbiota alterations such as a low *Firmicutes* to *Bacteroidetes* ratio in both caecal and faecal matter (Newell et al., 2016). In addition, changes to a few bacterial taxa that are also sex-specific have been recently reported in the BTBR intestine (Coretti et al., 2017).

According to such evidence, we could hypothesise that dietary interventions could be effective in ASD and we could expect a change in the cognitive and behavioural ASD symptoms through the GBA alteration. However, the preclinical and clinical studies performed in ASD, in which the intervention involved a microbiota modulation (faecal transplant, probiotics, symbiotics, or prebiotics) and their effects on behaviour symptoms, is highly limited (Table 2).

*INSERT TABLE 2 AROUND HERE*

*Preclinical studies*

The only preclinical study found to our knowledge in an animal model of ASD was that performed by Hsiao et al. (2013) who administrated *Bacteroides infantis* on a daily basis. Their results showed correct gut permeability and alterations in microbial composition and ameliorates ASD-related defects in communicative, stereotypic, anxiety-like and sensorimotor behaviours. These results support a gut-microbiome-brain connection in

ASD and identify a potential probiotic therapy for GI and behavioural symptoms in autism. Although there are other studies in which the effect of microbiota in the ASD is assessed, as far as we know, no one assesses the effect of a microbiota modulation in ASD symptoms.

*Clinical studies*

There has also been some interesting progress in humans in this field. The main intervention studied in the clinical studies that implicate a microbiota modulation is through the probiotic administration and there is one research that used faecal transplantation. In the study performed by Sichel, Roberts, Sichel, and Sichel (2013), the administration of a probiotic mixture during 6 months in 33 children diagnosed by ASD showed a decrease in diarrhoea severity and constipation severity (n=25). Overall, 88% reported a decrease in total ATEC score, signifying an improvement of ASD symptoms. Mean ATEC values decreased from 72.8 prior to treatment to 58.3 following treatment initiation. Participants also had significant improvements in all ATEC domains (speech/language/communication, sociability, sensory/cognitive awareness, and health/physical/behaviour). However, these results are not significant due to the study design not extrapolating the results.

Another microbiota modulation studied is antibiotic administration. After 8 weeks of Vancomycin treatment, the authors reported a clear improvement in communication and behaviour (Sandler et al., 2000). After the treatment period with Vancomycin, the authors administered a probiotic mixture during 4 weeks. The gains observed during the antibiotic treatment did not endure during the probiotic phase, contrary as could be expected. These results were explained in the difficulty in disguising the taste and the poor adherence or compliance to the probiotic treatment (Sandler, 2000). Similar results were reported in a probiotic-placebo controlled cross-over designed study, in which after 3 weeks of probiotic (*Lactobacillus plantarum* WCFS1) administration did not show any effect on the psychological assessment as compared to the placebo group (Parracho et al., 2010).

Results from recent small open-label study developed by Kang et al. (2017) suggest that microbiota transplantation could be a safe intervention in ASD children (7-16 years). This intervention shows significant improvement in GI and ASD-related symptoms, that improvements were maintained at least during 8 weeks. In addition, a significant negative correlation between changes in GSRS and PGI-II were reported by the authors,

suggesting that GI symptoms worsen directly with ASD behaviours, and that these can be altered via microbiota transplantation. Regarding ASD-related symptoms, the data showed an improvement in irritability, hyperactivity, lethargy, stereotypy and aberrant speech, as well as in adaptive behaviours (communication, daily living skills and socialisation).

With regard to the type of intervention, there are no data from microbiota transplantation previous to 7-year-old participants. As Kang et al. (2017) detailed, the US Food and Drug Administration (FDA) limited their participants to children ages 7–17 years, since most faecal microbiota transplantation studies have been conducted on adults, and there was very limited data and knowledge of the impact and usage of faecal microbiota transplant for younger children.

Although the studies evaluating the role of microbiota in the modulation of cognitive symptoms of ASD are limited, other research has pointed out this effect in other related clinical populations (Roman, Abalo, et al., 2018). In that sense, probiotic supplementation (*Lactobacillus Rhamnosus* GG) during the first 6 months of life may reduce the risk of neuropsychiatric disorder development (attention deficit hyperactivity disorder or Asperger syndrome) later in childhood, possible by mechanisms not limited to gut microbiota composition (Pärtty et al., 2015). Another study performed in very premature babies concluded that the administration of a probiotic combination (*Bifidobacterium infantis*, *Streptococcus thermophilus* and *Bifidobacterium lactis*) soon after birth until discharge home or term-corrected age did not adversely affect neurodevelopment or behaviour in early childhood (Jacobs et al., 2017).

A recent study performed in myalgic encephalomyelitis or chronic fatigue syndrome show large effect size estimates in several cognitive outcomes after administration during 4 weeks of an antibiotic (Erythromycin) and probiotic (*Lactobacillus rhamnosus*, *Bifidobacterium lactis*, *Bifidobacterium breve*, *Bifidobacterium longum*) therapy taken on alternate weeks. The cognitive outcome suggested an improvement in sustained attention from baseline to post, and also indicated improvement in processing speed, cognitive flexibility, story memory and verbal fluency (Wallis et al., 2018). Another recent study conducted on a similar clinical population, fibromyalgia patients, concluded that the daily administration of probiotics (*Lactobacillus Rhamnosus* GG, *Casei*, *Acidophilus*, and *Bifidobacterium Bifidus*) during 8 weeks improves impulsivity and decision-making in these patients (Roman, Estévez, et al., 2018).

Another population in which the probiotic has shown a positive effect is on HIV patients, who were treated during 6 months with a multi-strain probiotic (*Lactobacillus plantarum, Streptococcus thermophilus, Bifidobacterium breve, Lactobacillus paracasei, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus acidophilus, Bifidobacterium longum, and Bifidobacterium infantis*). The authors specifically reported an improvement of short and long memory and abstract reasoning (Ceccarelli et al., 2017).

Concerning memory cognitive dimension, Benton et al. (2007) found an unexpected and possibly coincidental finding: a poorer performance on two measures of memory after the administration of *Lactobacillus Shirota* over 3 months on healthy subjects (Benton et al., 2007).

However, other studies have not reported a positive effect or superior to probiotics in comparison to the placebo on cognitive improvement, for example, the administration of *Lactobacillus rhamnosus* (JB-1) in healthy males during 8 weeks was not superior to placebo in visuospatial memory performance, attention switching, and rapid visual information (Kelly et al., 2017). However, this probiotic had shown a positive effect in preclinical studies, and the actual challenge is translating the promising preclinical studies to healthy human participants. Whereas the administration of *Bifidobacterium longum* on healthy male volunteers during 4 weeks showed a subtle improvement in visuospatial memory performance (Allen et al., 2016).

The aim of this review is focused on the role of gut-brain axis in ASD and, specifically, the role of the gut-brain axis on executive functions. In the first section, we have provided a brief view in relation to the link between cognitive symptoms and GI symptoms in ASD. This relation makes us think about an altered gut microbiota and the possible relation through the gut-brain axis. Thus, in the second section, we reported bacterial and microbial metabolites changes reported in ASD patients and their relation with cognitive modulation. Finally, in the third section we detailed all the evidence, to our knowledge, available concerning the different microbiota modulation (antibiotic, probiotic, faecal transplantation, and others).

This review has detailed extensive and different hypotheses in which the ASD gut-microbiota is altered, however, it is not clear if the microbiota altered is a consequence of ASD symptoms or vice versa, so then more research is needed to disclose this important aspect and to allow, or not defined, ASD as a related dysbiosis intestinal pathology

(Mayer et al., 2014; Hooks & O'Malley, 2017). Therefore, preclinical and clinical studies are designed to specifically evaluate the causal relationship between these factors and to obtain a better understanding of the role of the GBA in ASD. In that sense, studies with animal models of ASD analysed the gut phenotypic. The use of appropriate behavioural and experimental assays is required.

According to the findings translated by preclinical studies to clinical studies and the role of gut microbiome alteration in ASD pathophysiology, more research is needed in this field to disclose the positive effects of different interventions and to disclose the possibility for novel treatment approaches, so there is an urgent need for large-scale, highly controlled, longitudinal human studies showing the causes and effects of dysbiotic gut states. One of the most considerable interventions could be probiotics, as a safer alternative to elimination diets, with their ability to target multiple physiological areas (Doenyas, 2018). In this sense, future studies must consider aspects such as the following: study design, intervention, adherence, safety assessment, strain specificity, bacterial quantification and microbial metabolites (Shane et al., 2010; Welch et al., 2011).

Taking into consideration all of the aforementioned evidence, probiotics among other microbiota interventions could improve ASD symptoms, especially in executive symptoms, among others. These new perspectives would open a promising therapeutic perspective, however, nowadays clinical evidence is very limited, and it is necessary to perform more clinical trials in which authors explore the possible effects of such intervention on ASD patients.

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**Table 1. Altered gut microbiota in ASD.**

| Altered gut microbiota | Reference |
|---|---|
| ↑*Clostridium* | (Parracho et al., 2005; Song et al., 2004) |
| ↑*Sutterella* spp.; ↑*Ruminococcus torques*; ↓*Akkermansia muciniphila* | (Wang et al., 2011, 2013) |
| ↑*Clostridium*; ↑*Sutterelaceae*; ↑*Enterobacteriacae*; ↓*Bifidobacterium* | (De Angelis et al., 2013) |
| ↑*Desulfovibrio*; ↑*Bacteroides vulgatus*; ↑*Ruminococcus*; ↓*Bifidobacterium* | (Finegold et al., 2010) |
| ↑*Collinsella*; ↑*Corynebacterium*; ↑*Dorea*; ↑*Lactobacillus*; ↓*Alistipes*; ↓*Bilophila*; ↓*Dialister*; ↓*Parabacteroides*; ↓*Veillonella* | (Strati et al., 2017) |
| ↓*Bifidobacter; ↑Lactobacillus* | (Adams, 2011) |
| ↓*Bacteroidetes; ↑Betaproteobactera* | (Williams et al., 2011) |
| ↑*Sutterella* | (Williams et al., 2012) |
| ↓*Prevotella; ↓Coprococcus; ↓Veillonellacea* | (Kang et al., 2013) |
| ↑*Clostridia; ↑Ruminococcus; ↑Bacteroidetes; ↑Clostridium difficile* | (Finegold et al., 2002) |
| ↑*Lactobacillaceae; ↑Bifidobacteraceae; ↑Veillonellaceae* | (Pulikkan et al., 2018) |

**Table 2. Preclinical and clinical studies about microbiota intervention and their effects on ASD behavioral symptoms.**

***Preclinical studies***

| Reference | Intervention | Population | Variables | Results |
|---|---|---|---|---|
| (Hsiao, 2013) | Probiotic (*Bacteroides fragilis*) (6 days) | Pregnant C57BL/6N mice | *Behavioral testing* (pre-pulse inhibition, open field exploration, marble burying, social interaction and adult ultrasonic vocalizations) *Microbial outcomes* *Metabolomics screening* | *Bacteroides fragilis* corrects gut permeability, alters microbial composition and ameliorates ASD-related defects in communicative, stereotypic, anxiety-like and sensorimotor behaviors |

***Clinical studies***

| Reference | Intervention | Population | Variables | Results |
|---|---|---|---|---|
| (Sandler, 2000) | Vancomycin (8 weeks) – Probiotic mixture (*Lactobacillus acidophilus, L bulgaricus, and Bijidobacterium bifidum*) (4 weeks) | 11 children with regressive-onset ASD | *Psychologic Evaluations* (assesment for behavior, communication, and social skills) *Microbial outcomes* | During the probiotic administration, most parents reported substantial behavioral deterioration within 2 weeks of discontinuance of vancomycin treatment. Because of difficulty in disguising the taste, probiotic treatment compliance was very poor in several children. Behavioral deterioration appeared to occur whether or |

| | | | | |
|---|---|---|---|---|
| | | | | not the child was compliant with the probiotic therapy regimen. |
| (Parracho, 2010) | Probiotic (*Lactobacillus plantarum* WCFS1) (3 weeks) | 39 ASD children | *Microbiological analysis*<br>*Bowel function and GI symptoms*<br>*Psychological analysis (*DBC, TBPS*)* | No significant effect as compared with placebo group |
| (Sichel, 2013) | Probiotic Delpro® (*Lactocillus acidophilus, Lactobacillus casei, Lactobacillus delbruecki, Bifidobacteria longum, Bifidobacteria bifidum*) (6 months) and immunomodulator Delpro® | 33 ASD children | *21-day stool frequency* diary prior and 21-day stool frequency diary following completion of treatment<br>*ATEC tool*<br>(speech/language/communication, sociability, sensory/cognitive awareness, and health/ physical/ behavior) | Probiotic and immunomodulator Delpro® may have significant benefit in the treatment of GI distress and other ATEC signs and symptoms among this population |
| (Kang, 2017) | Microbiota Transfer (18 week-tratment) | 18 ASD-diagnosed children (7 - 16 years) | CARS<br>GSRS<br>DSR<br>PGI-R<br>ABC<br>SRS<br>VABS-II | <u>GSRS:</u> approximately 80% reduction of GI symptoms at the end of treatment.<br>Improvements in symptoms of constipation, diarrhea, indigestion, and abdominal pain<br><br><u>Behavioral ASD symptoms.</u><br>improved significantly and remained improved 8 weeks after treatment ended.<br><br>Results were maintained 8 weeks after the treatment. |

ABC: Aberrant Behavior Checklist; ADI-R: Autism Diagnostic Interview-Revised; ADOS 2: Autism Diagnostic Observation Schedule- Second Edition; ATEC: Autism Treatment Evaluation Checklist; CARS: Childhood Autism Rating Scale; CBCL 1.5-5 Child Behavior Checklist 1.5-5;

DBC: Development Behaviour Checklist ;DSR: Daily Stool record; GIRS: Gastrointestinal Symptom Rating Scale; GISI: Gastro-intestinal Severity Index; GMDS-ER: Griffiths Mental Development Scale-Extended Revised; Mc Arthur-CDI: MacArthur-Bates Communicative Development Inventories; PGI-R: Parent Global Impressions; RBS-R: Repetitive Behavior Scale-Revised; SCQ: Social Communication Questionnaire; SRS: Social Responsiveness Scale; TBPS: Total Behaviour Problem score; VABS-II: Vineland Adaptive Behavior Scale II.